Md. Shahidul Islam
Editor

Islets of Langerhans

Second Edition

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With 170 Figures and 34 Tables

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ISBN 978-94-007-6685-3 ISBN 978-94-007-6686-0 (eBook)
ISBN 978-94-007-6687-7 (print and electronic bundle)
DOI 10.1007/978-94-007-6686-0
Springer Dordrecht Heidelberg New York London

Library of Congress Control Number: 2014950662

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

"Dedicated to the living memory of
Matthias Braun, M.D., Ph.D. 1966–2013"

Foreword

The tiny islets of Langerhans receive an extraordinary amount of attention from a variety of interested parties, many of whom will enthusiastically welcome publication of the second edition of the "Islets of Langerhans," ably edited by Md. Shahidul Islam, M.D., Ph.D., of the Karolinska Institute, Stockholm, Sweden. The amount of attention paid to islets is well deserved because the failure of their β cells to produce sufficient amounts of insulin results in diabetes, with its climbing prevalence worldwide and devastating complications. In type 1 diabetes the β cells are almost completely decimated by the vicious process of autoimmunity. With the far more common type 2 diabetes, the insulin resistance associated with obesity and our sedentary life style is linked to reduced β cell mass and function. The simplest view is that the β cells die because they are stressed by overwork, resulting in reduction of insulin secretion, which allows glucose levels to rise enough to cause further impairment of secretion through a process called glucotoxicity. Thus there is a loss of both β cell mass and function, resulting in the concept of decreased functional mass. Most people with insulin resistance never develop type 2 diabetes, which leads to the conclusion the β cell failure is the sine qua non for the development of the diabetic state.

Following from the above, the premise that β cell failure is the root cause of diabetes is conceptually very simple, which leads to the conclusion that the diabetic state should be reversed by administering insulin with injections, restoring β cell function with medication or by replenishment of the β cell deficit with transplantation or regeneration. Indeed, the all important proof-of-principle was achieved in the 1990s with the demonstration that both types 1 and 2 diabetes could be reversed with islet transplantation either as isolated islets placed in the liver or as whole organ pancreas transplants.

This second edition of "The Islets of Langerhans" is very timely, because in spite of the seeming simplicity of the basis of diabetes and progress with β cell replacement, we are still too far from our goal of providing these treatments for those in need. We need to understand islets on the most basic level so that preclinical therapeutic approaches can be explored and then taken to patients. The 49 chapters in "The Islets of Langerhans" provide up-to-date information on a carefully selected range of topics.

Important Unsolved Islet Puzzles

Knowing full well there are many opinions about which unsolved islet questions are most important, I will briefly mention a selection of issues that have captured my attention.

The islet as an organ The anatomy of islets is high organized with its cellular arrangements and islet-acinar portal blood flow. We know that β cell secretion has a major influence on glucagon secretion, but we have much to learn about the other interactions between beta, alpha and delta cells and how secretion from all of these influences downstream acinar cell development and maintenance. The role of the pancreatic polypeptide (PP) cells remains very much a mystery.

The mystery of glucose-stimulated insulin secretion (GSIS) For years we have had some understanding of the so-called K_{ATP} pathway of GSIS, yet we have little understanding of the quantitatively important K_{ATP}-independent pathway. This remains a major unsolved problem in β cell biology.

Finding new pharmacologic targets for insulin secretion Many of the chapters focus on the cell biology of insulin secretion, and there is much to be learned about these very basic facets, such is glucose and fat metabolism, ion and other transporters, mitochondrial function, calcium handling, phosphorylation reactions, insulin biosynthesis and more. A key question is how much more insulin secretion can we get out of a β cell? Simply put, if the cell is depolarized and fully stimulated by cyclic AMP, what approaches can be used to generate more insulin secretion?

Dedifferentiation of β cells and islet cell plasticity The phenotype of β cells in the diabetic state is deranged and accompanied by dysfunctional insulin secretion, with evidence pointing to glucotoxicity as the major driving force responsible for these changes. Restoration of normal glucose levels reverse these changes, but questions remain as to whether these β cells dedifferentiate toward a pluripotent progenitor state or some other distinct phenotype. The field is now swirling with the concept of islet cell plasticity, such as the potential alpha and delta cells being converted to β cells. There is also a big question about the alpha cell hyperplasia seen when glucagon action is inhibited: what is the signal of alpha cell growth?

The need for more β cells The β cell deficiency of diabetes could be restored by regeneration of new β cells in the pancreas or by transplanting β cells from some other source. As described in several chapters, this is one of the main priorities in diabetes research. Adult human β cells replicate very slowly but there has been great progress in understanding cell cycle mechanisms, which could somehow be exploited. Exciting progress has also be made with making mature β cells from human embryonic stem cells and from induced pluripotent stem cells. There have also been advances in exploiting the potential of exocrine multipotent progenitor cells and in bioengineering. Porcine cells also remain on the list.

Why do β cells die and how can this be prevented? We know that β cells in type 1 diabetes are killed by the immune system, and have watched impressive advances in defining the interactions among effector T cells, T regulatory cells, B cells, and the innate immune system. The process is very aggressive and there is a great need to control it with minimal or no immunosuppression. The Holy Grail is

restoration of tolerance. An old approach receiving renewed attention is encapsulation of islets to protect them from immune killing. The new biomaterials and approaches are exciting but we cannot yet be confident about its eventual value. In the context of type 2 diabetes much has been written about how β cells die, with mechanisms receiving the most attention being oxidative stress, endoplasmic reticulum stress, toxicity from IAPP oligomers, and the general concept of "overwork." The reality is that the death rate is very low and we have little idea about which mechanisms are the most important.

Of course there are many other important questions, but this sampling fits well with the contents of this valuable new edition of "The islets of Langerhans." Its chapters contain important information about these key questions, which make it likely that hours spent reading this book should help our field connect the critical dots that will result in new treatments for people with diabetes.

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On Becoming an Islet Researcher

At the time of this writing, I have spent a quarter of a century in islet research, but the purpose of this article is not to share my journey with you. I do not want to bore you with anecdotes from my experiences, but it is impossible that my views will not be subjective.

Be Clear About Your Goal

Irrespective of whether you have started islet research recently, or you have spent almost a whole life in islet research, it is worthwhile to reflect upon your goals. Here is the big picture. About 194 million people in the world are suffering from some form of islet failure, and by 2025 this number may increase to 333 million. The β-cells of many young people and children are dead. To live a normal life, they need to take insulin injections daily, and they need to prick their finger tips for testing plasma glucose concentration, numerous times. In others, overwork of the β-cells caused by overeating leads to the failure, and eventually to the death of these cells. If you want to see the burden of islet failure, do not hesitate to visit a nearby diabetes clinic. This may open your eyes, or give you a much needed insight.

Your goal is to contribute to the discovery of something, so that this huge human tragedy can somehow be prevented, treated, or cured. Your goal is not primarily to publish papers or just to do some experiments solely to satisfy your own intellectual curiosity. Your goal is not just counting the numbers of your publications, and their impact factors, and not to secure a promotion, advance your own career, or receive prizes. You have a bigger goal, which you may or may not reach, within your lifetime, but if you are conscious of your ultimate goal, you are better prepared to work steadily towards that goal. You may then become the islet researcher that you dream to be.

If you wish not to have a clear goal and prefer to see your scientific journey as the goal, then it is up to you. I think it is important to have visions and goals, perhaps some small goals, if not a big goal to start with.

Become the Finest Islet Researcher

The making of an islet researcher is not easy. Becoming a good islet researcher can be a long process. Educate yourself, keeping in mind that it is never too early or too late to start learning anything new. Through a choice of an unconventional path of education, you may become a specialist in more than one subject, and may thus be better prepared. You may first become a molecular biologist, and then educate yourself as a chemist. Numerous other combinations are possible. Enrich yourself with the necessary knowledge, and the skills from whatever source you need to. You may need to move to the environments that promote creativity, that have better infrastructures, and traditions for good research. To do this, you may need to leave your home country, and then struggle hard to adapt yourself to the new environments.

You almost certainly need to acquire a broad base of knowledge before you focus on some special areas. At the same time, you must also be able to filter out as much unnecessary information and distractions as possible. In an age of information pollution, your ability to decide what to filter out, and to filter those out effectively, may determine how intelligent you are. Clearly, you will not be able to do many things, at least not at the same time.

Start with asking one of the most important questions in the field of islet research, keeping in mind that you are expected to discover things that you are not aware of beforehand. Do not waste time in rediscovering the wheel. If you are not asking an important question, then it does not matter how sophisticated instrument or advanced method you are using.

Identify your strength, strength of your institution, and that of your network if you wish to. Once you have identified the strengths, use those. Do what you think is the right thing to do without fear of being judged by others, but resist the temptation to work on many projects at the same time; take the one you have started to completion. You do not need to compare yourself with others. You do not need to think that you are less talented than others. Do not give up when the going forward seems tough. Dig as deep as possible or change the direction based on your sound judgment. See mistakes as valuable learning experiences. If you have time, get inspirations by reading the life histories of other great scientists. From such readings, you may get important insights about how to develop your own intuition and creativity, and about how to get clues about the so called "unknown unknowns."

Depending on your question and the nature of the project, you may find it useful to work alone or with a small dedicated team, or you may need to network personally with a handful of scientists, including some who are not conventional islet researches. You may benefit more if you attend meetings that do not deal with islet research or if you read papers that do not deal with islet research. If you can bring a small piece of new knowledge from the fields that are very distant from the contemporary islet research, and apply that knowledge to solve some of the common questions in the field of islet research, that may contribute to a breakthrough.

Islet research is not just about science, it is a way of life. You have to make difficult choices during your journey. You are sincere about your purpose in life. At times you may have to juggle with too many bolls in the air. It will affect your social life and your relationships with your near and dear ones. Set your priorities right. You have decided to spend your life for the benefit of people who have islet failure. You are not after money, fame, glamour or festivities. You are a genuine islet researcher.

The Ecosystem of Islet Research

Unfortunately, it is not enough that you have developed yourself as one of the finest islet researchers, and that you have clear visions and goals. The chances of breakthroughs in islet research will depend on what we can call the ecosystem of islet research. The ecosystem of islet research will determines the growth, survival, and creativity of the type of islet researchers that I have alluded to. Important components of this ecosystem include the educational and research enterprises, the funding agencies, the governments and policy makers, industries, publishers, and last but not the least, the patient organizations. The ecosystem of islet research, as well as the ecosystem of research in general, has changed over the past decades, and it will keep changing. For an individual islet researcher, it may be difficult to track these changes, and it may be impossible for them to adapt to the changes that are taking place rather rapidly. At first sight, it may appear that the ecosystem has worked well, and has ensured important discoveries at a steady rate. Islet researchers are not supposed to question the ecosystem; the only thing expected of them is to adapt to the changes for their own survival and earn their bread and butter.

Survival of the islets researchers depends on their ability to write grant applications, and their ability to convince the people who read those applications that their ideas are excellent and the goals are achievable. Islet researchers spend enormous amount of time, money, and energy on writing grants and in about 80 % of the cases, the applications are rejected. It is impossible to assess who is the most talented islet researcher. Since talent cannot be measured, an opportunistic way is to measure what islet researchers have published in the past and how many times those publications have been cited. Even if one is able to identify the most talented islet researchers based on their performances in the past, it is impossible that these selected islet researchers will perform equally well in the future. Some scientists think that the system we have is counter-productive, and wasteful of time and energy (Garwood 2011).

The ecosystem of research, in general, seems to have changed in such a way that it is possible for some academic psychopaths to fool the system. They will write in their grant applications whatever is needed, and they will do whatever else is necessary to manipulate the system in their favor. One of the most talented scientists in the world published in one of the world's most luxurious journal, one of the most exciting breakthroughs in stem cell research that turned out to be bogus (Normile 2009). In one investigation, a bogus manuscript, written by some bogus

authors, from some bogus universities was accepted for publication by many scientific journals (Bohannon 2013). The system has become so corrupted that it is apparently possible for some scientists to publish without doing any experiment (Hvistendahl 2013). Don Poldermans published more than 300 papers some of which were fraudulent. Changes in clinical practice based on these papers has caused death of numerous people (Chopra and Eagle 2012). In islet research also, data included in many papers published in elegant journals cannot be reproduced. Many islet researchers are putting their names on papers written by their students, colleagues, and friends with minimal intellectual contributions.

It is possible that the altered ecosystem of islet research is supporting the proliferation of a group of islet researchers who are aggressive bullies, and academic psychopaths, and it is leading to the extinction of the finest islet researchers, who are genuinely talented and sincere, but are unable to survive in the ecosystem which is perceived as unsupportive and hostile.

Final Remarks

There is no take home message in this article. I have been partially able to write part of what I have thought, and if you have read this, then I have perhaps been able to transfer my thoughts to you.

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References

Bohannon J (2013) Who's afraid of peer review? Science 342:60–65

Chopra V, Eagle KA (2012) Perioperative mischief: the price of academic misconduct. Am J Med 125:953–955

Garwood J (2011) The heart of research is sick. Lab Times 2:24–31

Hvistendahl M (2013) China's publication bazaar. Science 342:1035–1039

Normile D (2009) Scientific misconduct. Hwang convicted but dodges jail; stem cell research has moved on. Science 326:650–651

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The Comparative Anatomy of Islets

1

R. Scott Heller

Contents

Abstract

In the past 20 years, numerous publications on a variety of mammalian and non-mammalian species have appeared in the literature to supplement the excellent comparative work performed in the 1970s and 1980s by the Falkmer, Epple, and Youson groups. What emerges is that islets are much more complex than once thought and show a lot of similarities in rodents and higher primates. The diversity of lifestyles, metabolic demands, and diets has most likely influenced the great diversity in both structure and cell-type content of islets in lower vertebrate species. In this chapter, I try to provide an overview of the evolution from endocrine cell types in invertebrates to the higher mammals and focus on what has been reported in the literature and some of our own experiences and also include a description of other hormones reported to be found in islets.

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_2,

Keywords
Comparative hormones • Islets • Species • Structure • Pancreas • Anatomy • Comparative • Species • Insulin • Glucagon • Hormones

Introduction

During the past 30 years or so, we have seen emergence of data on islet architecture and cell type expand from just a few species into a broad diversity across many phyla. In three model organisms in which developmental biology studies of the pancreas have been conducted (*Oryzias latipes*, *Xenopus laevis*, and chicken), three buds materialize from the gut tube: two from its ventral side and one from the dorsal (Assouline et al. 2002; Kelly and Melton 2000; Kim et al. 1997). In mouse, while initially three buds exist that come from the gut tube (where there is contact between the endoderm and the endothelium), the pancreas only develops from two of these buds, one dorsal and one ventral (Lammert et al. 2003). This aspect of dorsal and ventral pancreas development of the pancreas has never been examined in species earlier in evolution that teleost fish. The differences in the development of the dorsal and ventral pancreas, which later fuse to form one organ in higher vertebrates, also likely explain the different composition of islets in the head (ventral derived) or tail (dorsal derived).

The islets of Langerhans have generally been described as round clusters composed mainly of insulin (β-cells) and glucagon (α-cells) and minor populations of somatostatin (δ-cells) and pancreatic polypeptide cells (PP) generally in the mantle or rim of the islets. As the chapter and species evolve, you will see there are many exceptions to this generalization. Recent times have shown that in most species during development and the early postnatal period, a unique fifth endocrine cell type, the ϵ-cell, which produces the hormone ghrelin, is found (Heller et al. 2005; Prado et al. 2004; Wierup et al. 2002). Other endocrine hormones found in the islets are also discussed (Table 1). One must remember that almost all of this knowledge has been gained by using immunocytochemical methods based on antisera raised primarily against rodent or human hormones and that differences in the structures of the hormones between different species may be the reason why some hormones are found in some species and not in others. Finally, I have taken a phylogenetic approach to the presentation of the different species discussed (Fig. 1).

Invertebrates

A substantial amount of literature exists on hormones of the pancreatic family in a number of different invertebrates like the silk worm, tobacco hornworm, and dipteran blowfly, in which hormones belonging to the insulin, glucagon, PP (started out as NPY), and somatostatin families have been demonstrated to exist (Falkmer and Ostberg 1985). In addition, a large amount of work on insulin peptides in the

Table 1 Other peptides found in islets

Peptide	Cell type	Species	References
CART	δ	Rat	Wierup et al. 2004
		Sheep	Arciszewski et al. 2008
	δ	Ice rat	Gustavsen et al. 2008a
CCK	α, β, δ	Spiny dogfish	Jönsson 1995
	β	Rat	Shimizu et al. 1998
	β	Ice rat	Gustavsen et al. 2008a
CGRP	δ	Rat	Fujimura et al. 1988
IGF	δ, PP	Lizards	Reinecke et al. 1995
	α, PP	Frogs, birds	Reinecke et al. 1995
PYY	PP	Cat, dog, pig	Böttcher et al. 1993
	PP, α	Mouse, rat	Böttcher et al. 1993
	α, β, δ	Rat	Jackerott et al. 1996
		Bullfrog, eel	Ding et al. 1997a
	α	Sea bream	Navarro et al. 2006
		Brazilian sparrow	Nascimento et al. 2007
	α, PP	Ice rat	Gustavsen et al. 2008a
	PP	Spiny mouse	Gustavsen et al. 2009
Secretin	Unique	Frogs	Lee et al. 2003; Fujita et al. 1981
TRH	α, β	Rat	Kawano et al. 1983
		Rat, guinea pig	Tsuruo et al. 1988

The references cited are mostly based on immunoreactivity

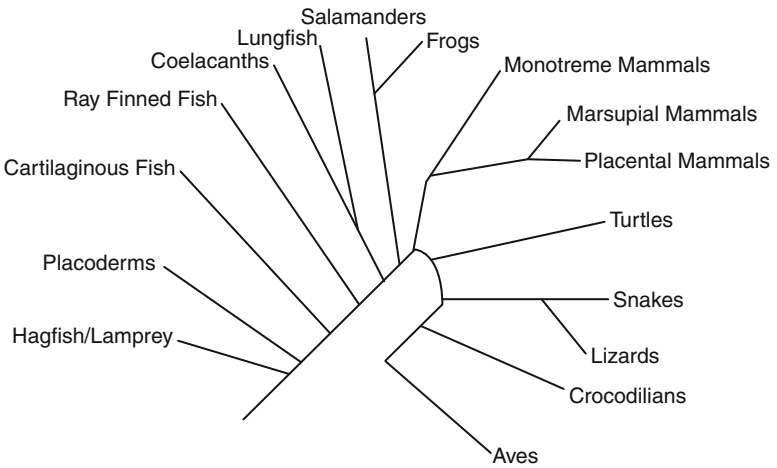

Fig. 1 Phylogenetic tree of vertebrates. The base of the phylogenetic tree represents the ancestral lineage, and the ends of the branches signify the descendents of that ancestor. When you move from the base to the ends, you are moving from the past to the present. When a lineage divides (speciation), it is demonstrated as branching on a phylogeny. When a speciation episode occurs, a single ancestral lineage gives rise to two or more daughter lineages (The figure was adapted from the Understanding Evolution website from the University of California Museum of Paleontology http://evolution.berkeley.edu)

phylum Mollusca has also been performed (Smit et al. 1998). Here I focus on the Drosophila, where some very important recent molecular studies have been performed that give a great insight into the evolution of the insulin- and glucagon-like peptides.

Pancreatic islets are not found in any invertebrate species, but surprisingly many regulatory peptides are found in the midgut of Drosophila, and Ilp3, the equivalent of the Drosophila insulin gene, is found surprisingly in the muscle and not the endocrine cells (Veenstra et al. 2008) (Fig. 2). The major source of insulin-like peptides (there are seven in Drosophila) is a group of neurons in the pars intercerebralis of the brain (Ikeya et al. 2002). Like insulin from islets, the insulin-like peptides in Drosophila are crucial for the regulation of glucose (actually trehalose, a disaccharide of two glucose molecules) levels in the hemolymph and energy metabolism (Rulifson et al. 2002). Ablation of the insulin-producing neurons generates growth-deficient and diabetic phenotypes. Interestingly, it has been demonstrated that the insulin-producing neurons make direct projections to communicate with the corpora cardiaca (CC) cells located at the heart, which produce glucagon-like peptides. Thus, information from insulin-producing cells to communicate with α-cells was established quite early (Rulifson et al. 2002).

The insect corpora cardiaca (CC) are clusters of endocrine cells in the ring gland. One of the principal peptides produced is adipokinetic hormone (AKH), which is surprisingly similar to mammalian glucagon, is found in dense core vesicles, is synthesized as a preprohormone, and has actions on the insect fat body to increase glycogenolysis and lipolysis, similar actions to mammalian glucagon (Veelaert et al. 1998; Van der Horst 2003). Injection of AKH in insects is sufficient to increase glucose in the hemolymph (Veelaert et al. 1998; Van der Horst 2003). A recent study demonstrated that ablation of the CC in Drosophila disrupts glucose homeostasis and that over expression of the AKH gene reverses the effects on hemolymph glucose, thus demonstrating that a glucagon-like peptide is critical to the regulation of glucose levels even in invertebrates (Kim and Rulifson 2004). In addition, like in mammalian islets, the CC cells are in direct contact with the vasculature. Interestingly, the CC cells arise during development from delamination from epithelia that give rise to the gut (DeVelasco et al. 2004). Kim et al. speculate that CC and neuroendocrine regulatory cells that are important for metabolism may have come from an ancient energy-sensing cell and that β-cells may have actually come from ancient α-cells. This is a very interesting and intriguing idea but will require more research to prove the hypothesis.

If we look back to the stem of vertebrate evolution and examine the primitive chordates urochordates (tunicates) and cephalochordates (Branchiostoma-Amphioxus), we find that peptides related to somatostatin, glucagon, and PP like (primitive NPY) are localized in the tunicate brain, while insulin uniquely moved to the gastrointestinal tract (GI) mucosa (Ali-Rachedi et al. 1984; Galloway and Cutfield 1988). Thus, it appears that insulin is the first hormone to have left the nervous system for the gut. Amphioxus is the earliest species for which all four of the main endocrine cell types are found in the GI tract, but not yet organized into an islet organ (Epple and Brinn 1986) (Fig. 2).

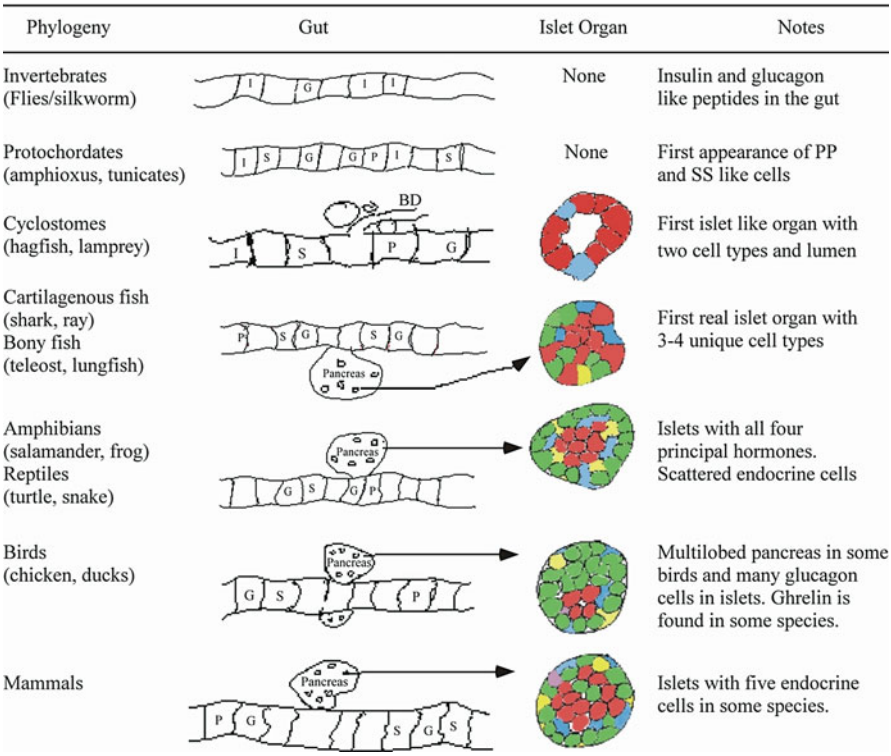

Fig. 2 Evolution of the islet organ from invertebrates to mammals. Considerable species variation occurs in all classes but the scheme is meant to be semi-representative. Family member cell types that still remain in the gut are represented by single letters: *I*, insulin; *G*, glucagon peptides; *SS*, somatostatin peptides; *P*, PP family peptides. The cyclostomes are the first species where islet-like clusters have migrated out of the gut tube into a separate cluster (islet) surrounding the common bile duct. It is with the cartilaginous and bony fish that the first real pancreas is formed with islets containing three and sometimes four hormones. These islets can lie within large islets (Brockmann bodies) or multiple islets within an exocrine pancreas. Reptiles and Amphibia are the first species with islets containing all four of the major hormones. Some species of Aves have multilobed pancreata and the islets tend to contain a lot of glucagon cells, and this is the first appearance of ghrelin cells in some species. Mammals have a diverse range of structures but are generally round and contain four or five islet hormones. Insulin (*red*), glucagon (*green*), somatostatin (*blue*), pancreatic polypeptide (*yellow*), ghrelin (*purple*). *BD*, bile duct

Agnatha-Cyclostomes: First Appearance of an Islet Organ

The hagfish is a very ancient fish and it has been demonstrated to have an islet organ, which consists of only insulin and somatostatin cells. It is located as a bulge in the intestine near to the exit of the common bile duct (Figs. 2 and 3). Scattered insulin cells are also found associated with the bile duct as is also found in higher vertebrates (Eberhard et al. 2008). No glucagon, PP, or ghrelin (Heller and Christensen 2009) cells have been identified in the structure

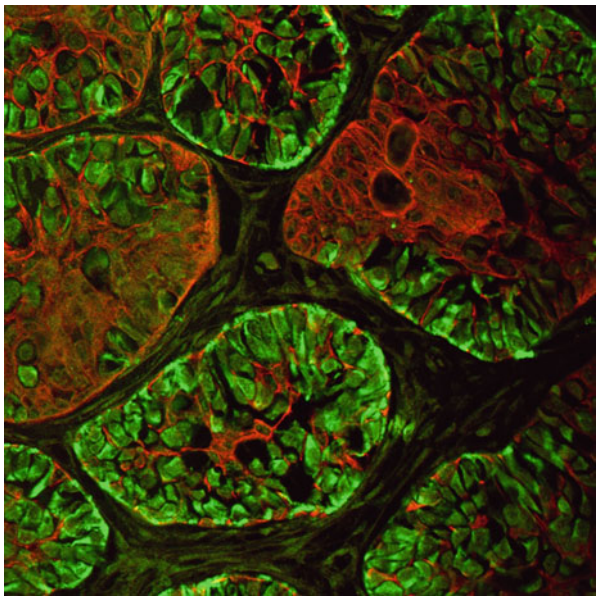

Fig. 3 The islets from the hagfish. The section was stained for insulin using the peroxidase staining protocol by Erna Pedersen (Hagedorn Research Institute). Insulin is in *red* (Image is taken at 200× magnification)

(Epple and Brinn 1986). The lamprey, a bottom-dwelling ocean fish relative of the hagfish, also has a distinct islet organ, which was described by August Epple as follicles of Langerhans due to its curious structure and that it was embedded in the submucosa of the intestine and features a duct-like lumen (Epple and Brinn 1986). It is comprised of insulin and somatostatin immunoreactive cells. Interestingly, it appears that many of the somatostatin cells from the gut have now migrated into the islet organ (Yui et al. 1988). One very interesting difference between the lamprey and hagfish is that removal of the islet organ in lamprey but not hagfish induced hyperglycemia (Epple and Brinn 1986; Epple et al. 1992).

Chondrichthyes (Jawed Fish)

Chondrichthyes is a large class consisting of rays, sharks, and skates. Here we see a large evolution in the islet organ as well as the appearance of some exocrine tissue associated with the islet tissue. Whether the islet organ in these ancient fish is derived from the dorsal or ventral pancreas or both is unknown. The glucagon cells have now migrated out of the GI tract and into the islet organ and are grouped together with insulin and somatostatin (Fig. 3) (Epple and Brinn 1975). The first appearance of the PP cells is found and some species such as the elephant fish have abundant PP cells (Kim and Rulifson 2004). The pancreas of the shark *Scyliorhinus stellaris* has large islets observed around small ducts. In addition, single islet cells or small groups of endocrine cells can also be observed to be incorporated into acini (Kobayashi and Syed 1981). Ghrelin has not been identified or examined for. Now, for the first time, we see an islet organ with juxtaposed exocrine and endocrine

tissue with the four main pancreatic cell types observed in most but not all Chondrichthyes. Interestingly, many glucagon, PP family, and somatostatin cells remain in the gut, a feature that remains with most higher vertebrates as these peptide families play important roles in GI physiology.

Osteichthyes (Lungfish and Teleost Fish)

Lungfish are unique when compared to the vast literature on the teleost fish. The anatomy of the pancreatic region is quite distinctive with a number of scattered encapsulated islets completely surrounded in the dorsal foregut wall. The stomach and intestine wrap around the organ and the spleen is also in close association (Brinn 1973; Briin and Epple 1976). For the first time, we see islets that are encapsulated by a collagenous-type connective tissue to exclude them from the exocrine tissue (Falkmer and Ostberg 1985). Glucagon, insulin, and somatostatin immunoreactive cells are localized in the islets, but few or no PP cells are found (Hansen et al. 1987; Youson 2007) (Fig. 2).

Hagfish Islets (Insulin)

The teleost are bony fish of the rayfin subclass and have been widely studied. Detailed developmental studies in zebrafish have demonstrated that the dorsal pancreas gives rise to the principal islet body often referred to as the Brockmann body (mainly found in the most advanced teleosts), while the ventral bud leads to the exocrine pancreas and associated smaller islets, also seen on other fish (Field et al. 2003). Islet structures vary broadly in this class of fish with some members having many islet structures scattered as clumps throughout the abdominal cavity with associated exocrine tissue (Youson et al. 2006). Generally, the islet organ is located in the mesentery that connects the stomach, intestine, liver, and gallbladder. Teleost fish tend to have islets that very much resemble mammalian islets with the insulin cells in the core surrounded by a mantle of glucagon, somatostatin, and PP cells, but not all teleost islets contain all four cell types (Youson and Al-Mahrouki 1999). Ghrelin cells have been detected in the pancreas of the catfish (Kaiya et al. 2005). Eels have been shown to have numerous peptide YY (PYY) cells in the islets (Table 1) (Lee et al. 2003).

Amphibia

Amphibia, which includes the urodeles (salamanders, newts) and anurans (frogs, toads), vary greatly in their islet structures. In some urodeles, the islets are not encapsulated, appear poorly innervated, and the cell types are more randomly distributed, while in others the islets appear as in most other tetrapods (Epple and Norris 1985). The literature on newts and salamanders is limited, but what has been reported shows that all four of the main cell types are found but are most often in clusters that do not show a distinct distribution, with insulin cells in the core

surrounded by the other cell types. It has been reported that the endocrine cells in the mudpuppy appear as groups of cells that are unencapsulated (Copeland and DeRoos 1971). Ghrelin has not yet been described in urodeles.

Frogs and toads have been more intensely studied and are the first species with five or even six unique cell types in the islets. In addition, Amphibia are the earliest vertebrates to show the classical islet structure of the β-cells in the center surrounded by the other cell types (Fig. 2). While the appearance of the islet structures in frogs is quite close to that of mammals, the cell composition is very different. In some frogs, there are equal numbers of insulin, glucagon, and PP cells and fewer somatostatin cells (Fig. 4). Interestingly, like hagfish, frog β-cells appear to lack Zn^{2+}. Also, like mammals, the splenic or tail portion of the pancreas often has larger islets. In addition to the four main cell types, single or small groups of secretin cells have been described in the red-bellied frog (Lee et al. 2003). While, Xenopus appear to have ghrelin cells in the islets, bullfrogs have the mRNA but not the immunoreactive peptide (Kaiya et al. 2008). Insulin-like growth factor-1 (IGF-1) has also been observed to colocalize with either PP or glucagon (Table 1) (Reinecke et al. 1995). PYY immunoreactive cells have also been described in the bullfrog pancreas (Ding et al. 1997b) (Table 1).

Reptilia (Turtles, Crocodiles, Lizards, Snakes)

While these animals were the first to make a complete transition from an aquatic to a terrestrial way of life and represent the animals that evolved into birds and mammals, very little is known about their islets compared to fish and amphibians. Reptiles in general have a distinct pancreas with exocrine tissue and islets. Perhaps not surprisingly, the Crocodilia and Squamata (lizards) are more similar than the turtles. They exhibit a compact pancreas with all four of the established pancreatic hormone cell types. In crocodiles, the insulin cells make up about 50 % of the islets, while in lizards, the glucagon cells are in abundance with a ratio of 4:5 for every insulin cell. Lizard islets also tend to be large and located in the tail of the pancreas (Norris 1980; Rhoten and Hall 1981). Interestingly, alligators have been reported to have a large number of somatostatin cells (Kim and Rulifson 2004). IGF-1 was shown to colocalize with either somatostatin or PP in lizards (*Lacerta viridis*, *Scincus officinalis*) (Table 1) (Ding et al. 1997b) and ghrelin has not been described in these species.

Chelonia (turtles) are the oldest in the class of reptiles and show a large diversity. A recent study in *Melanochelys trijuga* demonstrated that numerous scattered glucagon cells appear in the exocrine pancreas. In addition, small insulin islets of 3–20 cells are found, but when the cells are found together, they form islets with β-cells in the center and α-cells on the periphery and that the α-cells outnumber the β-cells (Chandavar and Naik 2008). In *Chrysemys picta*, it has been described that the duodenal (head) part of the pancreas contains scattered SS and PP cells that are distant from islets made of only glucagon and insulin cells and that the PP cells are found in an inverse relationship to the glucagon cells. Interestingly, in this species,

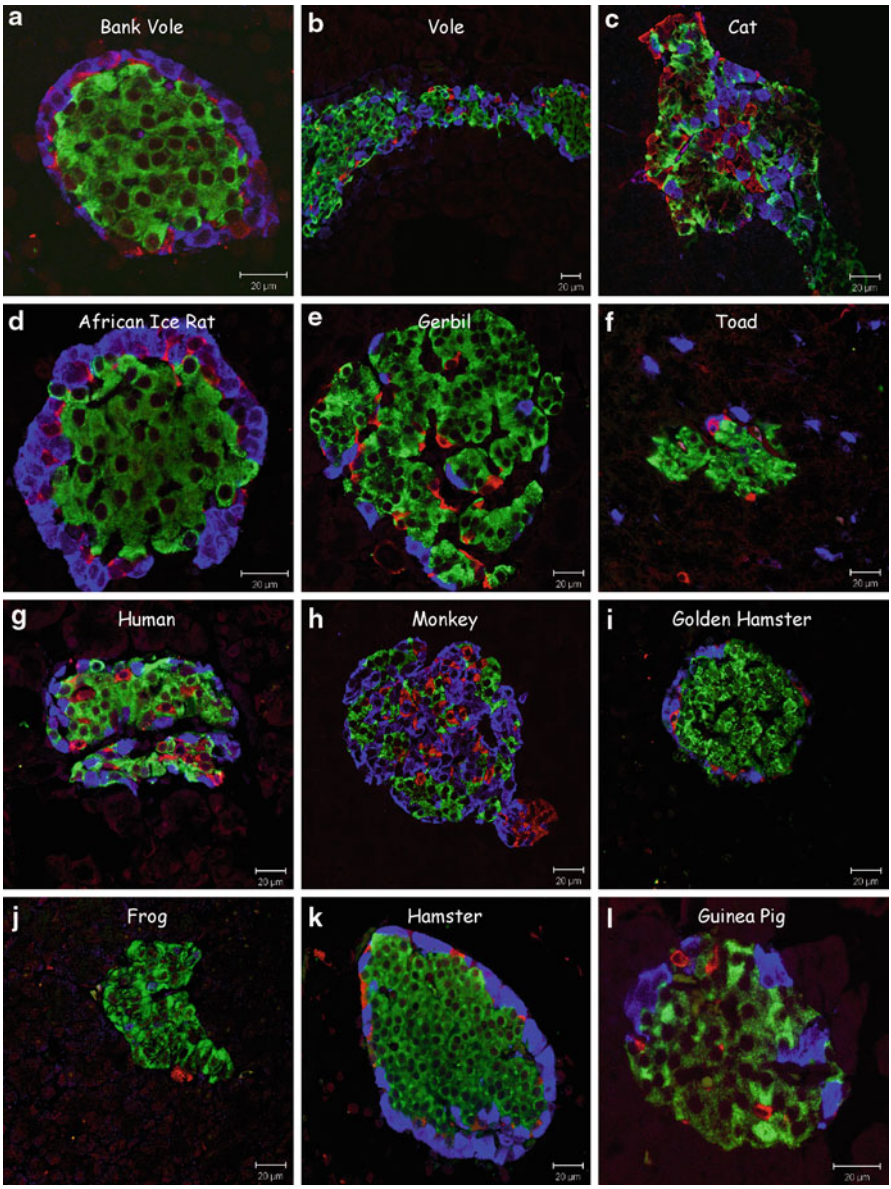

Fig. 4 The comparative anatomy of islets in 12 different species. Bank vole (**a**), vole (**b**), cat (**c**), African Ice Rat (**d**), gerbil (**e**), *Bufo* (toad) (**f**), human (**g**), monkey (**h**), golden hamster (**i**), Xenopus (frog)(**j**), hamster (**k**), guinea pig (**l**). Sections were stained for insulin (*green*), somatostatin (*red*), and glucagon (*blue*) and scanned with a Zeiss LSM 510 confocal microscope. Scale bar = 20 μM

insulin cells are still found in the gut, which is an evolutionary reverse predating the amphibians (Gapp et al. 1985). Not a lot of literature exists about other hormones in the islets, but ghrelin cells have been identified in the pancreas of the red-eared slider turtle (Kaiya et al. 2004) and IGF-1 in snakes (*Psamophis leniolatum*, *Coluber ravergieri*) in the glucagon or somatostatin cells (Reinecke et al. 1995; Table 1).

The endocrine pancreas in a few snakes has been reported, and it appears that in general, glucagon and somatostatin cells are found in the mantle, but also there are scattered somatostatin cells intermingled with the other endocrine cell types. Interestingly, in *Natrix*, there are more α-cells than β-cells but not in *Vipera*, where both appear to have about equal numbers of δ and β-cells, which is also quite different from mammals. Surprisingly, the authors did not find PP or gastrin/CCK family peptides in the islets (Masini 1988).

Aves

The avian pancreas has evolved as a multilobed and distinct pancreatic organ in the few species that have been studied. Almost all the data comes from chickens, ducks, quail, and pigeons, and all of these show quite a lot of similarity and are more closely related to what we observed in the Chelonia compared to the Crocodilia class. Early on it was observed that Aves have what is referred to as A and B islets, which consist of primarily glucagon or insulin cells with somatostatin cells as well as mixed islets (Falkmer and Ostberg 1980). More recently, these observations have been confirmed in the Japanese quail where it was observed that in the β-cell islets, the somatostatin cells were in the periphery, while in the α-cell islets, they were scattered throughout the islet (Simsek et al. 2008). Similar data were observed in the domestic duck, where A and mixed islets were more concentrated in the splenic lobe and decreased in number in the other lobes (Lucini et al. 1996). Two exceptions to these species appear to be the Australian eagle and the Houbara bustard, which were reported not to have A and B islets but only islets of the mixed type, and no reciprocal relationship between PP and glucagon was observed (Edwin and Leigh 1993; Mensah-Brown et al. 2000). More exceptions may well be observed as more species are studied, but one consistent finding is that birds tend to have large numbers of α-cells.

As we move further up the evolutionary scale, more and more different peptides have been localized in the islets. IGF-1 has been reported to colocalize with either SS or PP (Reinecke et al. 1995). Using specific non-cross-reacting antisera, PYY-specific cells have been observed in the chicken exocrine pancreas with rare cells in islets, which is also similar to what was observed in turtles (Ding et al. 1997c). Adrenomedullin has been described to be localized with the PP cells in chickens (López and Cuesta 2002). Ghrelin cells are found in adult domestic chickens (Nils Wierup, personal communication) but nothing is known in other Aves species (Table 1).

Mammals

Many more mammals have been investigated than lower vertebrate species and extensive literature is available. There are currently 5,400 species of mammals distributed in about 1,200 genera, 153 families, and 29 orders. This includes species from the Monotremes (echidnas and the platypus), Theriiformes (live-bearing mammals), marsupials, Anagalida (lagomorphs, rodents, and elephant shrews), Grand order Ferae (carnivorans, pangolins), Grand order Archonta (bats, primates, colugos, and tree shrews), Grand order Ungulata, Mirorder Eparctocyona [condylarths, whales, and artiodactyls (even-toed ungulates)], and Mirorder Altungulata: perissodactyls (odd-toed ungulates), elephants, manatees, and hyraxes (Fig. 1). Many of these have never been examined, but I will describe what has been reported.

By the time the mammals evolved, the basic structure of the pancreas, with multiple lobes and encapsulated islets, was really set. It is in mammals that we now have strong evidence that the islets are producing much more than the five main islet hormones (insulin, glucagon, somatostatin, pancreatic polypeptide, and ghrelin), and this includes a wide and diverse group of peptides and proteins, including islet amyloid polypeptide (IAPP), cholecystokinin (CCK), peptide YY (PYY), thyrotropin-releasing hormone (TRH), GABA, and cocaine-amphetamine-regulated transcript (CART) (Table 1).

Rodents

Forty percent of all mammals are rodents and this includes mice, rats, chipmunks, squirrels, gophers, hamsters, porcupines, beavers, guinea pigs, gerbils, degus, chinchillas, prairie dogs, and groundhogs. Out of all of these animals, the islet architecture and content has only been examined in mice, rats, hamsters, gerbils, and guinea pigs. In general, rodents such as mice, rats, and hamsters have fairly round islets with glucagon, somatostatin, and PP cells in the mantle and β-cells in the center (Wieczorek et al. 1998) (Figs. 2 and 4). We have recently examined the African Ice Rat (*Otomys sloggetti robertsi*) and observed that these animals have nearly equal numbers of α- and β-cells and the islets generally have two layers of glucagon cells surrounding the β-cells (Gustavsen et al. 2008a). In addition, we have fresh studies on several desert gerbils and have described their islet morphology (Gustavsen et al. 2008b). In gerbils of the Meriones family, we observed that like rats and mice, the β-cells are in the center of the islets and are surrounded by a ring of α-, δ-, and PP cells. We often observed colocalization of PYY with PP as well and this is seen in a number of mammalian species (Table 1). Cocaine-amphetamine-related transcript (CART) and CCK are also often colocalized with mostly δ-cells and β-cells, respectively (Table 1). Hamsters and guinea pigs also tend to have all the glucagon, somatostatin, and PP cells in the mantle, the core being only insulin cells (Fig. 4). We have recently examined two species of voles and found that in one the islets showed very similar morphology to other rodents, while the other had larger more elongated islets (Fig. 4).

Carnivora

Of the approximately 260 species, which includes dogs, foxes, bears, weasels, pandas, elephant seals, and cats, we only really have data on the domestic cat and dog and a few rare animals. The dog β-cells generally occupy the central portion of the islets but are also found as single cells in the exocrine pancreas (Wieczorek et al. 1998), while the α-cells are generally in the periphery but also found centrally in some islets. The δ-cells are generally mixed in the islets while PP cells appear as single cells or groups (Gapp et al. 1985; Redecker et al. 1992). The endocrine pancreas of the Cape fur seal showed very similar morphology to what is observed in other carnivorous species like the dog and cat, and this shows a central core of β-cells surrounded by glucagon, somatostatin, and PP cells. Like what we have seen in cats, they also observed scattered endocrine cells in the exocrine pancreas (Erasmus and Van Aswegen 1997). The red fox, *Vulpes vulpes*, was described to have small islets with insulin in the center surrounded by glucagon and somatostatin immunoreactive cells. The authors were unable to detect PP cells (Elvestad et al. 1984). Our experience with the examination of the domestic cat shows that these animals have very unusual islets with every shape you can imagine but not round islets and the endocrine cells can also be arranged in different sorts of clusters mixed together with groups of α-, β-, or δ-cells clustered together (Fig. 4). Whether this is a common occurrence in other cats is not known.

Artiodactyls (Even-Toed Ungulates)

The most widely studied even-toed ungulate is the pig. The minipig has been used in both type 1 and type 2 studies of diabetes (Larsen and Rolin 2004; Bellinger et al. 2006). The islets of the minipig have been described to have three types of islets: small with low numbers of β-cells, large islets with β-cells in the core, and large islets with β-cells in the periphery (Wieczorek et al. 1998). Interestingly, the left lobe of the pancreas was described to be high in α- cells and devoid of PP cells, while the δ-cells are mostly at the periphery of the islets or between acinar cells (Wieczorek et al. 1998). There has been one description in the literature on the morphology of the camel pancreas. In this paper, it was observed that the insulin immunoreactive cells were found in the central and peripheral parts of the islets of Langerhans as well as some solitary β-cells in the exocrine pancreas outside the islets. Glucagon immunoreactive cells were located in the periphery of the islets and were approximately 23 % of the total islet cells, while insulin immunoreactive cells were 67 % (Adeghate 1997). Little is known about other peptides in these species and the expression of but not colocalization of CART was recently described in sheep (Table 1).

Marsupials

The presence of the marsupium (distinctive pouch) is what characterizes this unique class of mammals. A few species have been examined. The fat-tailed dunnart, *Sminthopsis crassicaudata*, was shown to have all four of the major immunoreactive hormones clustered into islets as well as numerous PP cells scattered in the exocrine pancreas (Edwin et al. 1992). The same group has also looked at the Australian brush-tailed possum, *Trichosurus vulpecula* (Leigh and Edwin 1992).

They found that like the dunnart, the β-cells are in the middle of the islets, with the α-, δ-, and PP cells in the periphery, with numerous PP cells found in the exocrine pancreas. In the possum, *Trichosurus vulpecula*, it was described by another group that insulin cells were found in islets not only centrally but also in the periphery of islets, and in some islets the glucagon cells were the dominant cell type, found both centrally and in the mantle (Reddy et al. 1986). PP cells were quite rare with usually only one or two per islet, while somatostatin cells were mainly in the periphery. These data are similar to what was also observed in the opossum, *Didelphis virginiana* (Krause et al. 1989). A common feature in marsupials appears to be scattered PP cells in the exocrine pancreas. A recent study of the tammar wallaby, *Macropus eugenii*, showed that ghrelin cells were found in the developing pancreas up to day 10 but were not present 150 days after birth (Menzies et al. 2009). These data are the same as found in mice (Heller et al. 2005; Heller 2009).

Archonta: Bats, Primates, Tree Shrews

Archonta is the superorder which contains the bats, tree shrews, colugos, and primates (humans). A very interesting study was conducted on the fruit bat, *Rousettus aegyptiacus* (Michelmore et al. 1998). They found that the endocrine tissue makes up about 9 % of the pancreas, which is close to double of what is found in all other species studied so far, and this probably relates to the fact that these animals must absorb large amounts of glucose in very short periods of time. The endocrine cells were distributed in islets throughout the gland and also occurred as discrete cells in the exocrine ducts. The four major endocrine cell types were irregularly scattered throughout the islets with insulin (47.4 %) cells located throughout the islet and in between the glucagon cells (28.6 %). Somatostatin cells made up 7.8 % and pancreatic polypeptide (PP) cells 16.2 %, which is much higher than normal in other mammals. Interestingly, using pancreatic vascular casts of the common tree shrew (*Tupaia glis*), it was found that the α- and δ-cells appeared to occupy the core, whereas the β-cells were found at the periphery of the islets of Langerhans. This is quite unusual for a higher vertebrate (Bamroongwong et al. 1992).

The primates, which include monkeys, apes, and humans, have been widely studied morphologically. In general, there is a lot of similarity between monkeys and humans, with an intermingling of the major cell types (Fig. 4). In monkeys, it is not uncommon to see central groups of glucagon cells and large clusters of insulin cells that occupy specific sides including the mantle of the islet (Sujatha et al. 2004). The somatostatin cells are generally intermixed, while the PP and ghrelin cells are in the periphery of the islets (Wierup et al. 2002).

Conclusion

In conclusion, the islets of Langerhans have evolved from quite simple organs (Madsen et al. 2000) in the ancient fish to very complex organs in higher vertebrates, producing many hormones, neurotransmitters, and other signaling

molecules. Many of the variations of the standard map of the islet that we observe are likely to be related to the diet and environment of the animals, while the need to maintain blood glucose and regulate metabolism within a tight physiological range is an evolutionary pressure that is rarely altered. I think that as new immunocytochemical techniques such as whole mount immunoctyochemistry (Ahnfelt-Rønne et al. 2007; Jørgensen et al. 2007) and optical projection tomography (Alanentalo et al. 2007; Holmberg and Ahlgren 2008) become more widespread in islet research, we should see an expanded knowledge of how these important cellular clusters are localized, shaped, and function in different species and perhaps even reveal greater differences or more similarities than what has been appreciated from two-dimensional analysis.

Acknowledgments I would like to thank Nils Wierup, Carsten Godfredsen, and Yana Kvicerova for providing some of the tissue samples used in Fig. 4. I would also like to thank my graduate student Carsten Gustavsen for his excellent work in this area of comparative morphological analysis of different species and finally Jan Nygård Jensen, Ole Madsen for helpful comments on the chapter.

Cross-References

► Microscopic Anatomy of the Human Islet of Langerhans

References

Adeghate E (1997) Immunohistochemical identification of pancreatic hormones, neuropeptides and cytoskeletal proteins in pancreas of the camel (*Camelus dromedarius*). J Morphol 231(2):185–193

Ahnfelt-Rønne J, Jørgensen MC, Hald J, Madsen OD, Serup P, Hecksher-Sørensen J (2007) An improved method for three-dimensional reconstruction of protein expression patterns in intact mouse and chicken embryos and organs. J Histochem Cytochem 55(9):925–930

Alanentalo T, Asayesh A, Morrison H, Lorén CE, Holmberg D, Sharpe J, Ahlgren U (2007) Tomographic molecular imaging and 3D quantification within adult mouse organs. Nat Methods 4(1):31–33

Ali-Rachedi A, Varndell IM, Adrian TE, Gapp DA, Van Noorden S, Bloom SR, Polak JM (1984) Peptide YY (PYY) immunoreactivity is co-stored with glucagon-related immunoreactants in endocrine cells of the gut and pancreas. Histochemistry 80(5):487–491

Arciszewski MB, Całka J, Majewski M (2008) Cocaine-and amphetamine-regulated transcript (CART) is expressed in the ovine pancreas. Ann Anat 190(3):292–299

Assouline B, Nguyen V, Mahe S, Bourrat F, Scharfmann R (2002) Development of the pancreas in medaka. Mech Dev 117:299–303

Bamroongwong S, Chunhabundit P, Rattanchaikunsopon P, Somana R (1992) Pancreatic microcirculation in the common tree shrew (Tupaia glis) as revealed by scanning electron microscopy of vascular corrosion casts. Acta Anat (Basel) 143(3):188–194

Bellinger DA, Merricks EP, Nichols TC (2006) Swine models of type 2 diabetes mellitus: insulin resistance, glucose tolerance, and cardiovascular complications. ILAR J 47(3):243–258

Böttcher G, Sjöberg J, Ekman R, Håkanson R, Sundler F (1993) Peptide YY in the mammalian pancreas: immunocytochemical localization and immunochemical characterization. Regul Pept 43(3):115–130

Briin JE, Epple A (1976) New types of islet cells in a cyclostome, Petromyzon marinus. Cell Tissue Res 171:317–329

Brinn JE (1973) Te pancreatic islets of bony fish. Am Zool 13:652–656

Chandavar VR, Naik PR (2008) Immunocytochemical detection of glucagon and insulin cells in endocrine pancreas and cyclic disparity of plasma glucose in the turtle Melanochelys trijuga. J Biosci 33(2):239–247

Copeland PL, DeRoos R (1971) Effect of mammalian insulin on plasma glucose in the mud puppy, necturus maculosus. J Exp Zool 178:35–43

DeVelasco B, Shen J, Go S, Hartenstein V (2004) Embryonic development of the Drosophila corpus cardiacum, a neuroendocrine gland with similarity to the vertebrate pituitary, is controlled by sine oculis and glass. Dev Biol 274(2):280–294

Ding WG, Kimura H, Fujimura M, Fujimiya M (1997) Neuropeptide Y and peptide YY immunoreactivities in the pancreas of various vertebrates. Peptides 18(10):1523–1529

Eberhard D, Tosh D, Slack JM (2008) Origin of pancreatic endocrine cells from biliary duct epithelium. Cell Mol Life Sci 65(21):3467–3480

Edwin N, Leigh CM (1993) The endocrine pancreas in the Australian wedge-tailed eagle (Aquila audax) – an immunocytochemical study. Eur J Histochem 37(3):219–224

Edwin N, Yamada J, Leigh CM (1992) A light-microscopic immunocytochemical study of the endocrine pancreas in the Australian fat-tailed dunnart (Sminthopsis crassicaudata). Singap Med J 33(3):260–261

Elvestad K, Henriques UV, Kroustrup JP (1984) Insulin-producing islet cell tumor in an ectopic pancreas of a red fox (Vulpes vulpes). J Wildl Dis 20(1):70–72

Epple A, Brinn JE (1975) Islet histophysiology. Evolutionary correlations. Gen Comp Endocrinol 27:320–349

Epple A, Brinn JE (1986) Chap 10: Pancreatic islets. In: Vertebrate endocrinology. Fundamentals and biomedical implications, vol 1. Academic, New York

Epple A, Norris DA (1985) Vertebrate endocrinology. Lea and Febiger, Philadelphia

Epple A, Cake MH, Potter IC, Tajbakhsh M (1992) Impact of complete isletectomy on plasma glucose in the southern hemisphere lamprey Geotria australis. Gen Comp Endocrinol 86(2):284–288

Erasmus CP, Van Aswegen G (1997) The endocrine pancreas of the Cape fur seal, Arctocephaluspusillus (Schreber, 1776): an immunocytochemical study. Onderstepoort J Vet Res 64(3):239–242

Falkmer S, Ostberg Y (1980) The endocrine pancreas of birds Sitbon G, Mialhe P. J Physiol Paris 76(1):5–24

Falkmer S, Ostberg Y (1985) Chap 2: Comparative morphology of pancreatic islets in animals. In: Volk BW, Arquilla ER (eds) The diabetic pancreas. Plenum Press, New York

Field HA, Dong PD, Beis D, Stainier DY (2003) Formation of the digestive system in zebrafish. II. Pancreas morphogenesis. Dev Biol 261(1):197–208

Fujimura M, Greeley GH Jr, Hancock MB, Alwmark A, Santos A, Cooper CW, Reumont KJ, Ishizuka J, Thompson JC (1988) Colocalization of calcitonin gene-related peptide and somatostatin in pancreatic islet cells and inhibition of insulin secretion by calcitonin gene-related peptide in the rat. Pancreas 3(1):49–52

Fujita T, Yui R, Iwanaga T, Nishiitsutsuji-Uwo J, Endo Y, Yanaihara N (1981) Evolutionary aspects of "brain-gut peptides": an immunohistochemical study. Peptides 2(Suppl 2):123–131

Galloway SM, Cutfield JF (1988) Insulin-like material from the digestive tract of the tunicate *Pyura pachydermatina* (sea tulip). Gen Comp Endocrinol 69:106–113

Gapp DA, Kenny MP, Polak JM (1985) The gastro-entero-pancreatic system of the turtle, Chrysemyspicta. Peptides 3(6 Suppl):347–352

Gustavsen CR, Pillay N, Heller RS (2008a) An immunohistochemical study of the endocrine pancreas of the African ice rat, Otomys sloggetti robertsi. Acta Histochem 110(4):294–301

Gustavsen CR, Chevret P, Krasnov B, Mowlavi G, Madsen OD, Heller RS (2008b) The morphology of islets of Langerhans is only mildly affected by the lack of Pdx-1 in the pancreas of adult Meriones jirds. Gen Comp Endocrinol 159(2–3):241–249

Gustavsen CR, Kvicerova J, Dickinson H, Heller RS (2009) Acomys, the closest relatives to Gerbils do express Pdx-1 and have similar islet morphology. Islets 1(3):191

Hansen GN, Hansen BL, Jørgensen PN (1987) Insulin-, glucagon- and somatostatin-like immunoreactivity in the endocrine pancreas of the lungfish, *Neoceratodus forsteri*. Cell Tissue Res 248(1):181–185

Heller RS, Jenny M, Collombat P, Mansouri A, Tomasetto C, Madsen OD, Mellitzer G, Gradwohl G, Serup P (2005) Genetic determinants of pancreatic ε-cell development. Dev Biol 286(1):217–224

Holmberg D, Ahlgren U (2008) Imaging the pancreas: from ex vivo to non-invasive technology. Diabetologia 51(12):2148–2154

Ikeya T, Galic M, Belawat P, Nairz K, Hafen E (2002) Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in Drosophila. Curr Biol 12:1293–1300

Jackerott M, Oster A, Larsson LI (1996) PYY in developing murine islet cells: comparisons to development of islet hormones, NPY, and BrdU incorporation. J Histochem Cytochem 44(8):809–817

Jönsson AC (1995) Endocrine cells with gastrin/cholecystokinin-like immunoreactivity in the pancreas of the spiny dogfish, *Squalus acanthias*. Regul Pept 59(1):67–78

Jørgensen MC, Ahnfelt-Rønne J, Hald J, Madsen OD, Serup P, Hecksher-Sørensen J (2007) An illustrated review of early pancreas development in the mouse. Endocr Rev 28(6):685–705

Kaiya H, Sakata I, Kojima M, Hosoda H, Sakai T, Kangawa K (2004) Structural determination and histochemical localization of ghrelin in the red-eared slider turtle, *Trachemys scripta* elegans. Gen Comp Endocrinol 138(1):50–57

Kaiya H, Small BC, Bilodeau AL, Shepherd BS, Kojima M, Hosoda H, Kangawa K (2005) Purification, cDNA cloning, and characterization of ghrelin in channel catfish, Ictalurus-punctatus. Gen Comp Endocrinol 143(3):201–210

Kaiya H, Miyazato M, Kangawa K, Peter RE, Unniappan S (2008) Ghrelin: a multifunctional hormone in non-mammalian vertebrates. Comp Biochem Physiol A Mol Integr Physiol 149(2):109–128

Kawano H, Daikoku S, Saito S (1983) Location of thyrotropin-releasing hormone-like immunoreactivity in rat pancreas. Endocrinology 112(3):951–955

Kelly OG, Melton DA (2000) Development of the pancreas in Xenopus laevis. Dev Dyn 218(4):615–627

Kim SK, Rulifson EJ (2004) Conserved mechanisms of glucose sensing and regulation by Drosophila corpora cardiaca cells. Nature 431:316–320

Kim SK, Hebrok M, Melton DA (1997) Pancreas development in the chick embryo. Cold Spring Harb Symp Quant Biol 62:377–383

Kobayashi K, Syed AS (1981) Cell types of the endocrine pancreas in the shark Scyliorhinus stellaris as revealed by correlative light and electron microscopy. Cell Tissue Res 215(3):475–490

Krause WJ, Cutts JH 3rd, Cutts JH, Yamada J (1989) Immunohistochemical study of the developing endocrine pancreas of the opossum (Didelphis virginiana). Acta Anat (Basel) 135(1):84–96

Lammert E, Cleaver O, Melton D (2003) Role of endothelial cells in early pancreas and liver development. Mech Dev 120(1):59–64

Larsen MO, Rolin B (2004) Use of the Göttingen minipig as a model of diabetes, with special focus on type 1 diabetes research. ILAR J 45(3):303–313

Lee JH, Ku SK, Lee HS, Kitagawa H (2003) An immunohistochemical study of endocrine cells in the pancreas of the Red-bellied frog (*Bombina orientalis*). Eur J Histochem 47(2):165–172

Leigh CM, Edwin N (1992) A light-microscopic immunocytochemical study of the endocrine pancreas in the Australian brush tailed possum (Trichosurus vulpecula). Eur J Histochem 36(2):237–241

López J, Cuesta N (2002) Adrenomedullin as a pancreatic hormone. Microsc Res Tech 57(2):61–75

Lucini C, Castaldo L, Lai O (1996) An immunohistochemical study of the endocrine pancreas of ducks. Eur J Histochem 40(1):45–52

Madsen OD, Serup P, Jensen J, Petersen HV, Heller RS (2000) A historical and phylogenetic perspective of the understanding of islet cell development. In: Hussain MA, Miller CP, Habener JF (eds) Molecular basis of endocrine pancreas development and function. Kluwer, Boston

Masini MA (1988) Immunocytochemical localization of peptides in the endocrine pancreas of the snakes *Vipera aspis* and *Natrix maura*. Acta Histochem 84(2):111–119

Mensah-Brown EP, Bailey TA, Pallot DJ, Garner A (2000) Peptidergic hormones and neuropeptides, and aminergic neurotransmitters of the pancreatic islets of the Houbara bustard (*Chlamydotis undulata*). J Anat 196(Pt 2):233–241

Menzies BR, Shaw G, Fletcher TP, Renfree MB (2009) Early onset of ghrelin production in a marsupial. Mol Cell Endocrinol 299(2):266–273

Michelmore AJ, Keegan DJ, Kramer B (1998) Rousettus aegyptiacus. Immunocytochemical identification of endocrine cells in the pancreas of the fruit bat. Gen Comp Endocrinol 110(3):319–325

Nascimento AA, Sales A, Cardoso TR, Pinheiro NL, Mendes RM (2007) Immunocytochemical study of the distribution of endocrine cells in the pancreas of the Brazilian sparrow species Zonotrichia Capensis Subtorquata (Swaison, 1837). Braz J Biol 67(4):735–740

Navarro MH, Lozano MT, Agulleiro B (2006) Ontogeny of the endocrine pancreatic cells of the gilthead sea bream, *Sparus aurata* (Teleost). Gen Comp Endocrinol 148(2):213–226

Norris DO (1980) Vertebrate endocrinology. Lea and Febiger, Philadelphia

Prado CL, Pugh-Bernard AE, Elghazi L, Sosa-Pineda B, Sussel L (2004) Ghrelin cells replace insulin-producing β cells in two mouse models of pancreas development. Proc Natl Acad Sci USA 101(9):2924–2929

Reddy S, Bibby NJ, Fisher SL (1986) Elliott RB Immunolocalization of insulin, glucagon, pancreaticpolypeptide, and somatostatin in the pancreatic islets of the possum, *Trichosurus vulpecula*. Gen Comp Endocrinol 64(1):157–162

Redecker P, Seipelt A, Jörns A, Bargsten G, Grube D (1992) The microanatomy of canine islets of Langerhans: implications for intra-islet regulation. Anat Embryol (Berl) 185(2):131–141

Reinecke M, Broger I, Brun R, Zapf J, Maake C (1995) Immunohistochemical localization of insulin-like growth factor I and II in the endocrine pancreas of birds, reptiles, and amphibia. Gen Comp Endocrinol 100(3):385–396

Rhoten WB, Hall CE (1981) Four hormones in the pancreas of the lizard, anolis carolinensis. Anat Rec 199:89–97

Rulifson EJ, Kim SK, Nusse R (2002) Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science 296:1118–1120

Shimizu K, Kato Y, Shiratori K, Ding Y, Song Y, Furlanetto R, Chang TM, Watanabe S, Hayashi N, Kobayashi M, Chey WY (1998) Evidence for the existence of CCK-producing cells in rat pancreatic islets. Endocrinology 139(1):389–396

Simsek N, Ozüdoğru Z, Alabay B (2008) Immunohistochemical studies on the splenic lobe of the pancreas in young Japanese quails (Coturnix c. japonica). Dtsch Tierarztl Wochenschr 115(5):189–193

Smit AB, van Kesteren RE, Li KW, Van Minnen J, Spijker S, Van Heerikhuizen H, Geraerts WP (1998) Towards understanding the role of insulin in the brain: lessons from insulin-related signaling systems in the invertebrate brain. Prog Neurobiol 54(1):35–54

Sujatha SR, Pulimood A, Gunasekaran S (2004) Comparative immunocytochemistry of isolated rat & monkey pancreatic islet cell types. Indian J Med Res 119(1):38–44

Tsuruo Y, Hökfelt T, Visser TJ, Kimmel JR, Brown JC, Verhofstadt A, Walsh J (1988) TRH-like immunoreactivity in endocrine cells and neurons in the gastro-intestinal tract of the rat and guinea pig. Cell Tissue Res 253(2):347–356

Van der Horst D (2003) Insect adipokinetic hormones: release and integration of flight energy metabolism. Comp Biochem Phys B 136:217–226
Veelaert D, Schoofs L, De Loof A (1998) Peptidergic control of the corpus cardiacum-corpora allata complex of locusts. Int Rev Cytol 182:249–302
Veenstra JA, Agricola HJ, Sellami A (2008) Regulatory peptides in fruit fly midgut. Cell Tissue Res 334(3):499–516
Wieczorek G, Pospischil A, Perentes E (1998) A comparative immunohistochemical study of pancreatic islets in laboratory animals (rats, dogs, minipigs, nonhuman primates). Exp Toxicol Pathol 50(3):151–172
Wierup N, Svensson H, Mulder H, Sundler F (2002) The Ghrelin cell: a novel developmentally regulated islet cell in the human pancreas. Regul Pept 107(1–3):63–69
Wierup N, Kuhar M, Nilsson BO, Mulder H, Ekblad E, Sundler F (2004) Cocaine-and amphetamine regulated transcript (CART) is expressed in several islet cell types during rat development. J Histochem Cytochem 52(2):169–177
Youson JH (2007) Peripheral endocrine glands. I. The gastoenteropancreatic endocrine system and the thyroid gland. Fish Physiol Primit Fish 26:381–425
Youson JH, Al-Mahrouki AA (1999) Ontogenetic and phylogenetic development of the endocrine pancreas (islet organ) in fish. Gen Comp Endocrinol 116(3):303–335
Youson JH, Al-Mahrouki AA, Amemiya Y, Graham LC, Montpetit CJ, Irwin DM (2006) The fish endocrine pancreas: review, new data, and future research directions in ontogeny and phylogeny. Gen Comp Endocrinol 148(2):105–115
Yui R, Nagata Y, Fujita T (1988) Immunocytochemical studies on the islet and the gut of the arctic lamprey, *Lampetra japonica*. Arch Histol Cytol 51(1):109–119

Microscopic Anatomy of the Human Islet of Langerhans

2

Peter In't Veld and Silke Smeets

Contents

Abstract

Human islets of Langerhans are complex microorgans responsible for maintaining glucose homeostasis. Islets contain five different endocrine cell types, which react to changes in plasma nutrient levels with the release of a carefully balanced mixture of islet hormones into the portal vein. Each endocrine cell type is characterized by its own typical secretory granule morphology, different peptide hormone content, and specific endocrine, paracrine, and neuronal interactions. During development, a cascade of transcription factors determines the formation of the endocrine pancreas and its constituting islet cell

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_1,

types. Differences in ontogeny between the ventrally derived head section and the dorsally derived head, body, and tail section are responsible for differences in innervation, blood supply, and endocrine composition. Islet cells show a close topographical relationship to the islet vasculature and are supplied with a five- to tenfold higher blood flow than the exocrine compartment. Islet microanatomy is disturbed in patients with type 1 diabetes, with a marked reduction in β-cell content and the presence of inflammatory infiltrates. Histopathological lesions in type 2 diabetes include a limited reduction in β-cell content and deposition of amyloid in the islet interstitial space.

Keywords
Pathology • Type 1 diabetes • Type 2 diabetes • Morphology • Anatomy • Insulitis • Amyloid • β-Cell • α-Cell • δ-cell • PP cell • Autoimmunity • Innervation • Vasculature • Non-endocrine cells

Introduction

The pancreas is an unpaired gland of the alimentary tract with mixed exocrine–endocrine function. It is composed of four functionally different but interrelated components: the exocrine tissue, the ducts, the endocrine cells, and the connective tissue. These elements are intimately related through ontogeny, anatomy, histology, and function. Because the scope of this chapter is the microscopic anatomy of the islet of Langerhans, the other components will only briefly be mentioned.

The Pancreas and the Islets of Langerhans

The adult human pancreas has a mean weight of approx 65 g (range 45–120 g) (Ogilvie 1937). It has an elongated shape and is composed of a head region attached to the duodenum, a tail region attached to the spleen, and an intervening body region. Part of the head region (uncinate process) forms a hook-like structure posterior to the mesenteric vessels. Macroscopically, the pancreas has a yellowish-pink aspect and a soft to firm consistency depending on the level of fibrosis and fat accumulation in the organ. It is composed of small lobules measuring 1–10 mm in diameter. Microscopically, these lobules are composed of ductules, acini, and well-vascularized endocrine cell clusters that reflect the two main functions of the pancreas: digestion and glucose homeostasis. Exocrine cells (98 % of the parenchyma) release a mixture of digestive enzymes and bicarbonate into the duodenum. They are organized into acini that open into intercalated ducts, to which they are connected via centro-acinar cells. The intercalated ducts fuse into intralobular ducts, interlobular ducts, and finally into the main pancreatic ductus of Wirsung, which, together with the common bile duct, opens into the duodenum at the papilla of Vater (papilla major).

The secondary ductus of Santorini ends in the papilla minor, a few centimeters above the papilla major. Endocrine cells (1–2 % of the parenchyma) release nutrient-generated hormones into the portal vein. Clusters of endocrine cells form islets of Langerhans, microorgans that lie scattered throughout the exocrine parenchyma in between the acini and ductal structures. The islets of Langerhans are of vital importance to the body as they produce insulin, a prime regulator of glucose homeostasis. The name 'islets of Langerhans' was coined by Edouard Laguesse (1861–1927), a histologist working at the University of Lille, who, in a seminal paper in 1893, correctly deduced that they are involved in endocrine secretion. He named them after Paul Langerhans (1849–1888), who was the first to describe these cell clusters in his doctoral thesis in 1869 but who was unable to attribute them with a specific function (Volk and Wellman 1985). The adult human islet of Langerhans has a mean diameter of 140 μm (Hellman and Hellerström 1969). It is pervaded by a dense network of capillaries (Goldstein and Davis 1968) and is (partly) surrounded by a thin collagen capsule (Hughes et al. 2006) and glial sheet (Smith 1975) that separates the endocrine cells from the exocrine component. Islets vary in size and range from small clusters of only a few cells to large aggregates of many thousands of cells. Depending on the definition of how many cells minimally constitute an "islet," the estimated islet number in the adult human pancreas varies from several hundred thousands to several millions. Total β mass appears highly variable between subjects, ranging from 500 to 1,500 mg (Rahier et al. 2008), corresponding to an estimated 10^9 β-cells and 1–2 % of mean pancreatic weight. Adult islets contain four major endocrine cell types: α-cells (also referred to as α-cells), β-cells (also referred to as β-cells), δ-cells (D, formerly also called A1), and PP cells (pancreatic polypeptide cells, formerly also called F- or D1-cells). A fifth cell type, the ε or ghrelin cell, has recently been described.

Embryology and Fetal Development

The pancreas is derived from two primordia in the distal embryonic foregut (Edlund 2002; Pictet and Rutter 1972). At 3–4 weeks of gestation, a dorsal primordium is formed opposite the hepatic diverticulum and a ventral primordium (sometimes bilobed) in close apposition to the diverticulum. At 6 weeks of gestation, the ventral pancreas rotates and fuses with the dorsal pancreas around week 7. The ventral primordium gives rise to part of the head region of the gland ("ventral head"), while the dorsal primordium gives rise to the dorsal head, the body, and the tail. This difference in ontogeny is reflected in significant differences in endocrine cell composition, vascularization, and innervation between the ventral and dorsal pancreas. The ventral head is supplied with blood via the mesenteric artery. The dorsally derived head, body, and tail are irrigated by the celiac artery. The differences in ontogeny are mirrored by major differences in islet cell composition (Bencosme and Liepa 1955; Orci et al. 1978).

Pancreas development is controlled by a complex cascade of transcription factors (Cleaver and Dor 2012). Pancreatic and duodenal homeobox 1 (Pdx1)

induces early (primary) progenitor cells to expand and form duct-like outgrowths into the surrounding mesenchyme. In a second wave of differentiation (secondary transition), cells at the duct tips differentiate into acini, and cells in the duct walls give rise to endocrine cells, a process driven by another key transcription factor neurogenin 3 (Ngn3). Endocrine cells are first detected at 8–9 weeks at the basal side of the ductal epithelium where they grow out to primitive islets. Exocrine acini are observed from 10 to 12 weeks. Growth of the endocrine mass during fetal life follows that of the total gland, with endocrine tissue forming 2–5 % of the parenchyma (Stefan et al. 1983). Growth of β-cell mass in fetal and adult life appears to be partly by neogenesis from endogenous $Ngn3^+$ progenitor cells and partly by replication of existing β-cells (Kushner 2013). β-Cell replication peaks around 20 weeks of gestation after which replication levels decrease exponentially reaching near-zero values a few years after birth (Bouwens et al. 1997; Kassem et al. 2000; Meier et al. 2008). In the adult organ islet cells rarely show mitosis, although relatively high levels of replication are observed in selected patient populations, including young patients who are on prolonged life support (Veld et al. 2010).

During early development the percentage of the various endocrine cell types changes: at 8 weeks approximately 50 % of endocrine cells express glucagon, decreasing to 15–20 % in the adult. Similarly, the percentage of δ-cells decreases from 20 % to 25 % in neonates to approx 5 % in adults (Clark and Grant 1983; Like and Orci 1972; Orci et al. 1979; Rahier et al. 1981).

In the developing human pancreas, cells coproducing insulin and glucagon are present. It has been suggested that these cells are precursors to α cells in the mature pancreas (Riedel et al. 2012).

Endocrine Cell Types

Adult human islets contain at least five different endocrine cell types. α- and β-cells were both first described in 1907 by Lane (1907) on the basis of their histochemical staining characteristics, while δ-cells were first recognized by Bloom in 1931 (Bloom 1931). Both PP cells (Kimmel et al. 1971) and ghrelin cells (Wierup et al. 2002) were discovered with the aid of immunocytochemistry.

α-Cells

α-Cells secrete glucagon, a 29-amino-acid peptide with hyperglycemic action (Murlin et al. 1923). The peptide is derived from proglucagon (180 amino acids) through proteolytic cleavage. Other cleavage products that can be derived from the precursor are GLP-1, GLP-2, and glicentin (Bell et al. 1983; Vaillant and Lund 1986). Glucagon is stored in secretory granules that have a typical morphology with an electrondense core and a grayish peripheral mantle (Deconinck et al. 1971). Glucagon was immunohistochemically localized to the α-cells by

Table 1 Cell types in the adult human endocrine pancreas

	Cell type				
	A	B	D	PP	ε
Peptide hormone	Glucagon	Insulin	Somatostatin	Pancreatic polypeptide	Ghrelin
Molecular weight	3,500	5,800	1,500	4,200	3,400
Number of amino acids	29	51	14	36	28
Volume % (adult)					
Dorsal	15–20	70–80	5–10	<1	1
Ventral	<1	10–20	2	80	1
Total	15–20	70–80	5–10	15–25	1

Baum et al. (1962). The number of α-cells is estimated at 15–20 % (Rahier et al. 1983; Stefan et al. 1982), although the relative volume taken up by α-cells can vary significantly between islets with some islets containing up to 65 % of α-cells (Brissova et al. 2005). α-Cells are most prominent in the dorsally derived part of the pancreas and virtually absent in the ventrally derived part (Table 1).

β-Cells

β-Cells form the bulk of the pancreatic endocrine cell mass. Depending on the morphometric techniques that were used, the type of samples analyzed, and the extent of the analysis, a relative islet β-cell mass was found between 50 % and 80 % (Brissova et al. 2005; Cabrera et al. 2006; Rahier et al. 1983; Stefan et al. 1982). β-Cells secrete insulin, a 51-amino-acid peptide with strong hypoglycemic action. Insulin is essential for cellular nutrient uptake and thus for the survival of the organism. Its isolation and immediate successful clinical application in 1923 by Banting, Best, and Collip was one of the major medical breakthroughs of the twentieth century (Banting and Best 1922; Bliss 1982). Like virtually all peptide hormones, insulin is proteolytically derived from a precursor molecule, proinsulin. This biologically inactive precursor is split into three parts, an A and a B chain, which remain connected by two sulfur bridges, thus forming the biologically active insulin molecule, and a C chain (Connecting peptide), which is released together with insulin in a 1:1 molar ratio (Orci 1986). The β-cell also co-secretes islet amyloid polypeptide (IAPP, also called amylin), a 37-amino-acid peptide related to calcitonin gene-related peptide (CGRP) (Johnson et al. 1988). Under pathological conditions IAPP molecules may polymerize and form large intraislet amyloid deposits that are characteristic for type 2 diabetes and insulinoma but also occur in chronic type 1 DM and in the elderly in general (see below).

Insulin was first immunohistochemically localized to the β-cell by Lacy (1959). It is stored in cytoplasmic secretory vesicles that have a characteristic morphology with an electrondense core and a clear peripheral mantle (Fig. 1). Within the 350 nm granule, insulin (but not proinsulin) is complexed to zinc, forming insulin–zinc hexamers and crystalline granule cores. Depending on the maturation stage of

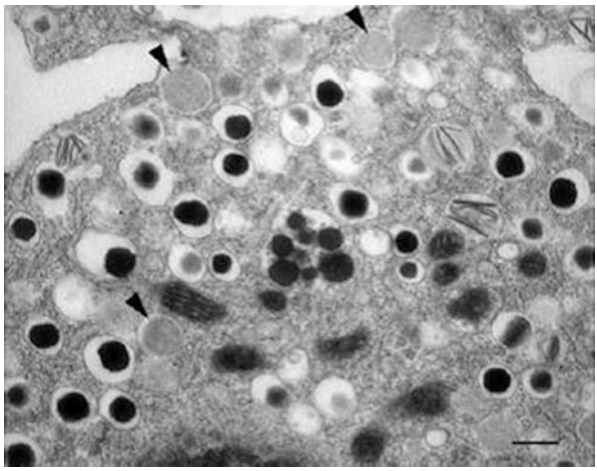

Fig. 1 Electron-microscopic image of an islet β-cell with mature dense-cored secretory granules and immature *gray* granules (*arrowheads*) (bar 300 nm)

the granule, the mantle may contain unprocessed proinsulin; when the proteolytic enzymes (prohormone convertases PC1–2, carboxypeptidase-H) present in the newly formed secretory granule have not yet resulted in sufficient cleavage of the precursor molecules, the granule core may be absent and typical immature "gray" granules are found (Lacy 1959). The biological reason for Zn complexation is not well understood, but its presence is of practical benefit in islet isolation procedures, where zinc-chelating dyes like dithizone (Maske 1957) are helpful in determining islet yield and purity.

A β-cell is estimated to contain 9–13,000 secretory granules (Dean 1973; Olofsson et al. 2002). With an average daily insulin requirement of 40 IU and an average insulin content per granule of 8 fg, it can be estimated that approx 10^{12} secretory granules are released from β-cells each day. Release may occur via a nutrient-regulated pathway or via a constitutive pathway. Nutrient-induced release is initiated via closure of ATP-dependent potassium-channels, membrane depolarization, opening of voltage-dependent calcium channels, and calcium-induced fusion of the secretory granules with the plasma membrane. The process of insulin release is complex and may partly consist of granule fusion with the plasma membrane and partly of temporary opening of small pores between the granule lumen and the extracellular milieu (Eliasson et al. 2008). A pool of granules is normally situated close to the plasma membrane. These docked and primed granules are ready for nutrient-induced exocytosis. They are considered to be responsible for the first phase of insulin release. Granules further down into the cytoplasma are considered to be responsible for second-phase insulin release. Under cholinergic stimulation, cytoplasmic secretory vesicles may fuse with each other, amplifying the insulin release process (Gaisano 2012; Orci and Malaisse 1980).

In addition to (pro)insulin, C-peptide, IAPP, zinc, and proteolytic enzymes, the secretory granule contains calcium, adenine nucleotides, biogenic amines, and a series of additional peptide (pro)hormones including chromogranin A and betagranin (Eiden 1987; Hutton et al. 1988). Several granule (membrane) proteins

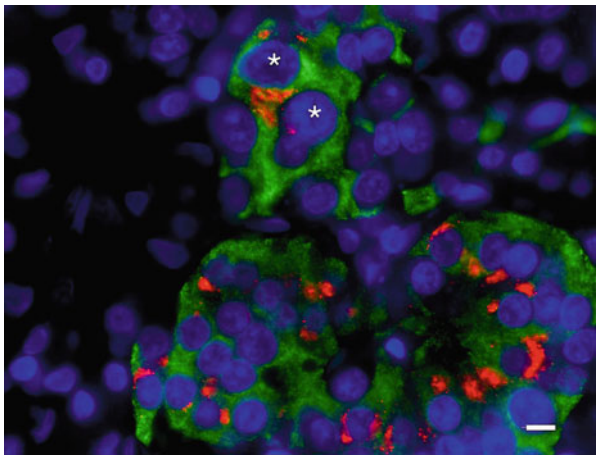

Fig. 2 Two-color fluorescent imaging for insulin (*green*) and proinsulin (*red*) of a human islet of Langerhans. Proinsulin has a predominantly perinuclear localization. Note the significant differences in nuclear size between islet β-cells (*asterisk*) (Bar 10 μm)

have been implicated in humoral autoimmunity in type 1 diabetes, like the zinc transporter ZnT8 (Wenzlau et al. 2007), insulinoma-associated protein 2 (IA-2; ICA-512) (Lan et al. 1996), and glutamic acid decarboxylase (GAD65) (Arvan et al. 2012; Baekkeskov et al. 1990).

β-Cells in the human pancreas may show marked variation in granulation, cell size, and size of the nuclei (Fig. 2). Differences in granulation and cell size may reflect a heterogeneity in glucose responsiveness and biosynthetic activity (Schuit et al. 1988), while differences in nuclear size may reflect polyploidy with nuclear DNA content of up to 8n being relatively common (Ehrie and Swartz 1974).

β-Cells in the aging human pancreas display multiple prominent lysosomes with lipid-like content (Fig. 3). These strongly autofluorescent organelles resemble the lipofuscin inclusions in aging neurons and increase linearly with age (Cnop et al. 2000). The age-related increase in lipofuscin can be used to estimate the average life-span of β-cells in the human pancreas. It was found that a long-lived β-cell population is established by 20 years of age (Cnop et al. 2010, 2011). Studies using ^{14}C dating similarly show that β-cell "birth" occurs before the age of 30 (Perl et al. 2010). The apparent longevity of these cells is reflected by the very low levels of replication that are found in adult human islets (Meier et al. 2008; In't Veld et al. 2010).

δ-Cells

The D (or δ) cells release somatostatin (formerly called somatotropin release-inhibiting factor), first isolated from the hypothalamus (Brazeau et al. 1973). This peptide hormone is a potent inhibitor of glucagon and insulin release and was first immunohistochemically located to the δ-cell by Luft et al. (1974). The hormone exists in a 14-amino-acid form and in a 28-amino-acid form (Bloom and Polak 1987). Although all islet cells have neuron-like characteristics, the δ-cells resemble

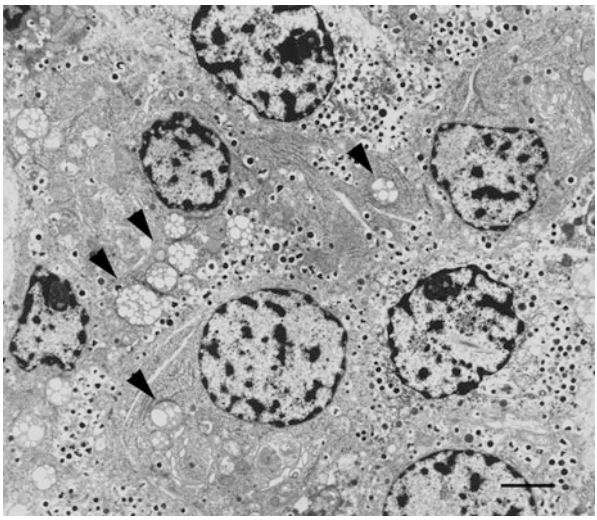

Fig. 3 Electron-microscopic image of aging human β-cells with multiple cytoplasmic inclusions (bar 5 μm)

small neurons most, as they often form long slender processes with a secretory-granule-rich knob-like ending near a capillary suggesting focal and possibly paracrine secretion (Grube and Bohn 1983). δ-cells form 5–10 % of islet volume (Table 1).

PP Cells

The least well studied of the islet hormones is PP, secreted by the PP cell. The peptide has been found immunocytochemically in two morphologically distinct cell types: PP immunoreactive cells (formerly designated as F-cells), characterized by round to angular secretory granules, were found in the ventrally derived head of the pancreas, while cells with small granules, formerly called D1-cells, were found in the dorsally derived part (Larsson et al. 1974). In the human pancreas the relative PP cell mass in the ventral pancreas is considerable, constituting up to 80 % of the cells (Table 1).

ε-Cells

The latest cell type that was added is the ε or ghrelin cell. The hormone ghrelin was first isolated from rat stomach and later localized to a specific cell type in the adult human islet (Wierup et al. 2002). Adult islets contain less than 1 % ε cells. The hormone is thought to be of importance in growth hormone release, metabolic regulation, and energy balance, but its exact role in islet cells has yet to be established. Recent data in rodents indicate that ghrelin expression defines a multipotent progenitor lineage giving rise to α-cells, PP cells, and rare β-cells (Arnes et al. 2012).

Islet Anatomy

Endocrine cells in the pancreas form aggregates of various sizes and microscopic aspect. Larger aggregates, the islets of Langerhans, form small, ellipsoid, or spherical structures dispersed throughout the exocrine part. The islet size and number of β-cells increases from birth to adulthood (Meier et al. 2008). In fetuses, islets are in close contact with ducts, but they become more separated from the ducts in neonates and adults. In adults, 50 % of the islets remain close to the ducts (Watanabe et al. 1999). Size and distribution of islets vary widely from individual to individual, but without recognizable pattern, except that their number seems to increase towards the tail of the pancreas (Saito et al. 1978; Wittingen and Frey 1974). In light microscopy, the epithelial cells of the islets of Langerhans form trabecular structures, separated by a dense network of anastomosing capillaries (Goldstein and Davis 1968). Two architecturally different types of islets are recognized: the diffuse islet and the compact islet. In the posterior–inferior (ventral) head of the pancreas, the islets are of the "diffuse" type, because the trabeculae seem more loosely arranged than in the islets occurring in the rest of the pancreas and which are known as "compact islets." The diffuse islets are very rich in PP cells and are larger than the compact islets. They also contain substantially less α-, β-, and δ-cells (Orci et al. 1976; Wang et al. 2013) than the compact islets which are primarily found in the body and tail and have sizes ranging from 50 to 280 μm. Compact islets are well circumscribed and separated by a thin layer of collagen from the surrounding acini. This is less the case in the diffuse islets, which are often irregular. Though occasional islets can measure 1–2 mm in diameter, compact islets larger than 250 μm are generally considered hyperplastic (Klimstra et al. 2007).

In humans, the endocrine cells are distributed throughout the islets without apparent organization; this contrasts with murine islets, which show a clear topographical separation of β- and α-cell mass. It cannot be excluded that such topographical differences between human and rodent islets are paralleled by differences in endocrine and paracrine islet cell interactions. The cytoarchitecture of the human islet, with its apparently random islet cell distribution, does not support functional islet domains in which the direction of blood flow determines intraislet endocrine signaling (Cabrera et al. 2006).

The relative proportion of the various endocrine cell types in the human islets can vary considerably; in one study (Brissova et al. 2005) the percentage of β-cells ranged from 28 % to 75 %, that of α-cells from 10 % to 65 %, and that of somatostatin cells from 1.2 % to 22 %. Not all endocrine cells in the pancreas occur in classical islet structures: 15 % of all β-cells are found in units with a diameter of $<$20 μm (1–3 cells) and without associated glucagon, somatostatin, or PP cells (Bouwens and Pipeleers 1998). These units, referred to as "single β-cells," are equally distributed throughout the whole gland and in close association with acini and ductules; they are significantly smaller than β-cells located in larger islets. It has been speculated that these cells are an early stage in the formation of new islets, although recent studies in rodents using β-cell lineage tracing were unable to confirm this (Dor et al. 2004).

The different islet cell types can be distinguished with special stains. Nowadays immunohistochemistry is used almost exclusively, but several cell-type-specific histochemical stains are available as well. The best known are Gomori's aldehyde fuchsin for β-cells (Gomori 1939; Grimelius and Strand 1974) and Hellman–Hellerstrom for δ-cells (Hellerström and Hellman 1960). The Mallory–Azan stain distinguishes between the three major cell types.

Non-endocrine Islet Cells

Between the islet cell trabeculae, small amounts of connective tissue are present, with blood vessels being most prominent. Other non-epithelial elements present in the islet are nerve fibers, Schwann cells, pericytes (Tang et al. 2013), macrophages (de Koning et al. 1998), and dendritic cells; the latter express major histocompatibility complex (MHC) class II molecules on their cell surfaces, which may play a role in graft rejection and the initiation of type 1 diabetes.

Pancreatic lymphatics are found in the interlobular septa of the exocrine portion but are seldom in contact with the islets (Morchoe 1997).

Islet Vasculature

The islet vasculature is critical for adequate glucose homeostasis, not only because of the high oxygen consumption of pancreatic β-cells but also because of timely responses to changes in plasma glucose concentration and the release of islet hormones into the circulation. Islet perfusion is mediated by neural, hormonal, and circulatory signals (Ballian and Brunicardi 2007). The islet capillary network has a density five times higher than the exocrine capillary network (Zanone et al. 2008): 1–3 afferent arterioles provide the islet with oxygenated blood, which leaves through efferent venules emptying into exocrine capillary networks or collecting venules that in turn empty directly into larger veins. The islet endothelium contains 95 nm fenestrations closed by a diaphragm and arranged into sieve plates (Fig. 4). Islet capillaries display up to tenfold more fenestrations than exocrine capillaries (Henderson and Moss 1985). VEGF-A released from pancreatic β-cells was shown to be a determining factor in inducing islet capillaries and their fenestrated endothelial cells (Lammert et al. 2003). Islet β-cells are usually bordered by at least one capillary and show polarity in their cytoplasm with the secretory granules at the apical pole towards the blood vessel (Bonner-Weir 1988). Islet capillaries are surrounded by a double basement membrane, each characterized by its own laminin subtypes.

One basement membrane is derived from a peri-islet membrane that accompanies the capillary along its winding path throughout the islet; the endothelial basement membrane constitutes the other. This situation differs from that in rodents where only a single basement membrane was found (Virtanen et al. 2008). The peri-islet basement membrane is suggested to constitute a barrier to infiltrating leukocytes in type 1 diabetes (Korpos et al. 2013).

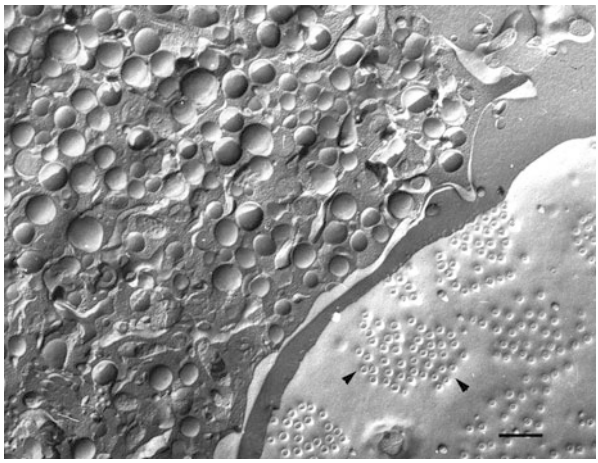

Fig. 4 Freeze fracture replica of a rat islet showing a fenestrated capillary with fenestrations arranged into sieve plates (*arrowheads*). Adjacent to the capillary is an endocrine cell with multiple secretory granules in the cytoplasm (bar 300 nm)

Innervation

Islets have sympathetic, parasympathetic, and sensory innervation; the nerve fibers contain acetylcholine, noradrenaline, and several neuropeptides. The fibers accompany the vasculature and are embedded in nonmyelinating Schwann cells. They end blindly in the pericapillary space in close proximity to the islet cells; true synaptic contacts on islet cells have not been described. Confocal imaging of human islets indicates that sympathetic fibers preferentially innervate central islet blood vessels and that parasympathetic fibers are rare (Rodriguez-Diaz et al. 2011). The ventral and dorsal parts of the pancreas have different innervation, with the dorsal pancreas receiving its sympathetic innervation from the celiac ganglion and the ventral pancreas from the superior mesenteric ganglion. Insulin secretion is stimulated by the parasympathetic system and inhibited by the sympathetic system (Ahrén 1999). It has been postulated that the thin peri-islet Schwann cell sheets surrounding the human islets may play a role in the initiation of type 1 diabetes (Tsui et al. 2008).

Islet in Type 1 Diabetes

Patients with recent-onset type 1 diabetes (DM1) usually present with a pancreas that is macroscopically normal in appearance and weight. This contrasts with findings in patients with chronic disease in whom the lack of endogenously released insulin leads to the atrophy of the acinar cells and a decrease in overall pancreatic weight (Gepts 1965; Löhr and Klöppel 1987).

The characteristic lesion in recent-onset DM1 is formed by the presence of inflammatory infiltrates in the islets of Langerhans. In a seminal study in 1965 (Gepts 1965), Willy Gepts described the presence of insulitis in 15/22 young patients with a duration of the disease of <6 months. He observed that the

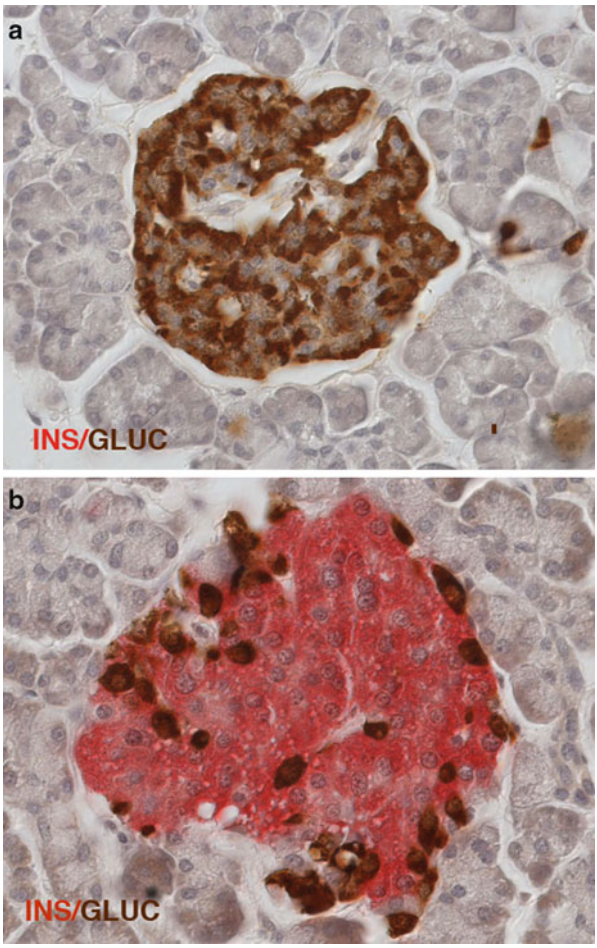

Fig. 5 Islets stained for insulin (*red*) and glucagon (*brown*). Islets from chronic type 1 diabetics are pseudoatrophic and consist primarily of α-cells (*top panel*), in contrast to islets from a normal control islet with both α- and β-cells

inflammatory lesions were limited to islets in which β-cells were still present (Fig. 5) and that most remaining islets were pseudoatrophic and contained only non-β-cells (Fig. 6), resulting in an overall decrease in β-cell mass to 10 % of normal values. He concluded that DM1 was probably the result of a protracted inflammatory disease of (auto)immune or viral etiology. Subsequent studies using immunohistochemical staining and precise morphometric methods have confirmed these initial histopathological findings (Foulis et al. 1986), but the use of more sensitive techniques also indicated that residual β-cells are still present many years after clinical onset, especially in older individuals. Our knowledge of the disease processes leading to overt diabetes is still fragmentary due to the fact that only very few patients with recent-onset diabetes could be studied by autopsy and this often under conditions that precluded extensive molecular and immunological studies (In't Veld 2011; Pipeleers and Ling 1992). The current view on the disease process is that a $CD8^+$ T-cell-mediated autoimmune reaction against islet β-cell antigens

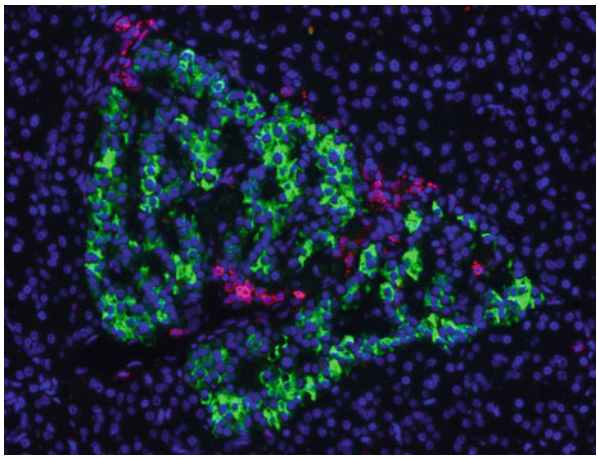

Fig. 6 Insulitis in an islet of Langerhans from a type 1 diabetic patient. Insulin-containing β-cells are stained in *green* and infiltrating $CD45^+$ leukocytes are stained in *red* (40×)

occurs in genetically susceptible individuals and that this process appears to be initiated by environmental triggers (Roep 2003). The intensity of the disease process appears to vary between patients and is often more severe in children. At clinical onset, most patients still retain a significant β-cells mass (averaging 10–30 % of normal values), but many islets have lost their β-cell component and only contain α-, D-, and PP cells; these islets are usually referred to as (pseudo)atrophic islets. A small fraction of islets still contain both β-cells and non-β-cells in normal proportions. Such β-cell containing islets may contain an inflammatory infiltrate that predominantly consists of CD8-positive T-cells and macrophages (In't Veld 2011; Pipeleers and Ling 1992; Willcox et al. 2009). Neither the mechanism leading to the leukocytic infiltration nor the antigen toward which the immune response is directed has been unequivocally identified. Studies using tetramer staining have indicated that islet infiltrating $CD8^+$ T-cells are directed against several different epitopes (Coppieters et al. 2012).

In addition to the cellular response, a humoral response is observed in both prediabetics, recent-onset cases and chronic cases (Bottazzo et al. 1985). Studies of the early phases leading to overt diabetes have indicated that positivity for autoantibodies directed against islet cell antigens often predate the disease by many years. The presence of multiple autoantibodies in combination with a susceptible HLA-DQ genotype was shown to have a predictive value of >70 % in relatives of DM1 patients (Bingley et al. 1993). As the effector phase of the disease appears to be cell mediated, the presence of autoantibodies may function as surrogate markers for islet cell destruction. Histopathological studies in nondiabetic adult organ donors with positivity for multiple autoantibodies and a susceptible HLA-DQ genotype showed that only a minor part (<10 %) of the islets presented with insulitis or other histopathological lesions (Fig. 6). As such islets also showed high levels of β-cell replication, it cannot be excluded that the clinical outcome of autoimmune attack depends on the balance between β-cell replication and autoimmune β-cell destruction (In't Veld et al. 2007). Evidence that such

regenerative processes may also occur in young patients with recent onset of the disease is found in the early cases described by Gepts, where islet hyperplasia was observed in a 2-year-old child that died 60 days after diagnosis in ketoacidosis. In this patient a single lobe of the gland showed marked hyperplasia of insulin-containing islets in a pancreas that was devoid of β-cells in the remaining part (Gepts 1965).

Additional evidence that β-cell regeneration may play a role in disease progression comes from studies where β-cell apoptosis was found in patients with long-standing DM1 (Butler et al. 2007), indirectly suggesting that β-cells are still being replenished many years after the onset of the disease. The mechanism underlying β-cell regeneration in the diabetic pancreas is unknown and may either involve neogenesis or replication. Evidence of β-cell replication in recent-onset patients is somewhat contradictory, with some studies indicating that no increased replication is observed (Meier et al. 2005), while others indicate an increase (Willcox et al. 2010). Although the bulk of the evidence favors an (auto)immune etiology of the disease, it is likely that at least some cases of DM1 have a viral origin as the Coxsackie B4 enterovirus could be isolated from a small series of recent-onset DM1 patients characterized by a non-destructive islet inflammation consisting of natural killer cells (Dotta et al. 2007).

Islets in Type 2 Diabetes

Type 2 DM occurs in predisposed individuals when the adaptive capacity of the endocrine pancreas fails. It is considered a disease of both insulin resistance and insulin deficit, with genetic and environmental factors playing an important role. No single characteristic histopathological lesion exists in the human endocrine pancreas, but both amyloid deposition and a decreased β cell mass are often observed.

The majority of type 2 diabetic subjects show deposition of non-AA amyloid in at least some of their islets (Fig. 7). However, not all DM2 subjects show amyloid deposition and islet amyloid can be found in nondiabetics (Clark et al. 1990; Opie 1901; Westermark et al. 1987; Westermark 1973) and in some patients with chronic DM1 (Keenan et al. 2010). The precursor of amyloid in DM2 is islet amyloid polypeptide (IAPP) or amylin, a 37-amino-acid peptide which is present in β-cell secretory granules and is co-secreted with insulin. Its function in normal physiology and in the pathogenesis of diabetes is still being debated (Westermark et al. 2011). The histochemical staining properties of islet amyloid are the same as for the other forms of amyloid with Congo Red being the stain generally used. It is obvious from a morphologist's point of view that once islets are almost completely invaded by amyloid, they can hardly function correctly and this can result in failure to secrete hormones into the bloodstream and failure to get sufficient nutrients to the islet cells. However, the number of islets affected in this way is minimal in most diabetics and therefore this does not seem to play a major role in the pathogenesis of DM2 (Sempoux et al. 2001).

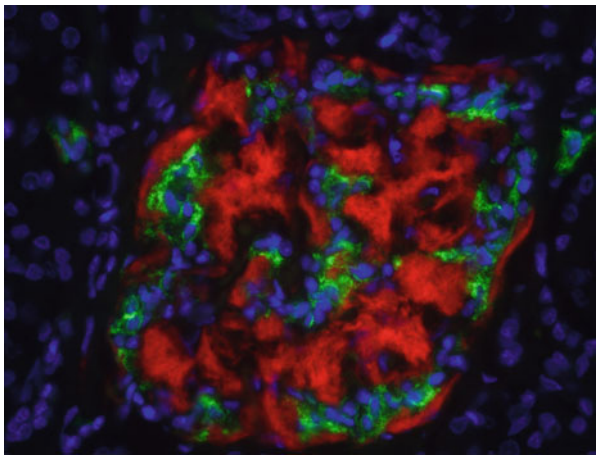

Fig. 7 Two-color fluorescent imaging for insulin (*green*) and amyloid deposition (*red*) of a human islet of Langerhans from a type 2 diabetic subject (40×)

Most authors agree that in DM2, the β-cell mass is reduced (Butler et al. 2003; Maclean and Ogilvie 1955; Rahier et al. 2008), whereas the average α-cell mass is comparable to nondiabetic subjects (Henquin and Rahier 2011). However, the reduction in β-cell mass in early disease seems insufficient to be a major causative factor. In pancreatectomy, diabetes only develops after a reduction in β cell area of approximately 65 % (Meier et al. 2012).

Acknowledgments PV and SS are supported by a grant from the FWO-Vlaanderen (G019211N).

Cross-References

- ▶ Exocytosis in Islet β-Cells
- ▶ Inflammatory Pathways Linked to β Cell Demise in Diabetes
- ▶ The Comparative Anatomy of Islets

References

Ahrén B (1999) Regulation of insulin secretion by nerves and neuropeptides. Ann Acad Med Singap 28:99–104

Arnes L, Hill JT, Gross S et al (2012) Ghrelin expression in the mouse pancreas defines a unique multipotent progenitor population. PLoS One 7:e52026

Arvan P, Pietropaolo M, Ostrov D, Rhodes CJ (2012) Islet autoantigens: structure, function, localization, and regulation. Cold Spring Harb Perspect Med 2:a007658

Baekkeskov S, Aanstoot HJ, Christgau S et al (1990) Identification of the 64 k autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 347:151–156

Ballian N, Brunicardi FC (2007) Islet vasculature as a regulator of endocrine pancreas function. World J Surg 31:705–714

Banting FG, Best CH (1922) The internal secretion of the pancreas. J Lab Clin Med 7:465–480

Baum J, Simmons BE, Unger RH, Madison LL (1962) Localization of glucagon in the α-cells in the pancreatic islet by immunofluorescence. Diabetes 11:371–374
Bell GI, Santerre RF, Mullenbach GT (1983) Hamster preproglucagon contains the sequence of glucagon and two related peptides. Nature 302:716–718
Bencosme SA, Liepa E (1955) Regional differences of the pancreatic islet. Endocrinology 57:588–593
Bingley PJ, Bonifacio E, Gale EAM (1993) Can we really predict IDDM? Diabetes 42:213–220
Bliss M (1982) The discovery of insulin. University of Chicago Press, Chicago
Bloom W (1931) A new type of granular cell in the islets of Langerhans of man. Anat Rec 49:363–371
Bloom SR, Polak JM (1987) Somatostatin. Br Med J 295:288–290
Bonner-Weir S (1988) Morphological evidence for pancreatic polarity of β cell within islets of Langerhans. Diabetes 37:616–621
Bottazzo GF, Dean BM, McNally JM et al (1985) In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulitis. N Engl J Med 313:353–360
Bouwens L, Pipeleers DG (1998) Extra-insular β cells associated with ductules are frequent in adult human pancreas. Diabetologia 41:629–633
Bouwens L, Lu WG, De Krijger R (1997) Proliferation and differentiation in the human fetal endocrine pancreas. Diabetologia 40:398–404
Brazeau P, Vale W, Burgus R et al (1973) Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 179:77–79
Brissova M, Fowler MJ, Nicholson WE et al (2005) Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. J Histochem Cytochem 53:1087–1097
Butler AE, Janson J, Bonner-Weir S et al (2003) β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 52:102–110
Butler AE, Galasso R, Meier JJ et al (2007) Modestly increased β cell apoptosis but no increased β cell replication in recent-onset type 1 diabetic patients who died of diabetic ketoacidosis. Diabetologia 50:2323–2331
Cabrera O, Berman DM, Kenyon NS et al (2006) The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proc Natl Acad Sci USA 103:2334–2339
Clark A, Grant AM (1983) Quantitative morphology of endocrine cells in human fetal pancreas. Diabetologia 25:31–35
Clark A, Saad MF, Nezzer T et al (1990) Islet amyloid polypeptide in diabetic and non-diabetic Pima Indians. Diabetologia 33:285–289
Cleaver O, Dor Y (2012) Vascular instruction of pancreas development. Development 139:2833–2843
Cnop M, Grupping A, Hoorens A et al (2000) Endocytosis of low-density lipoprotein by human pancreatic β cells and uptake in lipid-storing vesicles, which increase with age. Am J Pathol 156:237–244
Cnop M, Hughes SJ, Igoillo-Esteve M et al (2010) The long lifespan and low turnover of human islet β cells estimated by mathematical modelling of lipofuscin accumulation. Diabetologia 53:321–330
Cnop M, Igoillo-Esteve M, Hughes SJ et al (2011) Longevity of human islet α- and β-cells. Diabetes Obes Metab 13(Suppl 1):39–46
Coppieters KT, Dotta F, Amirian N et al (2012) Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. J Exp Med 209:51–60
De Koning EJ, van den Brand JJ, Mott VL et al (1998) Macrophages and pancreatic islet amyloidosis. Amyloid 5:247–254
Dean MP (1973) Ultrastructural morphometry of the pancreatic β cell. Diabetologia 9:115–119
Deconinck JF, Potvliege PR, Gepts W (1971) The ultrastructure of the human pancreatic islets. I. The islets of adults. Diabetologia 7:266–282

Dor Y, Brown J, Martinez OI, Melton DA (2004) Adult pancreatic β-cells are formed by self-duplication rather than stem-cell differentiation. Nature 429:41–46
Dotta F, Censini S, van Halteren AG et al (2007) Coxsackie B4 virus infection of β cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. Proc Natl Acad Sci USA 104:5115–5120
Edlund H (2002) Pancreatic organogenesis-developmental mechanisms and implications for therapy. Nat Rev Gen 3:524–532
Ehrie MG, Swartz FJ (1974) Diploid, tetraploid and octaploid β cells in the islets of Langerhans of the normal human pancreas. Diabetes 23:583–588
Eiden LE (1987) Is chromogranin-A a prohormone? Nature 325:301
Eliasson L, Abdulkader F, Braun M et al (2008) Novel aspects of the molecular mechanisms controlling insulin secretion. J Physiol 586:3313–3324
Foulis AK, Liddle CN, Farquharson MA et al (1986) The histopathology of the pancreas in type 1 (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom. Diabetologia 33:290–298
Gaisano HY (2012) Deploying insulin granule-granule fusion to rescue deficient insulin secretion in diabetes. Diabetologia 55:877–880
Gepts W (1965) Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes 14:619–633
Goldstein MB, Davis EA (1968) The three dimensional architecture of the islets of Langerhans. Acta Anat 71:161–171
Gomori G (1939) A differential stain for cell types in the pancreatic islets. Am J Pathol 15:497–499
Grimelius L, Strand A (1974) Ultrastructural studies of the argyrophil reaction in α1 cells in human pancreatic islets. Virchows Arch A Pathol Anat Histol 364:129–135
Grube D, Bohn R (1983) The microanatomy of human islets of Langerhans, with special reference to somatostatin (D-) cells. Arch Histol Jap 46:327–353
Hellerström C, Hellman B (1960) Some aspects of silver impregnation of the islets of Langerhans in the rat. Acta Endocrinol (Copenh) 35:518–532
Hellman B, Hellerström C (1969) Histology and histophysiology of the islets of Langerhans in man. In: Pfeiffer EF (ed) Handbook of diabetes mellitus. Lehmanns V, Munich, pp 90–118
Henderson JR, Moss MC (1985) A morphometric study of the endocrine and exocrine capillaries of the pancreas. Q J Exp Physiol 70:347–356
Henquin JC, Rahier J (2011) Pancreatic α cell mass in European subjects with type 2 diabetes. Diabetologia 54:1720–1725
Hughes SJ, Clark A, McShane P et al (2006) Characterisation of collagen VI within the islet exocrine interface of the human pancreas: implications for clinical islet isolation? Transplantation 81:423–426
Hutton JC, Peshavaria M, Johnston CF et al (1988) Immunolocalization of betagranin: a chromogranin A-related protein of the pancreatic β-cell. Endocrinology 122:1014–1020
In't Veld P, De Munck N, Van Belle K et al (2010) β cell replication is increased in donor organs from young patients after prolonged life support. Diabetes 59:1702–1708
In't Veld P (2011) Insulitis in human type 1 diabetes: the quest for an elusive lesion. Islets 3:131–138
In't Veld P, Lievens D, De Grijse J et al (2007) Screening for insulitis in adult autoantibody-positive organ donors. Diabetes 56:2400–2404
Johnson KH, O'Brien TD, Hayden DW et al (1988) Immunolocalization of islet amyloid polypeptide (IAPP) in pancreatic β cells by means of peroxidase-antiperoxidase (PAP) and protein A-gold techniques. Am J Pathol 130:1–8
Kassem SA, Ariel I, Thornton PS et al (2000) β cell proliferation and apoptosis in the developing normal human pancreas and in hyperinsulinism of infancy. Diabetes 49:1325–1333
Keenan HA, Sun JK, Levine J et al (2010) Residual insulin production and pancreatic β-cell turnover after 50 years of diabetes: Joslin medalist study. Diabetes 59:2846–2853

Kimmel JR, Pollock HG, Hazelwood RL (1971) A new pancreatic polypeptide. Fed Proc (USA) 30:1318 (abstr)
Klimstra DS, Hruban RH, Pitman MR (2007) In: Mills SE (ed) Histology for pathologist, 3rd edn. Williams & Wilkins, Baltimore
Korpos E, Kadri N, Kappelhoff R et al (2013) The Peri-islet basement membrane, a barrier to infiltrating leukocytes in type 1 diabetes in mouse and human. Diabetes 62:531–542
Kushner JA (2013) The role of aging upon β-cell turnover. J Clin Invest 123:990–995
Lacy PE (1959) Electron microscopic and fluorescent antibody studies on islets of Langerhans. Exp Cell Res 7:296–308
Lammert E, Gu G, McLaughlin M et al (2003) Role of VEGF-A in vascularization of pancreatic islets. Curr Biol 13:1070–1074
Lan MS, Wasserfall C, Maclaren NK, Notkins AL (1996) IA-2, a transmembrane protein of the protein tyrosine phosphatase family, is a major autoantigen in insulin-dependent diabetes mellitus. Proc Natl Acad Sci USA 93:6367–6370
Lane MA (1907) The cytological characteristics of the areas of Langerhans. Am J Anat 7:409–422
Larsson LI, Sundler F, Håkanson R et al (1974) Localization of APP, a postulated new hormone, to a pancreatic endocrine cell type. Histochemistry 42:377–382
Like AA, Orci L (1972) Embryogenesis of the human fetal pancreatic islets: a light and electron microscopic study. Diabetes 21:511–534
Löhr M, Klöppel G (1987) Residual insulin positivity and pancreatic atrophy in relation to duration of chronic type 1 (insulin-dependent) diabetes mellitus and microangiopathy. Diabetologia 30:757–762
Luft R, Efendic S, Hökfelt T et al (1974) Immunohistochemical evidence for the localization of somatostatin–like immunoreactivity in a cell population of the pancreatic islets. Med Biol 52:428–430
Maclean N, Ogilvie RF (1955) Quantitative estimation of the pancreatic islet tissue in diabetic subjects. Diabetes 4:367–376
Maske H (1957) Interaction between insulin and zinc in the islets of Langerhans. Diabetes 6:335–341
Meier JJ, Bhushan A, Butler AE et al (2005) Sustained β cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? Diabetologia 48:2221–2228
Meier JJ, Butler AE, Saisho Y et al (2008) β-cell replication is the primary mechanism subserving the postnatal expansion of β-cell mass in humans. Diabetes 57:1584–1594
Meier JJ, Breuer TGK, Bonadonna RC et al (2012) Pancreatic diabetes manifests when β cell area declines by approximately 65 % in humans. Diabetologia 55:1346–1354
Morchoe CC (1997) Lymphatic system of the pancreas. Microsc Res Tech 37:456–477
Murlin JR, Clough HG, Gibbs CB, Stokes AM (1923) Aqueous extracts of pancreas. I. Influence on the carbohydrate metabolism of depancreatized animals. J Biol Chem 56:253
Ogilvie RF (1937) A quantitative estimation of the pancreatic islet tissue. Q J Med 6:287–300
Olofsson CS, Göpel SO, Barg S et al (2002) Fast insulin secretion reflects exocytosis of docked granules in mouse pancreatic β cells. Pflugers Arch 444:43–51
Opie EL (1901) The relation of diabetes mellitus to lesions of the pancreas. Hyaline degeneration of the islands of Langerhans. J Exp Med 5:527–540
Orci L (1986) The insulin cell: its cellular environment and how it processes (pro)insulin. Diabetes Metab Rev 2:71–106
Orci L, Malaisse W (1980) Hypothesis: single and chain release of insulin secretory granules is related to anionic transport at exocytotic sites. Diabetes 29:943–944
Orci L, Baetens D, Ravazzola M et al (1976) Pancreatic polypeptide and glucagon: non-random distribution in pancreatic islets. Life Sci 19:1811–1815
Orci L, Malaisse-Lagae F, Baetens D, Perrelet A (1978) Pancreatic-polypeptide-rich regions in human pancreas. Lancet 2:1200–1201
Orci L, Stefan Y, Malaisse-Lagae F, Perrelet A (1979) Instability of pancreatic endocrine cell populations throughout life. Lancet 1:615–616

Perl S, Kushner JA, Buchholz BA et al (2010) Significant human β-cell turnover is limited to the first three decades of life as determined by in vivo thymidine analog incorporation and radiocarbon dating. J Clin Endocrinol Metab 95:E234–E239
Pictet R, Rutter WJ (1972) Development of the embryonic endocrine pancreas. In: Steiner DF, Freinkel N (eds) Handbook of physiology. Section 7: endocrinology. vol 1: endocrine pancreas. Williams & Wilkins, Baltimore, pp 25–66
Pipeleers D, Ling Z (1992) Pancreatic cells in insulin-dependent diabetes. Diabetes Metab Rev 8:209–227
Rahier J, Wallon J, Henquin JC (1981) Cell populations in the endocrine pancreas of human neonates and infants. Diabetologia 20:540–546
Rahier J, Goebbels RM, Henquin JC (1983) Cellular composition of the human diabetic pancreas. Diabetologia 24:366–371
Rahier J, Guiot Y, Goebbels RM et al (2008) Pancreatic β-cell mass in European subjects with type 2 diabetes. Diabetes Obes Metab 10(Suppl 4):32–42
Riedel MJ, Asadi A, Wang R et al (2012) Immunohistochemical characterisation of cells co-producing insulin and glucagon in the developing human pancreas. Diabetologia 55:372–381
Rodriguez-Diaz R, Abdulreda MH, Formoso AL et al (2011) Innervation patterns of autonomic axons in the human endocrine pancreas. Cell Metab 14:45–54
Roep BO (2003) The role of T-cells in the pathogenesis of type 1 diabetes: from cause to cure. Diabetologia 46:305–321
Saito K, Iwama N, Takahashi T (1978) Morphometrical analysis on topographical difference in size distribution, number and volume of islets in the human pancreas. Tohoku J Exp Med 124:177–186
Schuit F, In't Veld PA, Pipeleers DG (1988) Glucose recruits pancreatic β-cells to proinsulin biosynthesis. Proc Natl Acad Sci USA 85:3865–3869
Sempoux C, Guiot Y, Dubois D et al (2001) Human type 2 diabetes: morphological evidence for abnormal β-cell function. Diabetes 50(Suppl 1):S172–S177
Smith PH (1975) Structural modification of Schwann cells in the pancreatic islets of the dog. Am J Anat 144:513–517
Stefan Y, Orci L, Malaisse-Lagae F et al (1982) Quantitation of endocrine cell content in the pancreas of non-diabetic and diabetic humans. Diabetes 31:694–700
Stefan Y, Grasso S, Perrelet A, Orci L (1983) A quantitative immunofluorescent study of the endocrine cell populations in the developing human pancreas. Diabetes 32:293–301
Tang S-C, Chiu Y-C, Hsu C-T et al (2013) Plasticity of Schwann cells and pericytes in response to islet injury in mice. Diabetologia 56:2424 (published online 26 June 2013)
Tsui H, Winer S, Chan Y et al (2008) Islet glia, neurons, and β cells. Ann NY Acad Sci 1150:32–42
Vaillant CR, Lund PK (1986) Distribution of glucagon-like peptide I in canine and feline pancreas and gastrointestinal tract. J Histochem Cytochem 34:1117–1121
Virtanen I, Banerjee M, Palgi J et al (2008) Blood vessels of human islets of Langerhans are surrounded by a double basement membrane. Diabetologia 51:1181–1191
Volk BW, Wellman KF (1985) Historical review. In: Volk BW, Arquilla ER (eds) The diabetic pancreas. Plenum, New York, pp 1–16
Wang X, Zielinski MC, Misawa R et al (2013) Quantitative analysis of pancreatic polypeptide cell distribution in the human pancreas. PloS One 8:e55501
Watanabe T, Yaegashi H, Koizumi M et al (1999) Changing distribution of islets in the developing human pancreas: a computer-assisted three-dimensional reconstruction study. Pancreas 18:349–354
Wenzlau JM, Juhl K, Yu L et al (2007) The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci USA 104:17040–17045
Westermark P (1973) Fine structure of islets of Langerhans in insular amyloidosis. Virchows Arch A Patol Anat 359:1–18
Westermark P, Wilander E, Westermark GT, Johnson KH (1987) Islet amyloid polypeptide-like immunoreactivity in the islet B cells of type 2 (non-insulin-dependent) diabetic and non-diabetic individuals. Diabetologia 30:887–892

Westermark P, Andersson A, Westermark GT (2011) Islet amyloid polypeptide, Islet amyloid, and diabetes mellitus. Physiol Rev 91:795–826

Wierup N, Svensson H, Mulder H, Sundler F (2002) The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas. Reg Peptides 107:63–69

Willcox A, Richardson SJ, Bone AJ et al (2009) Analysis of islet inflammation in human type 1 diabetes. Clin Exp Immunol 155:173–181

Willcox A, Richardson SJ, Bone AJ et al (2010) Evidence of increased islet cell proliferation in patients with recent-onset type 1 diabetes. Diabetologia 53:2020–2028

Wittingen J, Frey CF (1974) Islet concentration in the head, body, tail and uncinate process of the pancreas. Ann Surg 179:412–414

Zanone MM, Favaro E, Camussi G (2008) From endothelial to β cells: insights into pancreatic islet microendothelium. Curr Diabetes Rev 4:1–9

Basement Membrane in Pancreatic Islet Function

3

Eckhard Lammert and Martin Kragl

Contents

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_8,

Abstract

Clinical treatment of diabetic patients by islet transplantation faces various complications. At present, in vitro expansion of islets occurs at the cost of their essential features, which are insulin production and release. However, the recent discovery of blood vessel/β-cell interactions as an important aspect of insulin transcription, secretion, and proliferation might point us to ways of how this problem could be overcome.

The correct function of β-cells depends on the presence of a basement membrane, a specialized extracellular matrix located around the blood vessel wall in mouse and human pancreatic islets. In this chapter, we summarize how the vascular basement membrane influences insulin transcription, insulin secretion, and β-cell proliferation. In addition, a brief overview about basement membrane components and their interactions with cell surface receptors is given.

Keywords

Basement membrane • β1-integrin • Laminin • Collagen • Blood vessels

Introduction

Basement membranes are imaged by transmission electron microscopy as sheetlike structures with an average thickness of 50–100 nm (Vracko 1972, 1974; Vracko and Benditt 1970, 1972). They are found in every tissue adjacent to epithelia, endothelia, peripheral nerve axons, and fat and muscle cells and are linked to the cytoskeleton via cell surface receptors (Paulsson 1992; Schittny and Yurchenco 1989). They serve important functions in conferring mechanical stability and compartmentalization in tissues as well as in regulating cell behavior (Paulsson 1992; Aumailley and Timpl 1986). In every organ, basement membranes exhibit different characteristics, which are vital for correct function. For example, the basement membrane encasing muscle fibers is specialized to support the fibers in response to the extreme mechanical forces, a feature that distinguishes it from basement membranes found in other organs. In contrast, in mouse pancreatic islets, a specialized basement membrane is largely formed by endothelial cells and is implicated in insulin production and release as well as β-cell proliferation (Fig. 1).

The most prominent components of basement membranes are collagen IV, laminins, heparan sulfate proteoglycans (HSPGs) such as perlecan and agrin, and nidogen/entactin (Paulsson 1992; Schittny and Yurchenco 1989; Yurchenco and Schittny 1990) (Fig. 2). These molecules can exist as different isoforms, which can be glycosylated in different manners. In addition, their tissue-specific combination in basement membranes is important for any given tissue (Cheng et al. 1997; Colognato and Yurchenco 2000; Hudson et al. 1993; Kalluri 2003).

Before addressing the role of the vascular basement membrane in β-cell function, we briefly introduce the molecules of the basement membrane and their cell surface receptors.

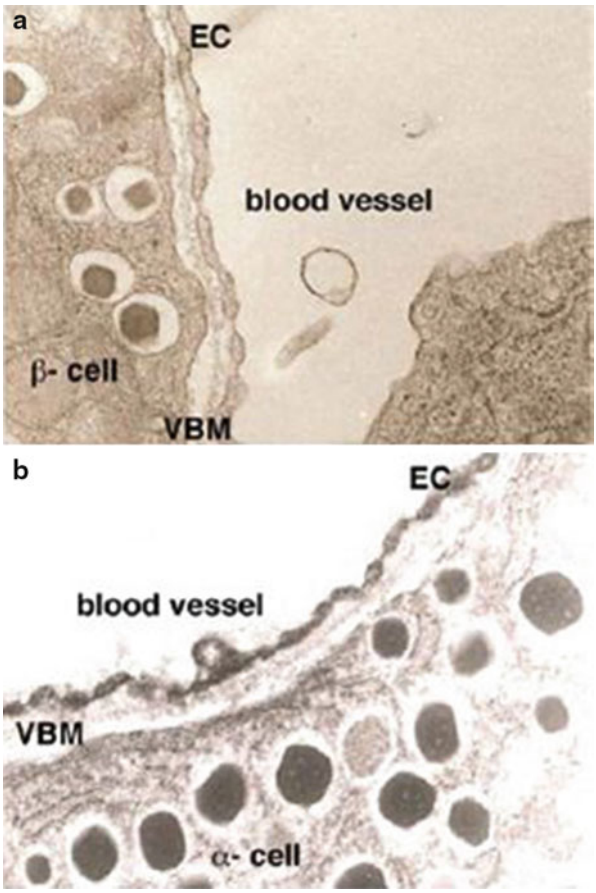

Fig. 1 Endocrine cells adjacent to a vascular basement membrane. (**a**) Electron micrograph of a β-cell next to a blood vessel. Endothelial cell (*EC*), β-cell, and vascular basement membrane (*VBM*) are indicated. (**b**) Electron micrograph of an α-cell next to a blood vessel. Endothelial cell (*EC*), α-cell, and vascular basement membrane (*VBM*) are indicated

Basement Membrane Components

Collagen IV

Collagen IV comprises a major part of all basement membranes and is also abundant in the vascular basement membrane of pancreatic islets (Kaido et al. 2004; Nikolova et al. 2006).

Collagen IV has been proposed to exist as a network of protomers in basement membranes (Timpl et al. 1981; Yurchenco and Furthmayr 1984). Protomers of collagen IV form from combinations of three α-chains. There are six genes coding for different α-chains, α1(IV)–α6(IV), and three different combinations of protomers have been identified in vivo so far: α1.α1.α2(IV), α3α4α5(IV), and α5α5α6(IV) (Hudson et al. 1993; Kalluri 2003; Boutaud et al. 2000; Yurchenco et al. 2002).

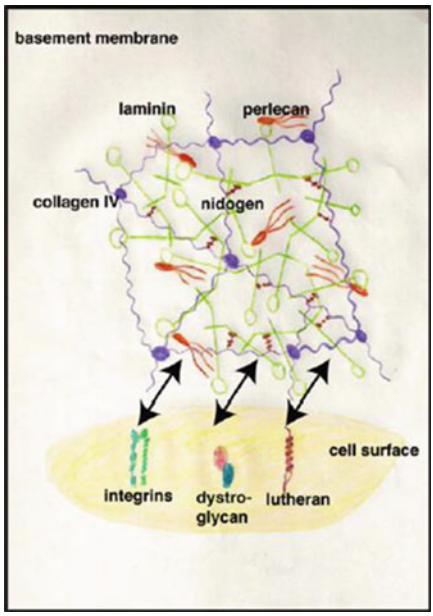

Fig. 2 Basement membrane components and their receptors. Basement membranes are sheetlike structures adjacent to epithelia, endothelia, nerves, muscle, or fat cells. They influence tissue stability and cell behavior via cell surface receptors. The scheme shows the major components of BMs: collagen IV, laminins, perlecan (a heparan sulfate proteoglycan), and nidogen, which exist as various glycosylated isotypes and isoforms that can potentially form networks and interact with cell surface receptors, such as integrins, dystroglycan, or lutheran

The major collagen IV isoform is α1.α1.α2(IV), and deletion of both α-chains causes early embryonic lethality due to defects in basement membrane stability (Poschl et al. 2004). In invertebrates, mutations or reduced expression of collagen IV-related genes is embryonic lethal due to the failure of muscle attachment to the basement membrane (Borchiellini et al. 1996; Gupta et al. 1997). In islets, collagen IV has been suggested to regulate insulin secretion (Kaido et al. 2004, 2006).

Laminin

Laminins are heterotrimeric glycoproteins that, according to the current model, assemble from an α-, β-, and γ-chain to form a trimer (Cheng et al. 1997; Chung et al. 1979; Miner and Yurchenco 2004; Timpl et al. 1979). In mammals, there are five genetically different α, 4 β, and three γ-chains, and 15 different laminin trimers have been found so far (Aumailley et al. 2005).

The different chain compositions define the nomenclature of laminin isoforms: for example, laminin-411 is composed of the α4, β1, γ1 chains, whereas laminin-511 is a trimer of the α5, β1, γ1 chains (Aumailley et al. 2005). Laminins have a cross- or T-like shape and bind other matrix components including collagen IV, nidogen-1, perlecan, and cell surface receptors (Chen et al. 1999; Sasaki and Timpl 2001; Timpl and Brown 1996; Yurchenco et al. 1992; Ettner et al. 1998; Mayer et al. 1998; Willem et al. 2002). Some laminin trimers such as laminin-111 and laminin-511 can undergo polymerization (Cheng et al. 1997, Chen et al. 1999; Yurchenco et al. 1992; Ettner et al. 1998; Mayer et al. 1998; Willem et al. 2002; Schittny and Yurchenco 1990).

Laminins are essential for vitality of an organism. For example, laminin α5-chain knockout mice are not viable; they die during embryogenesis at E16.5 with exencephaly, syndactyly, small or absent kidneys and eyes, defects in lung and tooth morphogenesis, and hair growth that come along with abnormalities in basement membrane assembly, structure, and integrity in these tissues (Fukumoto et al. 2006; Li et al. 2003; Miner and Li 2000). In contrast, laminin α4-chain knockout mice are viable. However, they display defects in vessels, neuromuscular junctions, and the peripheral nerve system (Patton et al. 2001; Thyboll et al. 2002; Wallquist et al. 2005).

In islets, laminin-411 and laminin-511 are expressed and have been suggested to play an important role in β-cell proliferation and insulin transcription (Nikolova et al. 2006).

Nidogen/Entactin

Nidogen is a component of basement membranes (Carlin et al. 1981) and exists as two isoforms: nidogen-1 and nidogen-2. Both are elongated molecules composed of three globular domains (G1, G2, and G3) connected by a flexible, protease-sensitive link, and a rigid rodlike domain (Fox et al. 1991; Kimura et al. 1998; Kohfeldt et al. 1998). Both nidogens are present in the vascular basement membrane of the islets (Irving-Rodgers et al. 2008). Several in vitro studies suggest that nidogen facilitates the interaction between collagen IV and laminin (Fox et al. 1991; Kohfeldt et al. 1998; Aumailley et al. 1993, 1989). Its in vivo role has been controversial, since nidogen-1 and nidogen-2 knockout mice did not display severe defects (Murshed et al. 2000; Schymeinsky et al. 2002). Interestingly, mice in which both isoforms were deleted developed until birth but died soon after birth with heart defects and impaired lung development, and deposition of basement membrane compounds in these organs appeared to be reduced. Surprisingly, defects in kidney development and glomerular basement membrane were less severe in these mutants (Bader et al. 2005).

At present, it is unknown whether nidogens are involved in β-cell function or not.

Heparan Sulfate Proteoglycans (HSPGs)

Most HSPGs are giant proteins with branched glycosyl residues and multiple binding sites for other matrix components and cell surface receptors. Due to their branched structure and charged sugar residues, they affect the distribution of FGFs (fibroblast growth factors), VEGFs (vascular endothelial growth factors), HGF (hepatocyte growth factor), and other molecules and their diffusion within the extracellular space (Hacker et al. 2005; Lin 2004; Strigini 2005).

One of the most abundant HSPG is perlecan (Iozzo 1998), which contains domains homologous to growth factors and cell adhesion molecules and interacts

with laminins and collagen IV (Ettner et al. 1998; Whitelock et al. 1999). Homozygous knockout mice die during embryogenesis due to BM defects (Arikawa-Hirasawa et al. 1999; Costell et al. 1999).

Although HSPGs have been poorly studied in the context of β-cell function, it is possible that they affect insulin transcription, secretion, or cell proliferation in islets. Perlecan is expressed in the intra-islet vascular basement membranes (Irving-Rodgers et al. 2008), and several growth factors whose diffusion and distribution is mediated by HSPGs have been reported to affect β-cell function, including VEGFs (Nikolova et al. 2006; Brissova et al. 2006; Lammert et al. 2003), FGFs (Hart et al. 2000; Kilkenny and Rocheleau 2008; Wente et al. 2006), and HGF (Dai et al. 2005; Lopez-Talavera et al. 2004).

Cell Surface Receptors

Integrins

Integrins were the first receptors identified to mediate BM/cell contacts in epithelium (Aumailley et al. 1991a, b; Hynes 1992; Sheppard 2000) and are also expressed on β-cells (Kaido et al. 2004; Nikolova et al. 2006).

Integrins are transmembrane proteins with large globular extracellular and smaller cytosolic domains. They undergo interactions with the BM as heterodimers of an α- and a β-integrin chain. The composition of the heterodimer defines specificity of the integrin for components of the basement membrane (Hynes 2002). To interact with extracellular factors, integrins need to become activated, either by intracellular or extracellular factors. Upon activation and ligand binding, integrins influence various cellular processes, such as cytoskeletal rearrangements, cell proliferation, and cell survival (Hynes 2002; ffrench-Constant and Colognato 2004; Legate et al. 2006) (Fig. 3a).

One of the most abundant integrin classes are those containing the β1-chain. Knockout of β1-integrin and members of some of its cytosolic partners resulted in embryonic lethality (Fassler and Meyer 1995; Li et al. 2005; Liang et al. 2005; Sakai et al. 2003; Stephens et al. 1995).

In islets, heterodimers containing β1-integrin have been suggested to affect insulin transcription and secretion as well as β-cell proliferation (Kaido et al. 2004; Nikolova et al. 2006).

Dystroglycan

Another cell surface receptor for basement membranes is dystroglycan. It is part of the dystrophin–glycoprotein complex in muscle fibers. However, it is also expressed in many other tissues (Durbeej et al. 1998).

Dystroglycan is a heterodimer composed of an extracellular α-subunit and a transmembrane β-subunit containing an intracellular signaling domain.

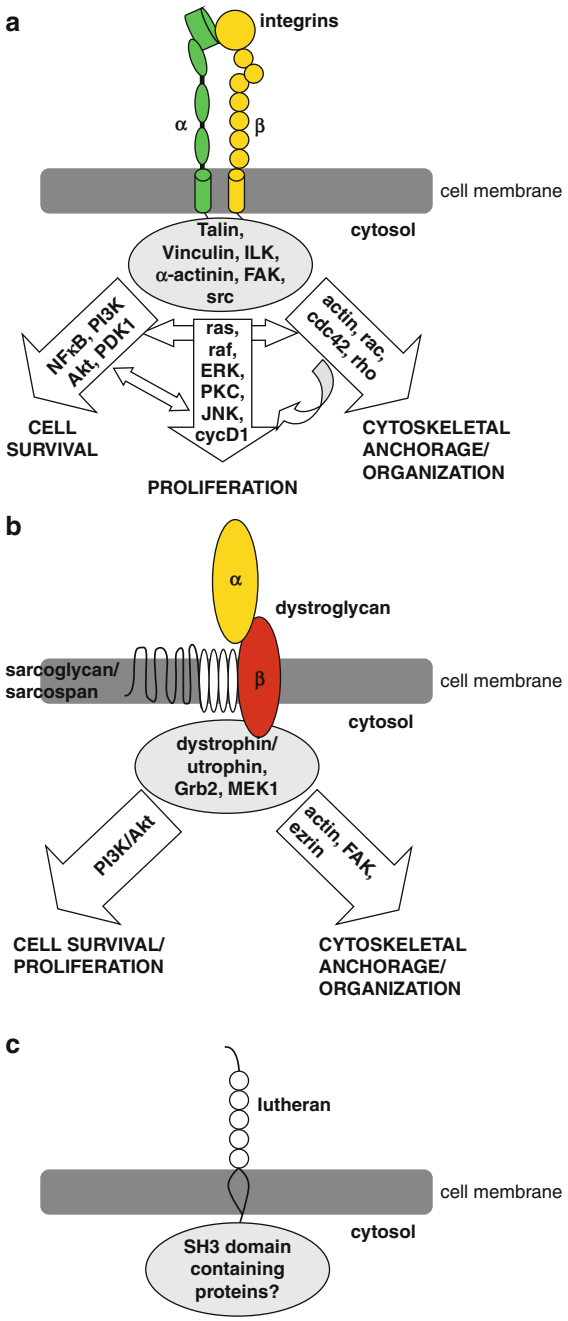

Fig. 3 Major cell surface receptors binding to BM components. (**a**) Integrins form heterodimers of an α- and β-chain and bind different forms of laminin, collagen IV, and perlecan. Integrins are upstream of various intracellular signaling pathways. Binding partners

Both subunits are encoded by one gene and are a product of posttranslational cleavage (Ibraghimov-Beskrovnaya et al. 1992, 1993).

The extracellular α-subunit is highly glycosylated. The glycosylation patterns differ among cell types, suggesting that glycosylation of the protein confers tissue-specific interactions between the basement membrane and the cell surface (Ervasti and Campbell 1993).

The extracellular α-subunit has been shown to interact with laminin-111 and laminin-211 as well as perlecan (Ervasti and Campbell 1993; Rudenko et al. 2001; Gee et al. 1993; Matsumura et al. 1993; Yamada et al. 1994), whereas the β-subunit binds to dystrophin in muscle fibers (Jung et al. 1995; Rentschler et al. 1999) or utrophin in other tissues (James et al. 2000), thus linking matrix components to the actin cytoskeleton. Dystroglycan has also been co-purified with Grb2 and FAK (Cavaldesi et al. 1999) and might be an adaptor for several other intracellular signaling molecules, including c-Src, Fyn, caveolin-1, MEK1, ERK, and ezrin (Sotgia et al. 2001, 2000; Spence et al. 2004a, b), suggesting that it might be involved in cell proliferation and cell motility (Fig. 3b).

Although dystroglycan has been suggested to play a role in laminin-induced β-cell differentiation (Jiang et al. 2001), this molecule has been poorly studied in the context of diabetes and β-cell function.

However, it has been shown to be an important regulator of interactions between basement membranes and cells. For example, abnormal glycosylation is associated with several congenital muscular dystrophies and impaired neural development (Durbeej and Campbell 2002; Haliloglu and Topaloglu 2004; Michele and Campbell 2003). Moreover, its targeted deletion in the brain resulted in a less organized extracellular matrix and a reduced laminin-binding activity (Moore et al. 2002). Thus, it might modulate the communication between β-cells and the vascular basement membrane.

Fig. 3 (continued) of the cytoplasmic tails (within the *gray oval circle*) link the integrins to these signaling pathways. For further details, readers are referred to several excellent reviews (Hynes 2002; Legate et al. 2006; Danen and Yamada 2001; Miranti and Brugge 2002; Schwartz and Ginsberg 2002; Wu and Dedhar 2001). (**b**) Dystroglycan consists of an extracellular α-subunit and a transmembrane β-subunit that undergo a non-covalent interaction. The α-subunit interacts with various BM components. Dystroglycan is upstream of various intracellular signaling pathways. Binding partners of the cytoplasmic tail of the β-subunit (within the *gray oval circle*) link dystroglycan to these pathways. For further details, readers are referred to several excellent reviews (Haenggi and Fritschy 2006; Sgambato and Brancaccio 2005; Winder 2001). (**c**) Lutheran is a cell surface receptor that belongs to the Ig superfamily. It has been mainly known as a blood group antigen. Apart from being expressed in red blood cells, it is also present in various tissues and is shown to be a laminin receptor. Its cytoplasmic tail contains an SH3 (Src homology 3)-binding domain, but its role in bridging the BM to the cytosol is currently unknown

Lutheran Glycoprotein

The lutheran glycoprotein, a member of the Ig superfamily, has been long known for being one of the blood group antigens in red blood cells. Recently, it has been shown to be a laminin receptor with a specific affinity for the laminin α5 chain (Eyler and Telen 2006; Kikkawa and Miner 2005; Kikkawa et al. 2002). Interestingly, this molecule is expressed in human pancreatic β-cells, while it is absent in the β-cells of rodents (Otonkoski et al. 2008; Virtanen et al. 2008).

There are two splice forms of the lutheran glycoprotein: one version has a short cytoplasmic domain containing an SH3-binding motif (Parsons et al. 2001, 1995) (Fig. 3c). Thus, proteins containing an SH3 domain, e.g., the tyrosine kinases c-Src and Fyn, might interact and provide a link between lutheran and important intracellular signaling processes. The shorter version of the protein (called B-CAM for basal cell adhesion molecule) lacks this domain. However, this short form is not expressed in human islets (Virtanen et al. 2008).

Although the role of lutheran in β-cell function has not been investigated, in vitro adhesion experiments with dispersed human β-cells revealed lutheran as one of the molecules that bind effectively to laminin-511 (Virtanen et al. 2008). It will be interesting to study the role of lutheran in β-cell function.

The Vascular Basement Membrane and Its Role in Pancreatic Islets

In islets, blood vessels play an important role, since they are required for forming the vascular basement membrane. Although most epithelial cells can form basement membrane, mouse pancreatic β-cells require blood vessels for basement membrane formation. The importance of blood vessels for β-cells is reflected by islet physiology: islets are highly vascularized (Konstantinova and Lammert 2004) (Fig. 4), and each β-cell is in contact with an endothelial cell-derived basement membrane. In contrast to rodents, blood vessels in human islets are surrounded by two layers of basement membrane – one probably derived from endothelial cells, the other one coming from another islet cell type, for example, pericytes or the β-cells themselves (Otonkoski et al. 2008; Virtanen et al. 2008).

A crucial factor for the communication between β-cells and endothelial cells is VEGF-A, which is secreted by β-cells. VEGF-A-depleted islets display a reduced degree of islet vasculature in a dose-dependent manner, and when transplanted into normal hosts, such islets were only inefficiently revascularized when compared to control islets (Brissova et al. 2006).

In addition, islets depleted of VEGF-A have a reduced number of capillaries and exhibit several defects in β-cell function, including insulin transcription (Nikolova et al. 2006), insulin content, and first-phase insulin secretion (Brissova et al. 2006; Jabs et al. 2008), glucose tolerance (Brissova et al. 2006; Lammert et al. 2003), and β-cell proliferation (Nikolova et al. 2006).

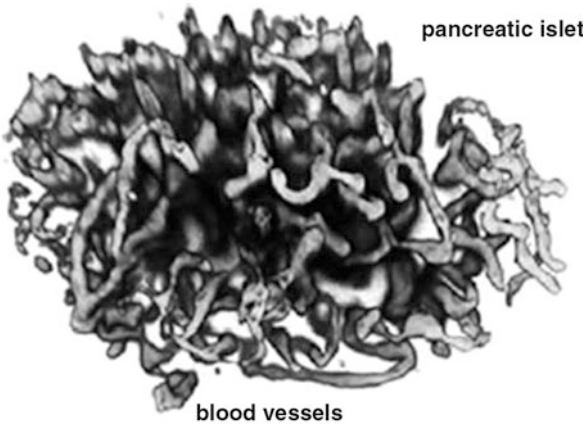

Fig. 4 Islets are highly vascularized. The 3D reconstruction of the vasculature in a mouse pancreatic islet reconstructed from images taken with a single plane illumination microscope (*SPIM*). The image shows the high density of blood vessels found within pancreatic islets (From Lammert (2008). Printed with permission of the Georg Thieme Verlag, Stuttgart)

It appears that the right dose of VEGF-A is important for the correct development and function of pancreatic islets. Transgenic mice overexpressing the VEGF-A gene in β cells display a significantly enhanced blood vessel density in their islets. But instead of a better function and proliferation of β cells, the increased number of endothelial cells leads to an impaired islet architecture, function, and mass (Cai et al. 2012; Agudo et al. 2012).

As mentioned above, endothelial cells are required for forming a basement membrane within the islets (Nikolova et al. 2006), and the lack of vascular basement membrane significantly contributes to impaired β-cell behavior in VEGF-A-deficient islets.

Control of β-Cell Function by Vascular Basement Membrane

Evidence for the vascular basement membrane being implicated in islet function came from in vitro experiments using purified rat β-cells plated on the so-called 804G-extracellular matrix (Bosco et al. 2000; Bosco and Kern 2004; Hammar et al. 2008, 2005; Parnaud et al. 2006). This matrix is formed by a rat bladder carcinoma cell line and contains essential basement membrane components such as collagens and laminins. Under these conditions, β-cells secreted more insulin in response to glucose and exhibited a better survival rate when compared to appropriate controls (Bosco et al. 2000).

Interestingly, it could also be shown that the 804G-matrix enhanced insulin secretion via NFκB as well as the Rho/ROCK pathway (Hammar et al. 2008, 2005). Furthermore, this matrix stimulated the activation of the ERK and Akt/PKB pathways, further suggesting that basement membrane components can influence cell survival and proliferation (Bosco et al. 2000; Bosco and Kern 2004).

Recent experiments using cultured pancreatic islets support the view of a beneficial effect of basement membrane components on islet function and survival. When rodent or human islets were incubated with matrix components, they

exhibited an improved glucose-stimulated insulin secretion and survival (Davis et al. 2012; Zhang et al. 2012; Sojoodi et al. 2013). Furthermore, islets kept in the presence of matrix components could improve blood glucose levels after their transplantation into diabetic mice (Vernon et al. 2012).

What are the specific basement membrane components and cell surface receptors that influence insulin production and secretion as well as β-cell proliferation? Parnaud et al. (2006) showed that interaction of laminin-332 and β1-integrin affects insulin secretion in this experimental system. In the following, we discuss more examples of basement membrane/integrin interactions implicated in β-cell function.

Specific Basement Membrane/Cell Surface Interactions That Control β-Cell Function

Laminin/α6β1-Integrin Interaction and Insulin Transcription

Nikolova et al. (2006) showed that laminins positively influence insulin transcription by performing rescue experiments on cultured VEGF-A$^{-/-}$ islets and in vitro studies using MIN6 cells, a mouse tumor cell line derived from pancreatic β-cells, plated on laminins (Miyazaki et al. 1990).

When MIN6 cells were plated on different basement membrane components, including laminins, collagen IV, or fibronectin, the transcriptional levels of both insulin genes were upregulated compared to controls. The strongest effect was observed, when cells were plated on laminin-111, laminin-411, and laminin-511. Specific knockdown of α6- or β1-integrin by siRNA and the use of a blocking antibody against β1-integrin showed that the α6/β1 integrin heterodimer is one laminin receptor that promotes insulin gene transcription.

Experiments on VEGF-A$^{-/-}$ islets, which do not harbor an intra-islet vascular basement membrane and exhibit reduced levels of insulin transcription, lead to a similar conclusion: soluble laminin-111 partially restored the transcriptional activity, and this rescue effect could be blocked by an antibody directed against β1-integrin (Nikolova et al. 2006).

Surprisingly, another study showed that the culture of primary human β-cells on collagen IV or vitronectin negatively affects insulin transcription, whereas laminin had neither a positive nor a negative effect (Kaido et al. 2006). This difference to the above experiments might be due to the differences between human and mouse cells or due to the fact that the mouse studies used intact islets, whereas the human β-cells were dissociated prior to culture.

Collagen IV/α1β1-Integrin Interaction and Insulin Secretion

Experiments on cultured primary human β-cells plated on various matrices showed that collagen IV could enhance insulin secretion (Kaido et al. 2004, 2006).

Furthermore, the use of a blocking antibody directed against the α1β1-integrin heterodimer abolished this effect, suggesting that the specific interaction between α1β1-integrin and collagen IV improves insulin secretion (Kaido et al. 2004).

Laminin and β-Cell Proliferation

Studies on MIN6 cells and VEGF-A$^{-/-}$ islets suggested that laminin positively influences β-cell proliferation. When plated on a laminin matrix, BrdU assays showed that the percentage of MIN6 cells undergoing S-phase was higher, when compared to cells plated on other matrices. This laminin effect could be blocked by the application of an antibody directed against β1-integrin or, alternatively, knock-down of β1-integrin.

Most importantly, soluble laminin-111 partially rescued the frequency of mitotic cells in VEGF-A$^{-/-}$ islets, further suggesting that laminin specifically supports β-cell proliferation (Nikolova et al. 2006).

A Role for Basement Membrane/β Cell Interaction In Vivo?

The experiments described here were performed on β cell lines or isolated pancreatic islets. It is, however, difficult to prove the importance of basement membrane components on β cell function, proliferation, and survival in vivo. Mice in which the VEGF-A gene is depleted from islets display an impaired glucose tolerance, but this could also be interpreted as a defect in glucose sensing and insulin release due to the absence of capillaries (Brissova et al. 2006). Also, β cell mass in islets without blood vessels is either slightly impaired or not affected at all, even after challenging these mice with a high-fat diet (Lammert et al. 2003; Toyofuku et al. 2009). However, the numerous in vitro experiments on basement membrane/β cell interaction offer a promising tool to expand functional islet mass in culture and improve β cell function after transplantation into patients, irrespective of whether there is an in vivo function or not.

Conclusion

Blood vessels are attracted to invade pancreatic islets via VEGF-A secreted by β-cells. The blood vessels, in turn, initiate the formation of the vascular basement membrane, a specialized extracellular matrix that controls β-cell function. Although we are far away from understanding the complex network of communication between the vascular basement membrane and the β-cells, a few specific basement membrane/cell surface receptor interactions could already be identified, which are implicated in insulin production and secretion as well as β-cell proliferation (Fig. 5).

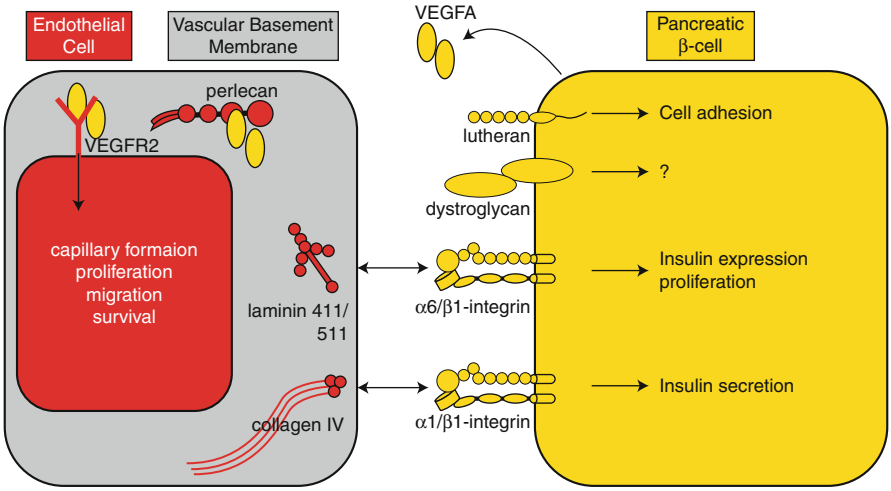

Fig. 5 Schematic view on the communication between blood vessels and β-cells and the role of the vascular basement membrane. VEGF-A, secreted by β-cells, attracts blood vessels to invade the islet. The presence of blood vessels in the islet is crucial for β-cell function as it depends on components of the specialized vascular basement membrane, which communicate with cell surface receptors on β-cells. The interaction between laminin and α6β1-integrin affects insulin gene transcription, whereas binding of collagen IV to α1β1-integrin influences insulin secretion. Laminin binding to β1-integrin receptors accounts for stimulation of β-cell proliferation

Outlook

It is important to understand the interactions between vascular basement membrane and β-cells in order to design more efficient diabetes therapies. In particular, inducing proliferation without affecting β-cell quality could improve the success of islet transplantation.

Various problems have been reported related to the transplantation of islets. One complication is that transplanted islets are opposed by the host immune system, leaving an insufficient number of islets to deal with glucohomeostasis. Therefore, restoring a proper basement membrane in islets may increase the viability of islets and at the same time lower their antigenicity. In addition, a few insulin-producing β-cells were observed in patients with diabetes (Meier et al. 2006). However, it is unknown how to expand these β-cells in these patients. Therefore, studies aiming to elucidate how the vascular basement membrane affects β-cell proliferation as well as autoimmune destruction of islets may help to improve the regenerative potential of islets.

Another problem of islet transplantation is to obtain sufficient numbers of healthy islets from donors, and a major goal is to culture and expand islets in vitro. However, it is difficult to stimulate β-cell proliferation without losing the β-cell's ability to secrete sufficient levels of insulin in response to glucose.

Therefore, understanding the molecular networks underlying the communication between the vascular basement membrane and β-cell surface receptors may help to reveal how the mass of functional insulin-producing and insulin-secreting β-cells can be increased.

Furthermore, it would be interesting to make artificial islets by generating a scaffold of basement membrane and populate this scaffold by β-cells or their progenitors. In this regard, it is noteworthy that a recent study showed that decellularized heart matrices could be repopulated by cardiocytes and endothelial cells (Ott et al. 2008). Thus, studies on the vascular basement membrane may open new avenues for generating artificial and functional islets.

Cross-References

- ▶ Apoptosis in Pancreatic β-Islet Cells in Type 1 and Type 2 Diabetes
- ▶ Islet Encapsulation
- ▶ Islet Structure and Function in the GK Rat
- ▶ Successes and Disappointments with Clinical Islet Transplantation

References

Agudo J, Ayuso E, Jimenez V, Casellas A, Mallol C, Salavert A, Tafuro S, Obach M, Ruzo A, Moya M, Pujol A, Bosch F (2012) Vascular endothelial growth factor-mediated islet hypervascularization and inflammation contribute to progressive reduction of β-cell mass. Diabetes 61(11):2851–2861

Arikawa-Hirasawa E, Watanabe H, Takami H, Hassell JR, Yamada Y (1999) Perlecan is essential for cartilage and cephalic development. Nat Genet 23:354–358

Aumailley M, Timpl R (1986) Attachment of cells to basement membrane collagen type IV. J Cell Biol 103:1569–1575

Aumailley M, Wiedemann H, Mann K, Timpl R (1989) Binding of nidogen and the laminin-nidogen complex to basement membrane collagen type IV. Eur J Biochem 184:241–248

Aumailley M, Specks U, Timpl R (1991a) Cell adhesion to type-VI collagen. Biochem Soc Trans 19:843–847

Aumailley M, Timpl R, Risau W (1991b) Differences in laminin fragment interactions of normal and transformed endothelial cells. Exp Cell Res 196:177–183

Aumailley M, Battaglia C, Mayer U, Reinhardt D, Nischt R, Timpl R, Fox JW (1993) Nidogen mediates the formation of ternary complexes of basement membrane components. Kidney Int 43:7–12

Aumailley M, Bruckner-Tuderman L, Carter WG, Deutzmann R, Edgar D, Ekblom P, Engel J, Engvall E, Hohenester E, Jones JC, Kleinman HK, Marinkovich MP, Martin GR, Mayer U, Meneguzzi G, Miner JH, Miyazaki K, Patarroyo M, Paulsson M, Quaranta V, Sanes JR, Sasaki T, Sekiguchi K, Sorokin LM, Talts JF, Tryggvason K, Uitto J, Virtanen I, von der Mark K, Wewer UM, Yamada Y, Yurchenco PD (2005) A simplified laminin nomenclature. Matrix Biol 24:326–332

Bader BL, Smyth N, Nedbal S, Miosge N, Baranowsky A, Mokkapati S, Murshed M, Nischt R (2005) Compound genetic ablation of nidogen 1 and 2 causes basement membrane defects and perinatal lethality in mice. Mol Cell Biol 25:6846–6856

Borchiellini C, Coulon J, Le Parco Y (1996) The function of type IV collagen during Drosophila muscle development. Mech Dev 58:179–191

Bosco DA, Kern D (2004) Catalysis and binding of cyclophilin A with different HIV-1 capsid constructs. Biochemistry 43:6110–6119

Bosco D, Meda P, Halban PA, Rouiller DG (2000) Importance of cell-matrix interactions in rat islet β-cell secretion in vitro: role of α6β1 integrin. Diabetes 49:233–243

Boutaud A, Borza DB, Bondar O, Gunwar S, Netzer KO, Singh N, Ninomiya Y, Sado Y, Noelken ME, Hudson BG (2000) Type IV collagen of the glomerular basement membrane. Evidence that the chain specificity of network assembly is encoded by the noncollagenous NC1 domains. J Biol Chem 275:30716–30724

Brissova M, Shostak A, Shiota M, Wiebe PO, Poffenberger G, Kantz J, Chen Z, Carr C, Jerome WG, Chen J, Baldwin HS, Nicholson W, Bader DM, Jetton T, Gannon M, Powers AC (2006) Pancreatic islet production of vascular endothelial growth factor–a is essential for islet vascularization, revascularization, and function. Diabetes 55:2974–2985

Cai Q, Brissova M, Reinert RB, Pan FC, Brahmachary P, Jeansson M, Shostak A, Radhika A, Poffenberger G, Quaggin SE, Jerome WG, Dumont DJ, Powers AC (2012) Enhanced expression of VEGF-A in β cells increases endothelial cell number but impairs islet morphogenesis and β cell proliferation. Dev Biol 367:40–54

Carlin B, Jaffe R, Bender B, Chung AE (1981) Entactin, a novel basal lamina-associated sulfated glycoprotein. J Biol Chem 256:5209–5214

Cavaldesi M, Macchia G, Barca S, Defilippi P, Tarone G, Petrucci TC (1999) Association of the dystroglycan complex isolated from bovine brain synaptosomes with proteins involved in signal transduction. J Neurochem 72:1648–1655

Chen MS, Almeida EA, Huovila AP, Takahashi Y, Shaw LM, Mercurio AM, White JM (1999) Evidence that distinct states of the integrin α6β1 interact with laminin and an ADAM. J Cell Biol 144:549–561

Cheng YS, Champliaud MF, Burgeson RE, Marinkovich MP, Yurchenco PD (1997) Self-assembly of laminin isoforms. J Biol Chem 272:31525–31532

Chung AE, Jaffe R, Freeman IL, Vergnes JP, Braginski JE, Carlin B (1979) Properties of a basement membrane-related glycoprotein synthesized in culture by a mouse embryonal carcinoma-derived cell line. Cell 16:277–287

Colognato H, Yurchenco PD (2000) Form and function: the laminin family of heterotrimers. Dev Dyn 218:213–234

Costell M, Gustafsson E, Aszodi A, Morgelin M, Bloch W, Hunziker E, Addicks K, Timpl R, Fassler R (1999) Perlecan maintains the integrity of cartilage and some basement membranes. J Cell Biol 147:1109–1122

Dai C, Huh CG, Thorgeirsson SS, Liu Y (2005) β-cell-specific ablation of the hepatocyte growth factor receptor results in reduced islet size, impaired insulin secretion, and glucose intolerance. Am J Pathol 167:429–436

Danen EH, Yamada KM (2001) Fibronectin, integrins, and growth control. J Cell Physiol 189:1–13

Davis NE, Beenken-Rothkopf LN, Mirsoian A, Kojic N, Kaplan DL, Barron AE, Fontaine MJ (2012) Enhanced function of pancreatic islets co-encapsulated with ECM proteins and mesenchymal stromal cells in a silk-hydrogel. Biomaterials 33(28):6691–6697

Durbeej M, Campbell KP (2002) Muscular dystrophies involving the dystrophin-glycoprotein complex: an overview of current mouse models. Curr Opin Genet Dev 12:349–361

Durbeej M, Henry MD, Ferletta M, Campbell KP, Ekblom P (1998) Distribution of dystroglycan in normal adult mouse tissues. J Histochem Cytochem 46:449–457

Ervasti JM, Campbell KP (1993) A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. J Cell Biol 122:809–823

Ettner N, Gohring W, Sasaki T, Mann K, Timpl R (1998) The N-terminal globular domain of the laminin α1 chain binds to α1β1 and α2β1 integrins and to the heparan sulfate-containing domains of perlecan. FEBS Lett 430:217–221

Eyler CE, Telen MJ (2006) The lutheran glycoprotein: a multifunctional adhesion receptor. Transfusion 46:668–677

Fassler R, Meyer M (1995) Consequences of lack of β1 integrin gene expression in mice. Genes Dev 9:1896–1908

ffrench-Constant C, Colognato H (2004) Integrins: versatile integrators of extracellular signals. Trends Cell Biol 14:678–686

Fox JW, Mayer U, Nischt R, Aumailley M, Reinhardt D, Wiedemann H, Mann K, Timpl R, Krieg T, Engel J et al (1991) Recombinant nidogen consists of three globular domains and mediates binding of laminin to collagen type IV. EMBO J 10:3137–3146

Fukumoto S, Miner JH, Ida H, Fukumoto E, Yuasa K, Miyazaki H, Hoffman MP, Yamada Y (2006) Laminin α5 is required for dental epithelium growth and polarity and the development of tooth bud and shape. J Biol Chem 281:5008–5016

Gee SH, Blacher RW, Douville PJ, Provost PR, Yurchenco PD, Carbonetto S (1993) Laminin-binding protein 120 from brain is closely related to the dystrophin-associated glycoprotein, dystroglycan, and binds with high affinity to the major heparin binding domain of laminin. J Biol Chem 268:14972–14980

Gupta MC, Graham PL, Kramer JM (1997) Characterization of α1(IV) collagen mutations in *Caenorhabditis elegans* and the effects of α1 and α2(IV) mutations on type IV collagen distribution. J Cell Biol 137:1185–1196

Hacker U, Nybakken K, Perrimon N (2005) Heparan sulphate proteoglycans: the sweet side of development. Nat Rev Mol Cell Biol 6:530–541

Haenggi T, Fritschy JM (2006) Role of dystrophin and utrophin for assembly and function of the dystrophin glycoprotein complex in non-muscle tissue. Cell Mol Life Sci 63:1614–1631

Haliloglu G, Topaloglu H (2004) Glycosylation defects in muscular dystrophies. Curr Opin Neurol 17:521–527

Hammar EB, Irminger JC, Rickenbach K, Parnaud G, Ribaux P, Bosco D, Rouiller DG, Halban PA (2005) Activation of NF-kappaB by extracellular matrix is involved in spreading and glucose-stimulated insulin secretion of pancreatic β cells. J Biol Chem 280:30630–30637

Hammar E, Tomas A, Bosco D, Halban PA (2008) Role of the Rho-rock (rho-associated kinase) signaling pathway in the regulation of pancreatic β cell function. Endocrinology 295:1277

Hart AW, Baeza N, Apelqvist A, Edlund H (2000) Attenuation of FGF signalling in mouse β-cells leads to diabetes. Nature 408:864–868

Hudson BG, Reeders ST, Tryggvason K (1993) Type IV collagen: structure, gene organization, and role in human diseases. Molecular basis of Goodpasture and Alport syndromes and diffuse leiomyomatosis. J Biol Chem 268:26033–26036

Hynes RO (1992) Integrins: versatility, modulation, and signaling in cell adhesion. Cell 69:11–25

Hynes RO (2002) Integrins: bidirectional, allosteric signaling machines. Cell 110:673–687

Ibraghimov-Beskrovnaya O, Ervasti JM, Leveille CJ, Slaughter CA, Sernett SW, Campbell KP (1992) Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. Nature 355:696–702

Ibraghimov-Beskrovnaya O, Milatovich A, Ozcelik T, Yang B, Koepnick K, Francke U, Campbell KP (1993) Human dystroglycan: skeletal muscle cDNA, genomic structure, origin of tissue specific isoforms and chromosomal localization. Hum Mol Genet 2:1651–1657

Iozzo RV (1998) Matrix proteoglycans: from molecular design to cellular function. Annu Rev Biochem 67:609–652

Irving-Rodgers HF, Ziolkowski AF, Parish CR, Sado Y, Ninomiya Y, Simeonovic CJ, Rodgers RJ (2008) Molecular composition of the peri-islet basement membrane in NOD mice: a barrier against destructive insulitis. Diabetologia 51:1680–1688

Jabs N, Franklin I, Brenner MB, Gromada J, Ferrara N, Wollheim CB, Lammert E (2008) Reduced insulin secretion and content in VEGF-a deficient mouse pancreatic islets. Exp Clin Endocrinol Diabetes 116(Suppl 1):S46–S49

James M, Nuttall A, Ilsley JL, Ottersbach K, Tinsley JM, Sudol M, Winder SJ (2000) Adhesion-dependent tyrosine phosphorylation of β-dystroglycan regulates its interaction with utrophin. J Cell Sci 113((Pt 10)):1717–1726

Jiang FX, Georges-Labouesse E, Harrison LC (2001) Regulation of laminin 1-induced pancreatic β-cell differentiation by α6 integrin and α-dystroglycan. Mol Med 7:107–114
Jung D, Yang B, Meyer J, Chamberlain JS, Campbell KP (1995) Identification and characterization of the dystrophin anchoring site on β-dystroglycan. J Biol Chem 270:27305–27310
Kaido T, Yebra M, Cirulli V, Montgomery AM (2004) Regulation of human β-cell adhesion, motility, and insulin secretion by collagen IV and its receptor α1β1. J Biol Chem 279:53762–53769
Kaido T, Yebra M, Cirulli V, Rhodes C, Diaferia G, Montgomery AM (2006) Impact of defined matrix interactions on insulin production by cultured human β-cells: effect on insulin content, secretion, and gene transcription. Diabetes 55:2723–2729
Kalluri R (2003) Basement membranes: structure, assembly and role in tumour angiogenesis. Nat Rev Cancer 3:422–433
Kikkawa Y, Miner JH (2005) Review: Lutheran/B-CAM: a laminin receptor on red blood cells and in various tissues. Connect Tissue Res 46:193–199
Kikkawa Y, Moulson CL, Virtanen I, Miner JH (2002) Identification of the binding site for the Lutheran blood group glycoprotein on laminin α5 through expression of chimeric laminin chains in vivo. J Biol Chem 277:44864–44869
Kilkenny DM, Rocheleau JV (2008) Fibroblast growth factor receptor-1 signaling in pancreatic islet β-cells is modulated by the extracellular matrix. Mol Endocrinol 22:196–205
Kimura N, Toyoshima T, Kojima T, Shimane M (1998) Entactin-2: a new member of basement membrane protein with high homology to entactin/nidogen. Exp Cell Res 241:36–45
Kohfeldt E, Sasaki T, Gohring W, Timpl R (1998) Nidogen-2: a new basement membrane protein with diverse binding properties. J Mol Biol 282:99–109
Konstantinova I, Lammert E (2004) Microvascular development: learning from pancreatic islets. Bioessays 26:1069–1075
Lammert E (2008) The vascular trigger of Type II Diabetes mellitus. Exp Clin Endocrinol Diabetes 116:S21–S25
Lammert E, Gu G, McLaughlin M, Brown D, Brekken R, Murtaugh LC, Gerber HP, Ferrara N, Melton DA (2003) Role of VEGF-A in vascularization of pancreatic islets. Curr Biol 13:1070–1074
Legate KR, Montanez E, Kudlacek O, Fassler R (2006) ILK, PINCH and parvin: the tIPP of integrin signalling. Nat Rev Mol Cell Biol 7:20–31
Li J, Tzu J, Chen Y, Zhang YP, Nguyen NT, Gao J, Bradley M, Keene DR, Oro AE, Miner JH, Marinkovich MP (2003) Laminin-10 is crucial for hair morphogenesis. EMBO J 22:2400–2410
Li S, Bordoy R, Stanchi F, Moser M, Braun A, Kudlacek O, Wewer UM, Yurchenco PD, Fassler R (2005) PINCH1 regulates cell-matrix and cell-cell adhesions, cell polarity and cell survival during the peri-implantation stage. J Cell Sci 118:2913–2921
Liang X, Zhou Q, Li X, Sun Y, Lu M, Dalton N, Ross J Jr, Chen J (2005) PINCH1 plays an essential role in early murine embryonic development but is dispensable in ventricular cardiomyocytes. Mol Cell Biol 25:3056–3062
Lin X (2004) Functions of heparan sulfate proteoglycans in cell signaling during development. Development 131:6009–6021
Lopez-Talavera JC, Garcia-Ocana A, Sipula I, Takane KK, Cozar-Castellano I, Stewart AF (2004) Hepatocyte growth factor gene therapy for pancreatic islets in diabetes: reducing the minimal islet transplant mass required in a glucocorticoid-free rat model of allogeneic portal vein islet transplantation. Endocrinology 145:467–474
Matsumura K, Yamada H, Shimizu T, Campbell KP (1993) Differential expression of dystrophin, utrophin and dystrophin-associated proteins in peripheral nerve. FEBS Lett 334:281–285
Mayer U, Kohfeldt E, Timpl R (1998) Structural and genetic analysis of laminin-nidogen interaction. Ann N Y Acad Sci 857:130–142
Meier JJ, Lin JC, Butler AE, Galasso R, Martinez DS, Butler PC (2006) Direct evidence of attempted β cell regeneration in an 89-year-old patient with recent-onset type 1 diabetes. Diabetologia 49:1838–1844

Michele DE, Campbell KP (2003) Dystrophin-glycoprotein complex: post-translational processing and dystroglycan function. J Biol Chem 278:15457–15460

Miner JH, Li C (2000) Defective glomerulogenesis in the absence of laminin α5 demonstrates a developmental role for the kidney glomerular basement membrane. Dev Biol 217:278–289

Miner JH, Yurchenco PD (2004) Laminin functions in tissue morphogenesis. Annu Rev Cell Dev Biol 20:255–284

Miranti CK, Brugge JS (2002) Sensing the environment: a historical perspective on integrin signal transduction. Nat Cell Biol 4:E83–E90

Miyazaki J, Araki K, Yamato E, Ikegami H, Asano T, Shibasaki Y, Oka Y, Yamamura K (1990) Establishment of a pancreatic β cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. Endocrinology 127:126–132

Moore SA, Saito F, Chen J, Michele DE, Henry MD, Messing A, Cohn RD, Ross-Barta SE, Westra S, Williamson RA, Hoshi T, Campbell KP (2002) Deletion of brain dystroglycan recapitulates aspects of congenital muscular dystrophy. Nature 418:422–425

Murshed M, Smyth N, Miosge N, Karolat J, Krieg T, Paulsson M, Nischt R (2000) The absence of nidogen 1 does not affect murine basement membrane formation. Mol Cell Biol 20:7007–7012

Nikolova G, Jabs N, Konstantinova I, Domogatskaya A, Tryggvason K, Sorokin L, Fassler R, Gu G, Gerber HP, Ferrara N, Melton DA, Lammert E (2006) The vascular basement membrane: a niche for insulin gene expression and β cell proliferation. Dev Cell 10:397–405

Otonkoski T, Banerjee M, Korsgren O, Thornell LE, Virtanen I (2008) Unique basement membrane structure of human pancreatic islets: implications for β-cell growth and differentiation. Diabetes Obes Metab 4(10 Suppl):119–127

Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, Taylor DA (2008) Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med 14:213–221

Parnaud G, Hammar E, Rouiller DG, Armanet M, Halban PA, Bosco D (2006) Blockade of β1 integrin-laminin-5 interaction affects spreading and insulin secretion of rat β-cells attached on extracellular matrix. Diabetes 55:1413–1420

Parsons SF, Mallinson G, Holmes CH, Houlihan JM, Simpson KL, Mawby WJ, Spurr NK, Warne D, Barclay AN, Anstee DJ (1995) The Lutheran blood group glycoprotein, another member of the immunoglobulin superfamily, is widely expressed in human tissues and is developmentally regulated in human liver. Proc Natl Acad Sci USA 92:5496–5500

Parsons SF, Lee G, Spring FA, Willig TN, Peters LL, Gimm JA, Tanner MJ, Mohandas N, Anstee DJ, Chasis JA (2001) Lutheran blood group glycoprotein and its newly characterized mouse homologue specifically bind α5 chain-containing human laminin with high affinity. Blood 97:312–320

Patton BL, Cunningham JM, Thyboll J, Kortesmaa J, Westerblad H, Edstrom L, Tryggvason K, Sanes JR (2001) Properly formed but improperly localized synaptic specializations in the absence of laminin α4. Nat Neurosci 4:597–604

Paulsson M (1992) Basement membrane proteins: structure, assembly, and cellular interactions. Crit Rev Biochem Mol Biol 27:93–127

Poschl E, Schlotzer-Schrehardt U, Brachvogel B, Saito K, Ninomiya Y, Mayer U (2004) Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. Development 131:1619–1628

Rentschler S, Linn H, Deininger K, Bedford MT, Espanel X, Sudol M (1999) The WW domain of dystrophin requires EF-hands region to interact with β-dystroglycan. Biol Chem 380:431–442

Rudenko G, Hohenester E, Muller YA (2001) LG/LNS domains: multiple functions – one business end? Trends Biochem Sci 26:363–368

Sakai T, Li S, Docheva D, Grashoff C, Sakai K, Kostka G, Braun A, Pfeifer A, Yurchenco PD, Fassler R (2003) Integrin-linked kinase (ILK) is required for polarizing the epiblast, cell adhesion, and controlling actin accumulation. Genes Dev 17:926–940

Sasaki T, Timpl R (2001) Domain IVa of laminin α5 chain is cell-adhesive and binds β1 and αVβ3 integrins through Arg-Gly-Asp. FEBS Lett 509:181–185

Schittny JC, Yurchenco PD (1989) Basement membranes: molecular organization and function in development and disease. Curr Opin Cell Biol 1:983–988

Schittny JC, Yurchenco PD (1990) Terminal short arm domains of basement membrane laminin are critical for its self-assembly. J Cell Biol 110:825–832

Schwartz MA, Ginsberg MH (2002) Networks and crosstalk: integrin signalling spreads. Nat Cell Biol 4:E65–E68

Schymeinsky J, Nedbal S, Miosge N, Poschl E, Rao C, Beier DR, Skarnes WC, Timpl R, Bader BL (2002) Gene structure and functional analysis of the mouse nidogen-2 gene: nidogen-2 is not essential for basement membrane formation in mice. Mol Cell Biol 22:6820–6830

Sgambato A, Brancaccio A (2005) The dystroglycan complex: from biology to cancer. J Cell Physiol 205:163–169

Sheppard D (2000) In vivo functions of integrins: lessons from null mutations in mice. Matrix Biol 19:203–209

Sojoodi M, Farrokhi A, Moradmand A, Baharvand H (2013) Enhanced maintenance of rat islets of Langerhans on laminin-coated electrospun nanofibrillar matrix in vitro. Cell Biol Int

Sotgia F, Lee JK, Das K, Bedford M, Petrucci TC, Macioce P, Sargiacomo M, Bricarelli FD, Minetti C, Sudol M, Lisanti MP (2000) Caveolin-3 directly interacts with the C-terminal tail of β-dystroglycan. Identification of a central WW-like domain within caveolin family members. J Biol Chem 275:38048–38058

Sotgia F, Lee H, Bedford MT, Petrucci T, Sudol M, Lisanti MP (2001) Tyrosine phosphorylation of β-dystroglycan at its WW domain binding motif, PPxY, recruits SH2 domain containing proteins. Biochemistry 40:14585–14592

Spence HJ, Chen YJ, Batchelor CL, Higginson JR, Suila H, Carpen O, Winder SJ (2004a) Ezrin-dependent regulation of the actin cytoskeleton by β-dystroglycan. Hum Mol Genet 13:1657–1668

Spence HJ, Dhillon AS, James M, Winder SJ (2004b) Dystroglycan, a scaffold for the ERK-MAP kinase cascade. EMBO Rep 5:484–489

Stephens LE, Sutherland AE, Klimanskaya IV, Andrieux A, Meneses J, Pedersen RA, Damsky CH (1995) Deletion of β1 integrins in mice results in inner cell mass failure and peri-implantation lethality. Genes Dev 9:1883–1895

Strigini M (2005) Mechanisms of morphogen movement. J Neurobiol 64:324–333

Thyboll J, Kortesmaa J, Cao R, Soininen R, Wang L, Iivanainen A, Sorokin L, Risling M, Cao Y, Tryggvason K (2002) Deletion of the laminin α4 chain leads to impaired microvessel maturation. Mol Cell Biol 22:1194–1202

Timpl R, Brown JC (1996) Supramolecular assembly of basement membranes. Bioessays 18:123–132

Timpl R, Rohde H, Robey PG, Rennard SI, Foidart JM, Martin GR (1979) Laminin—a glycoprotein from basement membranes. J Biol Chem 254:9933–9937

Timpl R, Wiedemann H, van Delden V, Furthmayr H, Kuhn K (1981) A network model for the organization of type IV collagen molecules in basement membranes. Eur J Biochem 120:203–211

Toyofuku Y, Uchida T, Nakayama S, Hirose T, Kawamori R, Fujitani Y, Inoue M, Watada H (2009) Normal islet vascularization is dispensable for expansion of β-cell mass in response to high-fat diet induced insulin resistance. Biochem Biophys Res Commun 383(3):303–307

Vernon RB, Preisinger A, Gooden MD, D'Amico LA, Yue BB, Bollyky PL, Kuhr CS, Hefty TR, Nepom GT, Gebe JA (2012) Reversal of diabetes in mice with a bioengineered islet implant incorporating a type I collagen hydrogel and sustained release of vascular endothelial growth factor. Cell Transplant 21(10):2099–2110

Virtanen I, Banerjee M, Palgi J, Korsgren O, Lukinius A, Thornell LE, Kikkawa Y, Sekiguchi K, Hukkanen M, Konttinen YT, Otonkoski T (2008) Blood vessels of human islets of Langerhans are surrounded by a double basement membrane. Diabetologia 51:1181–1191

Vracko R (1972) Significance of basal lamina for regeneration of injured lung. Virchows Arch A Pathol Pathol Anat 355:264–274

Vracko R (1974) Basal lamina scaffold-anatomy and significance for maintenance of orderly tissue structure. Am J Pathol 77:314–346

Vracko R, Benditt EP (1970) Capillary basal lamina thickening. Its relationship to endothelial cell death and replacement. J Cell Biol 47:281–285

Vracko R, Benditt EP (1972) Basal lamina: the scaffold for orderly cell replacement. Observations on regeneration of injured skeletal muscle fibers and capillaries. J Cell Biol 55:406–419

Wallquist W, Plantman S, Thams S, Thyboll J, Kortesmaa J, Lannergren J, Domogatskaya A, Ogren SO, Risling M, Hammarberg H, Tryggvason K, Cullheim S (2005) Impeded interaction between Schwann cells and axons in the absence of laminin α4. J Neurosci 25:3692–3700

Wente W, Efanov AM, Brenner M, Kharitonenkov A, Koster A, Sandusky GE, Sewing S, Treinies I, Zitzer H, Gromada J (2006) Fibroblast growth factor-21 improves pancreatic β-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways. Diabetes 55:2470–2478

Whitelock JM, Graham LD, Melrose J, Murdoch AD, Iozzo RV, Underwood PA (1999) Human perlecan immunopurified from different endothelial cell sources has different adhesive properties for vascular cells. Matrix Biol 18:163–178

Willem M, Miosge N, Halfter W, Smyth N, Jannetti I, Burghart E, Timpl R, Mayer U (2002) Specific ablation of the nidogen-binding site in the laminin gamma1 chain interferes with kidney and lung development. Development 129:2711–2722

Winder SJ (2001) The complexities of dystroglycan. Trends Biochem Sci 26:118–124

Wu C, Dedhar S (2001) Integrin-linked kinase (ILK) and its interactors: a new paradigm for the coupling of extracellular matrix to actin cytoskeleton and signaling complexes. J Cell Biol 155:505–510

Yamada H, Shimizu T, Tanaka T, Campbell KP, Matsumura K (1994) Dystroglycan is a binding protein of laminin and merosin in peripheral nerve. FEBS Lett 352:49–53

Yurchenco PD, Furthmayr H (1984) Self-assembly of basement membrane collagen. Biochemistry 23:1839–1850

Yurchenco PD, Schittny JC (1990) Molecular architecture of basement membranes. FASEB J 4:1577–1590

Yurchenco PD, Cheng YS, Colognato H (1992) Laminin forms an independent network in basement membranes. J Cell Biol 117:1119–1133

Yurchenco PD, Smirnov S, Mathus T (2002) Analysis of basement membrane self-assembly and cellular interactions with native and recombinant glycoproteins. Methods Cell Biol 69:111–144

Zhang Y, Jalili RB, Warnock GL, Ao Z, Marzban L, Ghahary A (2012) Three-dimensional scaffolds reduce islet amyloid formation and enhance survival and function of cultured human islets. Am J Pathol 181(4):1296–1305

Approaches for Imaging Pancreatic Islets: Recent Advances and Future Prospects

4

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_39,

Abstract

Many questions about the natural history of both type 1 and type 2 diabetes remain unanswered, mostly because our present knowledge is derived from either in vitro studies or quite indirect in vivo measurements. Methods to noninvasively and repeatedly evaluate the mass and function of β-cells in vivo, two parameters that are central to the study of diabetes, are expected to change our understanding of this disease. However, and in spite of remarkable progress in many imaging techniques, no method yet fulfills the minimal requirements required for such an imaging, because of a combination of anatomical, biological, and technological problems. Here, we briefly review the major optical methods, which have been applied in imaging of the pancreatic β-cells, as well as the nonoptical methods, which may become relevant for the clinical assessment of islets, with particular attention to the individual advantages and limits of each approach.

Keywords

β-cell imaging • PET-CT • MRI • CT • US • Optical imaging

Introduction

Diabetes mellitus (DM) is characterized by the chronic elevation of glucose in the blood, which leads to organ dysfunction. Despite intense efforts being made to understand and develop preventive and curative therapies, the incidence of diabetes is still rising in developed and developing countries and is reaching epidemic proportions worldwide (Danaei et al. 2011). The pancreatic β-cells, which produce and secrete insulin, are key players in most forms of diabetes. Of the two main types of diabetes, type 1 diabetes (T1D) is a complex multifactorial disease, accounting for roughly 5 % of worldwide cases of diabetes (Maahs et al. 2010), with a poorly understood pathogenesis which culminates in the autoimmune destruction of β-cell. Indirect evidence of the autoimmune origin of T1D is provided by the presence of insulitis (lymphocyte infiltration and inflammation) in most patients (Bottazzo et al. 1985; Imagawa et al. 2001), as well as by presenting autoantibodies against β-cell proteins (Barone et al. 2011; Orban et al. 2009). A major difficulty is that at the onset of clinical symptoms, the autoimmune process is already at an advanced stage. The amount and speed of destruction, leading to T1D, is not well known because of our lack of accurate methods to quantify β-cell mass (BCM). However, a destruction of >90 % of the BCM has been usually seen at the onset of T1D (Lebastchi and Herold 2012). The lack of clear knowledge about the progression of T1D impedes the development of therapies. Therefore, imaging of the BCM in the context of T1D would be a great clinical value.

Type 2 diabetes (T2D) is the most common form of diabetes, accounting for approximately 90 % of all cases. T2D is characterized by the presence of insulin

resistance, which results in inadequate glucose uptake into the cells in response to the insulin secreted into the blood. The lack of glucose clearance from the blood and the increased insulin secretion due to insulin resistance lead to hyperglycemia and hyperinsulinemia, up to a point where β-cells fail to function properly. This failure accentuates the sustained hyperglycemia, causing multiple associated secondary diseases, including kidney failure, cardiovascular disease, poor blood circulation, blindness, and neuropathies. The cellular and molecular mechanisms that underline the development of T2D are still unclear (Lin and Sun 2010; Wild et al. 2004).

One aspect, which is crucial in developing therapeutic treatments, is the early detection of the disease onset and the monitoring of its progression. It has been widely shown that β-cells may continue releasing sufficient amounts of insulin in spite of a significant decrease in the β-cell mass, making it difficult to provide an accurate diagnosis in a normoglycemic patient. The decrease in mass and/or function of the β-cells is only indirectly measured in clinical settings. Therefore, direct visualization of β-cells would be of great interest in both preclinical and clinical settings. However, there are several difficulties in imaging β-cells of the pancreatic islets, among which the most important are (i) the deep location of the pancreas inside the abdomen and the dispersed distribution of islets within the pancreas; (ii) the small size (50–600 μm in diameter) of these islets, which requires high-resolution imaging techniques; and (iii) the relatively small volume of the islets in the pancreas (1–3 % of the gland volume), which is largely composed of exocrine tissue. An ideal imaging method should allow to repeatedly and noninvasively assess the β-cells mass and/or function, without toxicity or discomfort to the patient.

In clinical practice, noninvasive imaging has now become an important tool for the diagnosis of many diseases. Today imaging systems provide anatomical and pathological information based on changes in tissue structure, i.e., at relatively late stages. Such imaging systems include ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI). When pathology induces physical changes in the tissue, imaging modalities can detect them, based on changes in the propagation/reflection of sonic waves for ultrasound, changes in density for CT, and changes in water content for MRI. Molecular imaging, i.e., the imaging of cellular and subcellular events, has also gained tremendous interest in the past few years (Weissleder 2006). Major advances in molecular biology, chemistry, and imaging methods have coalesced to develop innovative imaging strategies. In the clinics, molecular imaging, such as provided by positron emission tomography (PET) and single photon emission computed tomography (SPECT), is mostly used in nuclear medicine. In parallel, sizable advances have been made in MRI, CT, ultrasound, and optical imaging, allowing molecular imaging also in other medical fields (Kircher and Willmann 2012; Lange et al. 2008). Furthermore, molecular contrast media have been designed to specifically image cellular or subcellular events. There are numerous ways to synthetize molecular imaging contrast media, among them are the smart probes and the targeted contrast media, which are discussed below.

Smart Probes

These agents change their physical properties after specific molecular interaction and are sometimes referred to as "molecular beacons." The approach of using in vivo optical (near-infrared) smart probes has been pioneered to detect proteolytic activity (Bremer et al. 2001, 2005; Figueiredo et al. 2006; Jaffer and Weissleder 2004), which is based on a quenching/dequenching paradigm. Thus, the probes are optically silent in their native (quenched) state and become highly fluorescent after the enzyme-mediated release of fluorochromes, resulting in signal amplification up to several hundred folds, depending on the design.

Targeted Contrast Media

To overcome the limitations of specificity, developments of new targeted contrast media have been proposed. Such approaches consist of coupling targeting moieties to contrast media. The targeting moieties, such as an antibody, a sugar, or a peptide, have to specifically recognize the cellular component of interest, whereas the contrast media depend on the imaging modality chosen for detection. Thus, microbubbles are used for ultrasound imaging (Behm and Lindner 2006; Pochon et al. 2010; Willmann et al. 2008), nanoparticles for MRI, and fluorophores for optical imaging. A recent approach to imaging is to obtain a fusion of different contrast media, which would result in a multimodal contrast media that would be visible by different imaging modalities. This multimodal approach would result in a cross-platform contrast agent, which would integrate the results from different imaging techniques, improving both spatial and temporal resolutions. For example, in the case of fluorescent nanoparticles, one could image the whole body by MRI and then go to the cellular/subcellular level using fluorescence microscopy (Montet et al. 2006a, b).

In this chapter, we summarize the methods, which are currently under test for imaging pancreatic β-cells, taking care to explain the advantages and disadvantages of each imaging modality.

Optical Imaging

Optical imaging is the process of acquiring images obtained using optical devices to capture electromagnetic waves (above x-ray to below radio), from or through a sample. Although initially optical imaging was solely applied to in vitro models, their failure to reproduce the in vivo conditions led many to look for a way to image biological processes in live animals (e.g., intravital microscopy).

A typical imaging setup comprises a light source, such as a laser diode, a test stage or holder, and a recording device, such as a CCD camera. The light can come from an external source, such as a lamp or diode, and either passes through a sample for collection or can reflect on the sample and then be collected. The source could also be internal, that is, the sample emits light. The position and biophysical

properties of the collected light can be used to determine the functional and structural characteristics of the sample. While optical imaging is widely used in preclinical microscopic research for fixed and live tissue samples and cultured cells, advances in imaging technologies have made macroscopic live-animal, whole-body imaging possible, allowing cellular and molecular processes to be noninvasively monitored. The recent development of molecular probes and tags using bioluminescence, such as luciferase, and red fluorescence, such as from fluorescent proteins, has permitted deeper imaging penetration as well as more complex physiological process to be followed in real time. Progress in protein chemistry, proteomics, and genomics is responsible for the identification of new imaging targets. Hopefully these new targets will increase our understanding of the disease and of its progression.

A major advantage of optical imaging systems is that they are cost-effective and have short learning curves and short imaging times. Furthermore, unlike radioactive radiation, fluorescent and bioluminescent light can be used repeatedly and more frequently, which provides a greater opportunity for clinical and preclinical imaging protocols and modalities. This increases the safety of use with patients and animal subjects.

Two major drawbacks of optical imaging are that light is scattered, absorbed, or reflected as it passes through a tissue (Wang 2002) and that several components of tissues have autofluorescent properties (Monici 2005), leading to high background and poor contrast. For example, the probability of photon absorption is different in the blood than the skin, and in the skin it is different whether it is passing through fat, water, or cells rich in the UV-protective protein, melanin. The absorption is also dependent on the wavelength and the distance of propagation. Light scattering is the result of light interacting with various components in the tissue and cells, such as nuclei and mitochondria, which have different refractive indices resulting in the attenuation of the signal at increasing distances.

These factors are mainly responsible for the difficulties of using optical imaging for deep tissue visualization in a clinical setting. The light scattering and attenuation of the signal is the main reason why optical imaging is limited to shallow tissue depths and why, for proper resolution, the acquisition must be made near the site of interest. Further complications come from the autofluorescent components present in the heterogeneous layers of tissue that reduce the signal-to-noise ratio. Fluorescence is the emission of a photon by an excited electron as it relaxes back to its basal state. The electron can be excited by different forms of energy, including a source of light (photons) of higher energy (shorter wavelength), which will emit a lower-energy photon (longer wavelength). Autofluorescence is the fluorescence emitted by native proteins, metabolites, and organelles, such as NADPH and collagen fibers, in cells and extracellular spaces. Although there is interest in using autofluorescence in imaging of pathological tissues and potential diagnosis (Falk 2009), its wide emission spectrum limits the range of fluorophores that can be used in probes for target-specific imaging.

To target specific tissues or cells, techniques have been developed which require excitation of a probe or dye for detection. The nature of the incident light is important since it greatly influences the penetration and energy needed to image the specimen. Thus, ultraviolet (UV) to visible light (200–650 nm) can be absorbed

by blood and molecular components and may only penetrate about 1 mm of tissue, whereas near-infrared light can penetrate at centimeter depths, allowing access to tissues located deeper in the body. The advances in light source technology not only have provided more accurate imaging but have also decreased the equipment cost, resulting in widespread use of in vivo imaging technologies and rapid innovation in both probe development and light capturing devices.

There are typically three main light sources used in in vivo imaging systems: white light, light-emitting diodes (LEDs), and laser. White light is made up of the widest spectral range. This source is adaptable, easily accessible, and cheap but suffers from inefficient wavelength filtering. LED sources are typically more expensive, due to the rare-earth metals used and their manufacturing cost. LEDs have the advantage of having lower power consumption, higher luminous efficiency, longer life spans, and narrower spectral ranges. The third source is laser, which, in contrast to white light and LED, has a quite narrow spectrum, with little background and highly effective excitation of the dye, and thus higher signal-to-noise ratios. However, lasers have very high power consumption and high cost and can cause cellular damage. The current development of cheaper and efficient lasers, plus the attractiveness of using higher wavelength laser diodes, is making lasers very attractive for clinical applications.

Two optical approaches have been developed to image in vivo the insulin-producing cells; these are FLI (fluorescence) and BLI (bioluminescence). FLI can be used on transgenic mice expressing fluorescent proteins or after the delivery of fluorescently tagged probes. BLI is a technique based on the emission of light resulting from the catalysis of luciferin by the luciferase enzyme. Given that vertebrates do not express luciferase, the enzyme has to be expressed by transgenesis in the desired cell type. In FLI, the photon is emitted from an excited fluorophore expressed or attached on the specific cell type. In BLI, the photon emission is made cell specific by expressing a luciferase cDNA under control of a cell-specific promoter (the insulin promoter in the case of pancreatic β-cells) (Virostko et al. 2010). The common problems to both imaging modalities are the high photon scattering through tissues and the low penetration depth. This is because both technologies are based on the capture of emitted photons through a heterogeneous space, rendering the recording and quantification of the photon emission difficult (Virostko et al. 2004). Indeed, the more the target of interest is deeply located within the body, the more the photons have to pass through different organs and structures, resulting in few photons escaping scattering and absorption before leaving the body to be recorded by the camera.

Furthermore, the choice of the fluorophore can exacerbate its specific detection. Such can be the case for transgenic mice expressing green fluorescent proteins, such as GFP (Hara et al. 2006). Short-wavelength light has a higher propensity for absorption in tissues; thus the excitation and emission of green fluorescence suffer from poor efficiency. A simple rule to follow to limit the absorption would be to use longer emission wavelengths, for example, in the near infrared. This rule is also true for autofluorescence, which, in vivo, is significant in shorter wavelengths and could be mitigated using a fluorophore with a long-wavelength excitation and emission and

by feeding the animals prior to the imaging session with food devoid of highly fluorescent components. One advantage of BLI over FLI is that there is almost no background signal, making it possible to increase the exposure time in order to obtain higher signal-to-noise ratios. However, BLI can be only used in animals expressing luciferase, whereas FLI is adaptable to image both transgenic and non-transgenic mice, provided the latter have been injected or have ingested a fluorescent probe.

In Vivo Fluorescence Imaging (FLI)

Few studies have explored the development of a fluorescent probe to image β-cells. A study by Reiner et al. (2011) demonstrated the development of a fluorescent peptide, derived from exendin-4, which we modified to image native β-cells (Fig. 1). Within the pancreas, the peptide is quite specific. However, an optical fiber is required to record the emitted photons through an abdominal incision, making the technique invasive. Another study from Vats et al. (2012) showed the characterization of a novel antibody targeting the transmembrane protein TMEM27, which is highly expressed in β-cells. A fluorescent probe (coupled to an Alexa fluorophore), as well as a PET-compatible probe (coupled with 89Zr), was synthetized. The authors studied this new antibody on a mouse model of subcutaneous insulinoma and on a transgenic mouse overexpressing the TMEM27 under the control of a rat insulin promoter. As yet, however, no mouse featuring an altered BCM was monitored with this approach, which is required to evaluate the sensitivity of the new probe.

Kang and colleagues (2013) synthesized a red fluorescent compound named pancreatic islet yellow (PiY), which labels β-cells. The PiY was not toxic to mice and does not modify the insulin-secretory function of islets. There was a significant decrease in islet labeling upon STZ treatment, and furthermore, the probe was used to enrich β-cells from the pancreas by FACS. The probe weakly labels the stomach, gut, and brain; however, PiY strongly labeled the liver and heart, perhaps limiting the imaging modalities that can be used with this probe. Nevertheless, the clear targeting of β-cells over other islet cells with PiY provides an improved asset for future studies toward live imaging of β-cells in humans.

New technical developments, such as near-infrared optical projection tomography (Eriksson et al. 2013), may facilitate the in vivo application of FLI.

Optical Coherence Tomographic (OCT) Imaging of the Islets

Extended-focus optical coherence microscopy (Berclaz et al. 2012) has been described to image pancreatic islets ex vivo, without the need of any exogenous labeling (Fig. 2). Again, however, the in vivo application of the approach requires a preliminary surgery to bring the optics close by the abdominal pancreas. Because of this requirement, it is clear that, in the current state, these techniques are not yet easily usable in vivo and in the clinics.

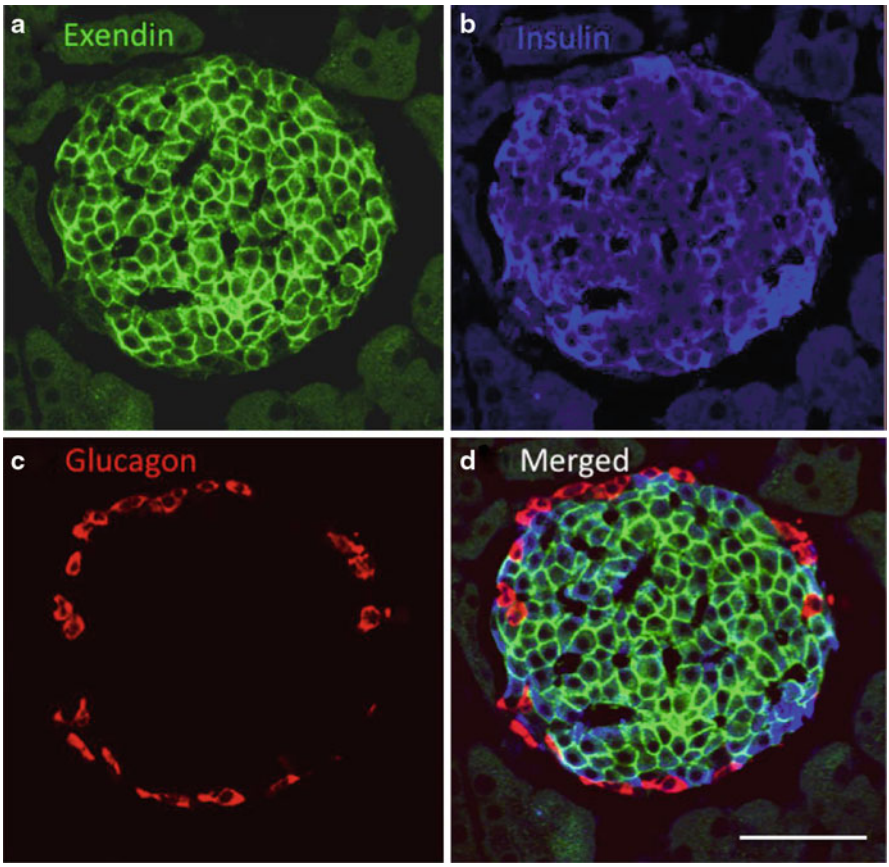

Fig. 1 Fluorescence imaging. One hour after the in vivo administration of exendin-4-FITC, a section of the pancreas was immunostained for FITC (*green*), insulin (*blue*), and glucagon (*red*). In all islets, exendin-4 was easily detected at the surface of virtually all β-cells, but not α-cells. Scale bar 100 μm

In Vivo Bioluminescence Imaging (BLI)

For the in vivo imaging of β-cells of animal models, BLI seems today to gain unqualified success over FLI and OCT, as judged by the number of publications. Indeed, BLI provides a linear correlation between photon emission and BCM, for example, in models of β-cell destruction by streptozotocin (Fig. 3) (Wang 2002; Hara et al. 2006). Thus, the method allows for an evaluation of the number of living (BLI is dependent both on the activity of the intracellular luciferase and on the blood supply to bring luciferin to the cells expressing the enzyme) islets after transplantation. Virostko et al. (2010) created transgenic mouse (MIP-Luc-VU) expressing luciferase under control of the mouse insulin promoter. The authors were able to quantify β-cell mass in the native pancreas of living animals after increased (high-fat diet) or decreased

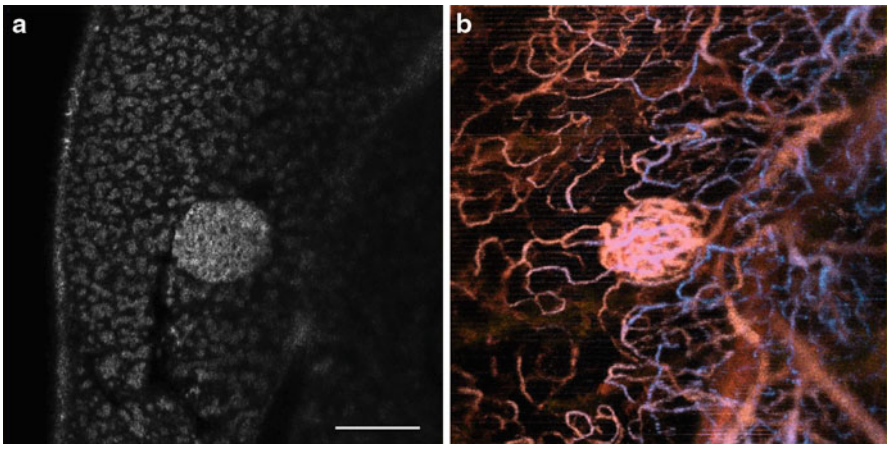

Fig. 2 Extended-focus optical coherence imaging of pancreas. Typical aspect of an islet is presented in (**a**) and islet vascularization in (**b**) (max z projection: *blue* above, *red* below). Scale bar: 100 μm (Courtesy of Prof. Lasser, EPFL, Laboratoire d'optique biomedicale, Lausanne, Switzerland)

(streptozotocin treatment) BCM and after islet transplantation. Furthermore, they repeatedly monitored BCM for more than 1 year. More recently (Virostko et al. 2013), the same authors generated a new model (MIP-Luc-VU-NOD) by expressing the luciferase protein in NOD mice. This model of type 1 diabetes allows for the monitoring of β-cell during the age-dependent development of autoimmune diabetes, including before overt hyperglycemia has developed. This model could be useful to test the efficacy of new therapies for T1D in a preclinical setting. In vivo BLI could also be used to evaluate β-cell regeneration. Indeed, Grossman et al. (2010) showed the spontaneous regeneration of β-cell after ablation and islets transplantation (Vats et al. 2012). More recently, Yin et al. (2013) investigated the efficacy of type 2 diabetic therapies on the regeneration of β-cell destroyed by streptozotocin, showing the interest of BLI for the repeated, longitudinal monitoring of BCM.

Imaging by Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT)

Positron emission tomography (PET) uses a radioactive isotope coupled with a metabolically active molecule. These tracers emit positrons, which, after annihilation with an electron, generate a pair of high-energy photons (511 keV), moving in nearly opposite directions. When a scintillator detects the photons, a burst of light is created, which is amplified by a photomultiplier tube, and used to generate the PET image. As the resolution of PET is around 1–2 mm, an anatomical image is often coupled with PET acquisition, usually in the form of a CT image, though MRI is now becoming an option. The most frequently used positron-emitting isotopes are ^{15}O, ^{13}N, ^{11}C, and ^{18}F. Most of these isotopes have relatively short half-lives, from 2 min for ^{15}O to 120 min for ^{18}F.

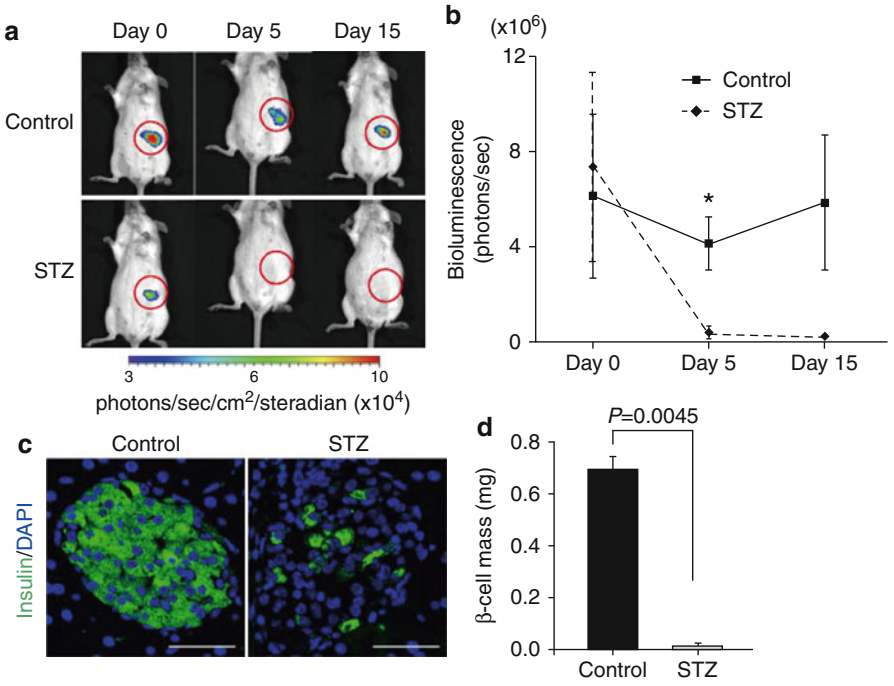

Fig. 3 Bioluminescence imaging of streptozotocin-treated mice. (**a**) Representative bioluminescence images of Ins1-luc BAC transgenic male mice before and after treatment with vehicle control or streptozotocin (*STZ*). *Circles* indicate the regions of interest. (**b**) Quantification of signal intensity in the control and STZ groups at 0, 5, and 15 days after the injection. $*P = 0.025$. (**c**) Immunohistochemistry for anti-insulin antibody in the islets of control and treated mice. Scale bars: 50 μm. (**d**) β-cell mass in the control and STZ-treated groups (From Katsumata et al. (2013))

This implies that the time for the biological process to be imaged has to be shorter than two to three half-lives for the signal to be detected. Today, the most used isotope is ^{18}F coupled to fluorodeoxyglucose (^{18}F-FDG), used to study glucose metabolism. Single photon emission computed tomography (SPECT) is another imaging method that uses gamma radiation and scintillation cameras to obtain images from multiple angles in order to create a tomographic image. The most frequently used gamma isotopes are ^{99}Tc, ^{111}In, ^{123}I, and ^{131}I. A common drawback of PET and SPECT is that most of the required isotopes are produced in a cyclotron, thus limiting their availability. Another consideration should be that if tracers reach the pancreatic islets in sufficiently high concentration, the localized radiation might become toxic, if not lethal, for β-cell.

Targeting the tracers to β-cells is still another unresolved issue. Targeting of the vesicular monoamine transporter 2 (VMAT2) by radiolabeled dihydrotetrabenazine (DTBZ) has shown potential to image BCM in vivo (Goland et al. 2009; Harris et al. 2013; Normandin et al. 2012; Singhal et al. 2011; Souza et al. 2006) and to detect the destruction of the β-cells after injection of streptozotocin (Simpson et al. 2006). VMAT2 is specifically expressed in human β-cells in the pancreas and neurons in the

brain. Goland and colleagues (2009) used ^{11}C-DTBZ to successfully image the pancreas in healthy and long-standing T1D patients. In this study, VMAT2 binding was quantified and correlated with insulin secretion following stimulation (Goland et al. 2009; Harris et al. 2013). However, the expression of VMAT2 in neurons innervating the pancreas, the highly nonspecific signal, and the constant expression of VMAT2 independently of the evolution of BCM (Harris et al. 2013; Judenhofer et al. 2008) compromise the use of DTBZ to target β-cell, at least in rodent models (Judenhofer et al. 2008). Another limitation of using VMAT2 as a target is that it is not expressed in rodents (Judenhofer et al. 2008), and the assessment of the imaging probe is therefore restricted to nonhuman primates, which hinders research developments.

Other interesting candidate probes for PET imaging are ^{111}In- and ^{68}Ga-exendin-3, an analog of glucagon-like peptide 1 that specifically binds the GLP-1 receptors (Brom et al. 2010). The drawback of using these probes is their strong binding to the kidneys, which could confound the signal obtained from the pancreas. Nevertheless, an in vivo study in rats and cynomolgus monkeys (in this case combined with CT) showed that ^{68}Ga-exendin-4 binding was specific to insulin-producing cells and that the signal was decreased upon β-cell ablation with STZ (Selvaraju et al. 2013). As shown by this study, ^{68}Ga-exendin-4 provides an acceptable, sensitive signal (Selvaraju et al. 2013), which could also be used to monitor transplanted islets. Exendin-4 labeled with ^{18}F resulted in encouraging results for the imaging of experimental insulinomas and models of transplanted islets (Wu et al. 2013).

The combination of PET with other imaging techniques, such as MRI, should improve both the poor spatial resolution of PET and the low sensitivity of MRI. Judenhofer and colleagues developed a machine combining 3D PET scanner with a 7-T magnet and showed that both techniques preserve their characteristics even when they are alternatively used. The combined PET-MRI could provide functional and morphological information in living mice (Judenhofer et al. 2008). Moreover, multimodal imaging from bioluminescence, x-ray CT, and PET was used to evaluate binding of different DTBZ ligands in mice (Virostko et al. 2011). The combination of these imaging techniques allowed for the unequivocal identification of the pancreas, as well as for the quantification of the signal emanating from the ligands. The approach demonstrated that several VMAT2 ligands do not specifically bind to murine insulin-producing β-cells (Virostko et al. 2011).

Ultrasound Imaging

Ultrasounds, or ultrasonography, use sound waves at high frequencies to penetrate tissues and obtain structural information from the echoes returned from the waves bouncing off tissues and organs. This imaging modality is relatively inexpensive, non-irradiating, and rapidly done (~15 min is needed for a classical abdominal scan) and is readily available in most clinical facilities. For these reasons, ultrasounds have become one of the most widely used imaging modalities in medicine. Ultrasounds allow for tomographic images to be obtained in real time, based on the interaction of the waves with the surrounding tissue (Hangiandreou 2003). Despite

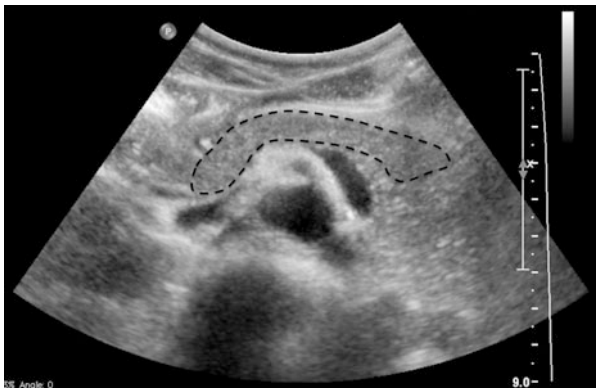

Fig. 4 Pancreas imaging by ultrasounds. When analyzed with a 3.5-MHz transducer, the pancreas (*dotted line*) appears slightly hyperechoic compared to neighboring abdominal organs. Individual islets cannot be visualized by this technique

all these advantages, the technique suffers from deep penetration problems, low resolution at the frequencies (2–18 MHz) used in the clinics, and lack of contrast between different anatomical structures. The use of contrast media (mainly bubbles containing gas) has interesting possibilities to enhance the vascularization of organs and/or pathology. Moreover, the quantity of contrast media needed to be detected is extremely low, in the order of 10^{-12} M (Klibanov et al. 2004). With respect to the pancreas, ultrasound is mainly used to search for pancreatic tumors or generalized inflammation (pancreatitis). An example of pancreas sonogram is presented in Fig. 4. Ultrasounds show the pancreatic gland in its entirety but are unable to differentiate between the exocrine and the endocrine pancreas because the biophysical properties of these two tissues are not sufficiently different. The potential use of targeted microbubbles of several dozen μm in diameter to enhance the islet signal seems difficult because the size of these microbubbles would preclude their passage through the vascular islet endothelium. An emerging approach, referred to as photoacoustic or optoacoustic imaging, could provide a useful alternative. Optoacoustic combines fluorescent optical imaging and ultrasounds, i.e., the generation of ultrasounds after absorption of light energy by either a fluorophore or gold or carbon nanoparticles. The acoustic waves can be detected with an array of ultrasonic detectors. The conversion of light energy into ultrasonic waves overcomes the scattering problems of photons in the tissue (≈1,000-folds less). While in its infancy, this approach promises an expansion of the field of molecular imaging and targeted biomarkers, opening possibilities of β-cell imaging research (Ntziachristos 2010; Wang and Hu 2012).

Computed Tomography (CT) Imaging

This technique uses x-rays and detectors rotating around the patient to create tomographic images, based on the absorption of x-rays by high z atomic number elements. In clinics, this imaging modality is relatively expensive and irradiating but allows for the rapid acquisition of images (only a few seconds is needed for an

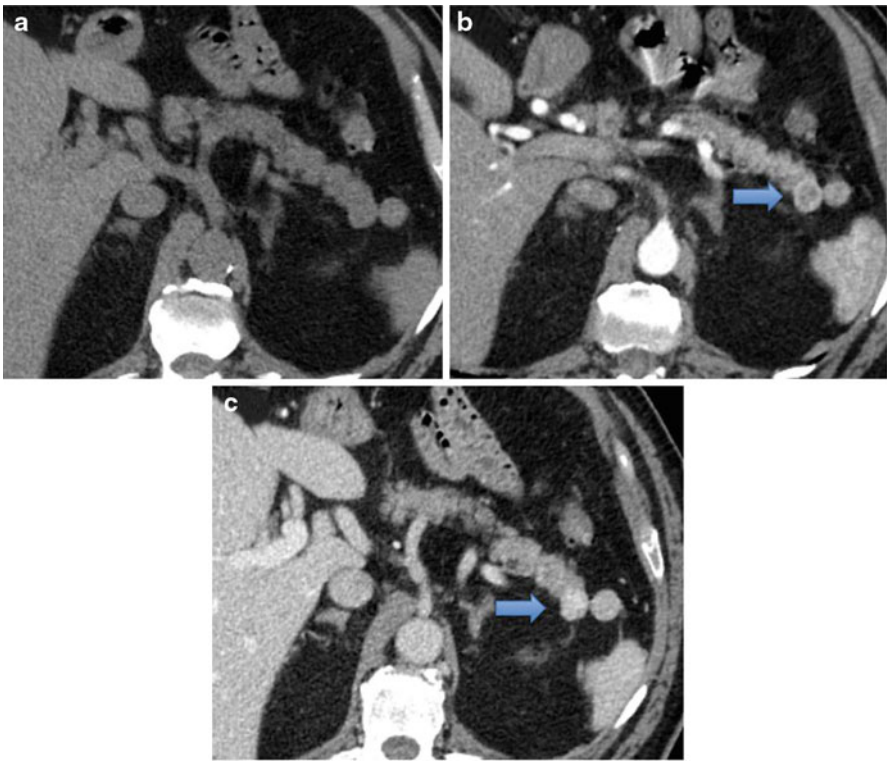

Fig. 5 Computed tomography (CT) of the pancreas. The pancreas is presented without contrast media (**a**), during the arterial (**b**) and venous phases (**c**). Note the presence of a lesion (*arrow*) presenting an enhancement at the arterial and venous phase (wash-in), corresponding to an insulinoma. The native islets cannot be resolved with CT

entire thoracoabdominal scan, the exact scan time depending on the number of detectors used) and is readily available. The clinical resolution is typically 400–800 μm, without limitation of penetration. As CT suffers from low contrast between soft tissues, the injection of iodinated contrast agents is often used to enhance the images, based on differences in wash-in/washout of the contrast in different organs (Fig. 5). The development of micro-CT, dedicated to small animal imaging, now allows for a much higher resolution (2–10 μm), but still suffers from low contrast between soft tissues. There are only a few reports on cellular imaging by micro-CT, using liposomes containing iodinated contrast media, mainly for targeting endovascular cells, given that the relatively large size of liposomes (~100–500 μm) does not allow extravasation (Danila et al. 2009; Wyss et al. 2009). Other nanoparticle-based contrast media have proven their ability to escape from the circulation (due to their smaller size) and to specifically targets receptors, which are overexpressed in cancers (Hill et al. 2010; Li et al. 2010; Reuveni et al. 2011).

Currently, there is no evidence that the small islets of Langerhans could be imaged with a CT technology. To do so, an islet-specific contrast medium must be

developed. However, due to the low sensitivity (10^{-1} to 10^{-2} M) of CT, imaging of the native islets will remain a challenge. In contrast, transplanted human islets can be easily imaged by loading them with contrast agents before implantation into the host body. Barnett et al. (2011) developed microcapsules that protect transplanted islets of Langerhans, which integrate contrast media, for the multimodal (CT, US, MRI) visualization of the graft.

Magnetic Resonance Imaging (MRI)

MRI is based upon the nuclear magnetic resonance (NMR) principle. When placed inside a static magnetic field, hydrogen nuclei will process at the Larmor frequency. As the Larmor frequency is linearly dependent of the local magnetic field, the addition of three gradients (in the x-y and z direction) allows for the exact position of the nuclei of interest to be determined and an image created. The first gradient, referred to as the slice selection gradient, allows for selection of a slice along the z axis, whereas the x and y positions are determined by a phase-encoding gradient and a frequency-encoding gradient, respectively. After the administration of a 90° radio-frequency (RF) pulse, the magnetic moment of each nucleus tilts the x-y plane. As soon as the RF is stopped, the magnetic moment of the nucleus starts to relax to regain its initial position in the static magnetic field. The time needed to relax will determine the T1 and T2 relaxation times, which differ depending on the anatomical structure and are the basis of the MRI contrast. Contrast media are used to further enhance the contrast between different organs, as well as between normal and pathological tissues. Some contrast media, like gadolinium and manganese, have a substantial effect on T1-weighted images, whereas others, like iron oxide, mostly affect T2-weighted images. It should be stressed that MRI does not directly visualize the contrast media, but rather their effects on the T1 and T2 relaxation times of water protons. The MRI signal increases with T1 contrast agents and decreases with T2 contrast agents.

Imaging Transplanted Islets

MRI contrast agents, such as superparamagnetic iron oxide nanoparticles (SPIO), function by reducing the MRI signal on T2-weighted sequences. In general, SPIOs show higher sensitivity than other contrast agents because of their crystal structure and the high number of iron atoms per nanoparticle (~8,000). Several studies used SPIOs to label human islets before transplantation to follow the fate of these transplanted islets over time (Leoni and Roman 2010). However, this approach does not distinguish between functioning and nonfunctioning, if not dead islets, given the remanence of SPIOs in cells and extracellular spaces for extended time

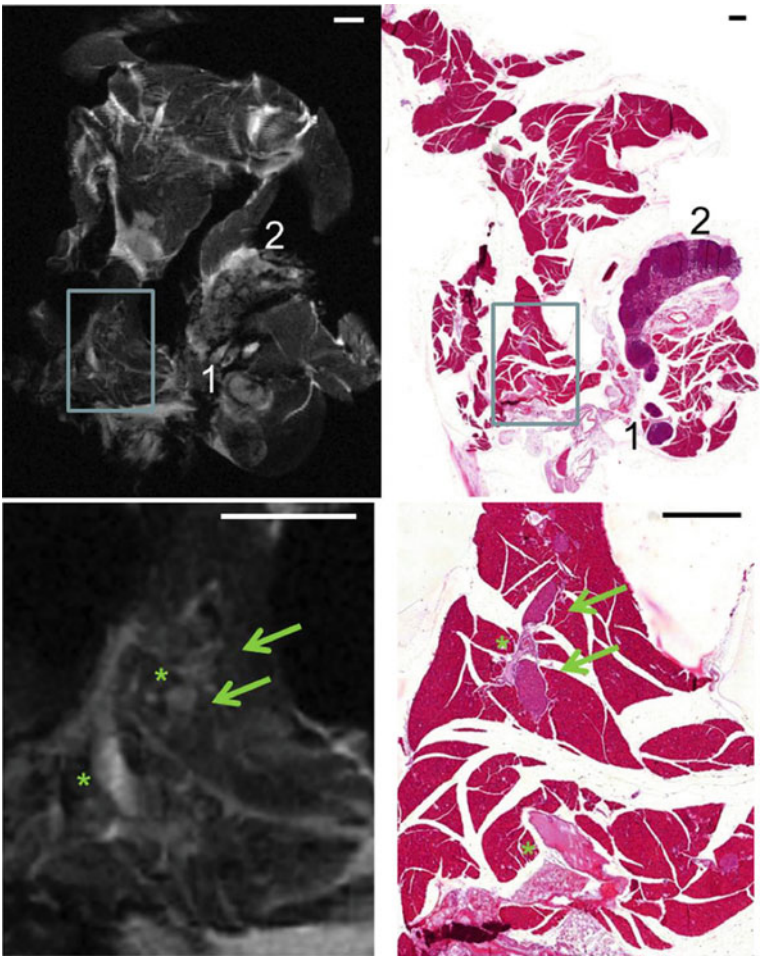

Fig. 6 Individual pancreatic islets are visualized by magnetic resonance imaging (MRI). Whole mouse pancreata (300-μm-thick slices) were imaged ex vivo, with a 60-μm in-plane resolution (TR/TE = 282/7 ms). *Left panels*, MR image showing enhanced contrast after i.v. infusion of MnCl combined with an i.p. injection of glucose. Under these conditions, MRI allows distinction of whitish tubular structures, as well as highly contrasted round-ovoid structures of various sizes, identified as islets by the histological analysis (*right panels*) of the same pancreas. MRI further differentiates the pancreatic parenchyma from intrapancreatic lymphatic ganglia (*1*) and the spleen (*2*). MR image (*lower*, *left*) shows a view at high magnification of the pancreas in the *upper left panel*, recorded with a 60-μm in-plane resolution. Small, round-ovoid whitish structures (pointed by *green arrows*) are seen dispersed within the pancreatic lobules. Histology (*right*) confirmed that these structures are pancreatic islets, dispersed within the exocrine parenchyma, between vessels and ducts (*green asterisk*). Scale bars: 0.5 mm

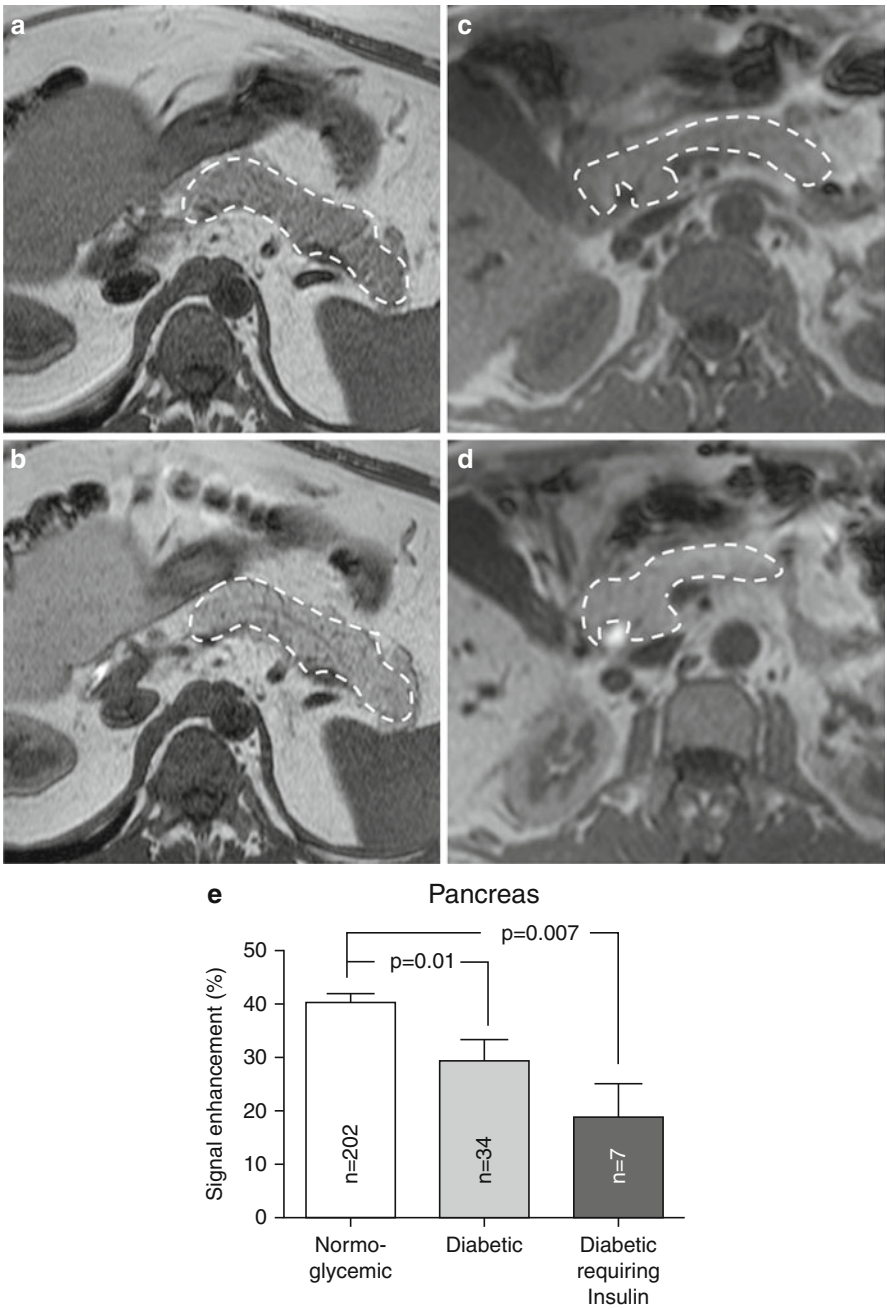

Fig. 7 Manganese-enhanced MRI (MEMRI) distinguishes human control and diabetic pancreas. The MRI signal enhancement of the pancreas is enhanced by manganese. T1-weighted magnetic resonance imaging showing the pancreas (*dashed line*) before (**a** and **c**) and 20 min after

periods. An alternative method using murine and human gadolinium-labeled islets showed successful in vitro and in vivo imaging, following iso- and xenotransplantation into the kidney capsule (Biancone et al. 2007).

Imaging Insulitis

Changes in the volume, flow, and permeability of microvessels have been described in several models of T1D. These changes have been imaged by MRI using a paramagnetic compound (Medarova et al. 2007) or by using superparamagnetic iron oxide (SPIO) nanoparticles in both animal models (Denis et al. 2004) and humans (Gaglia et al. 2011).

A second approach consists of targeting lymphocytes and following their pancreas infiltration as insulitis develops. Two different strategies could be used to load T lymphocytes with iron oxide nanoparticles. The first takes advantage of the Tat protein, the translocation peptide of the HIV virus. Coupling of the Tat protein to iron oxide nanoparticles allows for its incorporation by the cells into the nucleus. Iron oxide-loaded $CD8^+$ lymphocytes were used to track the immune reaction in a model of T1D (Moore et al. 2002). This study showed a progressive decrease in the T2 signal in a model of insulitis, as lymphocytes infiltrated the pancreas. A specific subpopulation of diabetogenic lymphocytes was then loaded with iron oxide nanoparticles specific for NRP-V7, which allows for the targeting of the T-cell receptor α on NRP-V7-reactive $CD8^+$ T cells (Moore et al. 2004). This approach again allowed for a real-time assessment of lymphocyte recruitment into the pancreas of NOD mice. Moreover, imaging approaches allowed to sort these non-obese diabetic (NOD) mice into groups that do or do not develop T1D (Fu et al. 2012) or respond to therapy (Turvey et al. 2005). Altogether, these studies have shown the possibility of noninvasive imaging of insulitis by MRI. Still, these approaches are not translatable to patients affected by T2D, nor can they evaluate the BCM of healthy patients. To this end, one needs to specifically target the native β-cell.

Manganese-Enhanced MRI (MEMRI)

MRI is extensively used in clinics to monitor most organs with a vast range of applications and magnetic fields (1.5 to 7 T). Lately, magnets were specifically developed to image small animals at even higher field strengths (up to 21 T),

Fig. 7 (continued) Mn-DPDP infusion (**b** and **d**). In both a normoglycemic (**a** and **b**) and a type 2 diabetic patient (**c** and **d**), the MRI signal of the pancreas was enhanced by the manganese infusion. (**e**) This enhancement was significantly higher in normoglycemic than in type 2 diabetic patients. Data are mean + SEM signal enhancement, expressed as % of the signal evaluated prior to the manganese infusion (From Botsikas et al. (2012))

resulting in a sufficient increase of in-plane resolution, to visualize pancreatic islets (Montet-Abou et al. 2010). High magnetic fields are also useful to improve imaging protocols and test putative contrast agents before clinical translation.

Still, this technological progress has not solved many of the issues limiting the islet and even more the β-cell imaging. Manganese ions (Mn^{2+}), which behave like Ca^{2+} and can enter the cells via Ca^{2+} channels, are well known MRI contrast agents that were successfully used to image activated neurons (Lin and Koretsky 1997) and cardiomyocytes (Montet-Abou et al. 2010). Taking advantage of this property, several studies (Antkowiak et al. 2009; Lamprianou et al. 2011; Mayo-Smith et al. 1998) have tested Mn^{2+} to label pancreatic islets. The labeling is not cell specific, given that Mn^{2+} enters several types of Ca^{2+} channels, including those of the non-β-cells of the islets and the acinar cells of the pancreas. However, the ability of a glucose bolus to enhance the uptake of Mn^{2+} in insulin-producing cells (Rorsman and Hellman 1982), which is consistent with the effect of the sugar on the opening of voltage-dependent Ca^{2+} channels, implied that it should be possible to detect changes in the Mn^{2+}-enhanced signal as a function of alterations in BCM and/or β-cell function (Antkowiak et al. 2009; Antkowiak and Epstein 2012; Gimi et al. 2006). Gimi et al. (2006) were the first to show that MEMRI could be applied to image β-cells (Gimi et al. 2006). Thus, the MRI signal could be enhanced after the incubation of murine islets in the presence of Mn^{2+}, specifically after glucose stimulation. More recently, Leoni et al. (2010) showed that this approach also applies to isolated human islets. Antkowiak et al. (2009) extended these results to in vivo conditions, by showing that the MRI signal of pancreas is enhanced after Mn^{2+} infusion and a glucose bolus. More recently, these authors showed that the technique can be used to noninvasively detect the progressive loss of BCM in living mice (Antkowiak et al. 2013).

Using a 14.1-T magnet, we have shown ex vivo that MEMRI can visualize individual islets of control and streptozotocin-treated mice, as confirmed by correlative histology (Fig. 6). Still, the technique displays several limitations including the sizable influence of the partial volume effects, which results in an apparent shift of the islet diameter toward larger values, impeding the identification of islets with a diameter smaller than 50 μm (Lamprianou et al. 2011). Moreover, following the complete ablation of β-cells by streptozotocin, MEMRI revealed the expected decrease in the relative number and volume densities of the islets. However, the change in the MEMRI signal (~50 %) contrasted with the larger decrease (>90 %) in β-cells, due to the persistent labeling by Mn^{2+} of islets mostly populated by α-cells (Lamprianou et al. 2011). Testing MEMRI in humans (Botsikas et al. 2012), we showed that the approach allows for the differentiation of normoglycemic and T2D patients, based on the signal of the pancreas after Mn^{2+} injection (Fig. 7). The results, which documented a selective decrease in the pancreas signal of about 30 %, are strikingly consistent with the change in BCM (also about 30 %) which has been documented by autopsy studies of human patients with T2D (Marchetti et al. 2012; Rahier et al. 2008). A limitation of MEMRI is that Mn^{2+} also alters the T2* relaxation time, which could complicate the monitoring of the signal due to the islets under fully noninvasive conditions. Therefore, a significant effort has begun to identify ligands, which specifically target membrane proteins localized at the β-surface.

Targeted Magnetic Resonance Imaging

The use of targeted contrast media in MRI has been described (Gupta and Weissleder 1996), but there are only a few examples dedicated to the pancreas (Montet et al. 2006b; Zhang et al. 2013). Exendin-4, an analog of glucagon-like peptide-1 (Zhang and Chen 2012), was coupled to iron oxide nanoparticles. The probe was used for imaging in vivo an insulinoma (Zhang and Chen 2012). Whether the native β-cells of the pancreas could be similarly imaged was not investigated in this study and remains a central question for future studies.

Conclusions

The approaches highlighted in this review give an overview of recent advances made in pancreatic islet live-animal imaging, those methodologies used to target β-cells, as well as to study the pathophysiology of diabetes. Molecular imaging is a fast-growing field, where almost all imaging modalities have been applied, including ultrasound, CT, PET-CT, SPECT-CT, MRI, and optical imaging. The rapid development of fluorescent and bioluminescent technologies, coupled to advanced targeting probes or contrast media, has opened up the possibilities for multimodal imaging and higher spatial-temporal resolution. Despite these efforts, the imaging of the native β-cells of the islets is still challenging in a clinical setting. As we have seen in this review, current technologies are oriented toward improving the specificity of probes or contrast media while maintaining a nontoxic and noninvasive approach that allows repeated monitoring of the same individual. We anticipate that as all these technologies mature and are joined by other fields of research, they will develop into invaluable tools in basic and translational research toward an understanding and treatment of diabetes.

Acknowledgments This work was supported by grants from the Swiss National Science Foundation (310000-109402, CR32I3_129987), the Juvenile Diabetes Research Foundation (40-2011-11, 99-2012-775), the European Union (BETAIMAGE 222980, IMIDIA 155055, BETATRAIN 289932), the Fondation Romande pour le Diabète, and the Boninchi Foundation.

Cross-References

► Calcium Signaling in the Islets
► Islet Xenotransplantation: Recent Advances and Future Prospect
► Pancreatic β Cells in Metabolic Syndrome

References

Antkowiak PF, Tersey SA, Carter JD, Vandsburger MH, Nadler JL, Epstein FH, Mirmira RG (2009) Noninvasive assessment of pancreatic β-cell function in vivo with manganese-enhanced magnetic resonance imaging. Am J Physiol Endocrinol Metab 296:E573–E578

Antkowiak PF, Vandsburger MH, Epstein FH (2012) Quantitative pancreatic β cell MRI using manganese-enhanced Look-Locker imaging and two-site water exchange analysis. Magn Reson Med 67:1730–1739

Antkowiak PF, Stevens BK, Nunemaker CS et al (2013) Manganese-enhanced magnetic resonance imaging detects declining pancreatic β-cell mass in a cyclophosphamide-accelerated mouse model of type 1 diabetes. Diabetes 62:44–48

Barnett BP, Ruiz-Cabello J, Hota P et al (2011) Fluorocapsules for improved function, immunoprotection, and visualization of cellular therapeutics with MR, US, and CT imaging. Radiology 258:182–191

Barone B, Dantas JR, Almeida MH et al (2011) Pancreatic autoantibodies, HLA DR and PTPN22 polymorphisms in first degree relatives of patients with type 1 diabetes and multiethnic background. Exp Clin Endocrinol Diabetes 119:618–620

Behm CZ, Lindner JR (2006) Cellular and molecular imaging with targeted contrast ultrasound. Ultrasound Q 22:67–72

Berclaz C, Goulley J, Villiger M et al (2012) Diabetes imaging-quantitative assessment of islets of Langerhans distribution in murine pancreas using extended-focus optical coherence microscopy. Biomed Opt Express 3:1365–1380

Biancone L, Crich SG, Cantaluppi V, Romanazzi GM, Russo S, Scalabrino E, Esposito G, Figliolini F, Beltramo S, Perin PC, Segoloni GP, Aime S, Camussi G (2007) Magnetic resonance imaging of gadolinium-labeled pancreatic islets for experimental transplantation. NMR Biomed 20:40–48

Botsikas D, Terraz S, Vinet L et al (2012) Pancreatic magnetic resonance imaging after manganese injection distinguishes type 2 diabetic and normoglycemic patients. Islets 4:243–248

Bottazzo GF, Dean BM, McNally JM et al (1985) In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulitis. N Engl J Med 313:353–360

Bremer C, Bredow S, Mahmood U et al (2001) Optical imaging of matrix metalloproteinase-2 activity in tumors: feasibility study in a mouse model. Radiology 221:523–529

Bremer C, Ntziachristos V, Weitkamp B et al (2005) Optical imaging of spontaneous breast tumors using protease sensing 'smart' optical probes. Invest Radiol 40:321–327

Brom M, Oyen WJ, Joosten L, Gotthardt M, Boerman OC (2010) 68Ga-labelled exendin-3, a new agent for the detection of insulinomas with PET. Eur J Nucl Med Mol Imaging 37:1345–1355

Danaei G, Finucane MM, Lu Y et al (2011) National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet 378:31–40

Danila D, Partha R, Elrod DB et al (2009) Antibody-labeled liposomes for CT imaging of atherosclerotic plaques: in vitro investigation of an anti-ICAM antibody-labeled liposome containing iohexol for molecular imaging of atherosclerotic plaques via computed tomography. Tex Heart Inst J 36:393–403

Denis MC, Mahmood U, Benoist C et al (2004) Imaging inflammation of the pancreatic islets in type 1 diabetes. Proc Natl Acad Sci USA 101:12634–12639

Eriksson AU, Svensson C, Hornblad A et al (2013) Near infrared optical projection tomography for assessments of β-cell mass distribution in diabetes research. J Vis Exp 17: e50238

Falk GW (2009) Autofluorescence endoscopy. Gastrointest Endosc Clin N Am 19:209–220

Figueiredo JL, Alencar H, Weissleder R et al (2006) Near infrared thoracoscopy of tumoral protease activity for improved detection of peripheral lung cancer. Int J Cancer 118:2672–2677

Fu W, Wojtkiewicz G, Weissleder R et al (2012) Early window of diabetes determinism in NOD mice, dependent on the complement receptor CRIg, identified by noninvasive imaging. Nat Immunol 13:361–368

Gaglia JL, Guimaraes AR, Harisinghani M et al (2011) Noninvasive imaging of pancreatic islet inflammation in type 1A diabetes patients. J Clin Invest 121:442–445

Gimi B, Leoni L, Oberholzer J, Braun M, Avila J, Wang Y, Desai T, Philipson LH, Magin RL, Roman BB (2006) Functional MR microimaging of pancreatic β-cell activation. Cell Transplant 15:195–203

Goland R, Freeby M, Parsey R et al (2009) ^{11}C-dihydrotetrabenazine PET of the pancreas in subjects with long-standing type 1 diabetes and in healthy controls. J Nucl Med 50:382–389

Grossman EJ, Lee DD, Tao J et al (2010) Glycemic control promotes pancreatic β-cell regeneration in streptozotocin-induced diabetic mice. PLoS One 5:e8749

Gupta H, Weissleder R (1996) Targeted contrast agents in MR imaging. Magn Reson Imaging Clin N Am 4:171–184

Hangiandreou NJ (2003) AAPM/RSNA physics tutorial for residents. Topics in US: B-mode US: basic concepts and new technology. Radiographics 23:1019–1033

Hara M, Dizon RF, Glick BS et al (2006) Imaging pancreatic β-cells in the intact pancreas. Am J Physiol Endocrinol Metab 290:E1041–E1047

Harris PE, Farwell MD, Ichise M (2013) PET quantification of pancreatic VMAT 2 binding using (+) and (−) enantiomers of [^{18}F]FP-DTBZ in baboons. Nucl Med Biol 40:60–64

Hill ML, Corbin IR, Levitin RB et al (2010) In vitro assessment of poly-iodinated triglyceride reconstituted low-density lipoprotein: initial steps toward CT molecular imaging. Acad Radiol 17:1359–1365

Imagawa A, Hanafusa T, Tamura S et al (2001) Pancreatic biopsy as a procedure for detecting in situ autoimmune phenomena in type 1 diabetes: close correlation between serological markers and histological evidence of cellular autoimmunity. Diabetes 50:1269–1273

Jaffer FA, Weissleder R (2004) Seeing within: molecular imaging of the cardiovascular system. Circ Res 94:433–445

Judenhofer MS, Wehrl HF, Newport DF, Catana C, Siegel SB, Becker M, Thielscher A, Kneilling M, Lichy MP, Eichner M, Klingel K, Reischl G, Widmaier S, Röcken M, Nutt RE, Machulla HJ, Uludag K, Cherry SR, Claussen CD, Pichler BJ (2008) Simultaneous PET-MRI: a new approach for functional and morphological imaging. Nat Med 4:459–465

Kang NY, Lee SC, Park SJ et al (2013) Visualization and isolation of Langerhans islets by a fluorescent probe PiY. Angew Chem Int Ed Engl 52:8557–8560

Katsumata T, Oishi H, Sekiguchi Y et al (2013) Bioluminescence imaging of β cells and intrahepatic insulin gene activity under normal and pathological conditions. PLoS One 8: e60411

Kircher MF, Willmann JK (2012) Molecular body imaging: MR imaging, CT, and US. Part I. Principles. Radiology 263:633–643

Klibanov AL, Rasche PT, Hughes MS et al (2004) Detection of individual microbubbles of ultrasound contrast agents: imaging of free-floating and targeted bubbles. Invest Radiol 39:187–195

Lamprianou S, Immonen R, Nabuurs C, Gjinovci A, Vinet L, Montet XC, Gruetter R, Meda P (2011) High-resolution magnetic resonance imaging quantitatively detects individual pancreatic islets. Diabetes 60:2853–2860

Lange N, Becker CD, Montet X (2008) Molecular imaging in a (pre-) clinical context. Acta Gastroenterol Belg 71:308–317

Lebastchi J, Herold KC (2012) Immunologic and metabolic biomarkers of β-cell destruction in the diagnosis of type 1 diabetes. Cold Spring Harb Perspect Med 2:a007708

Leoni L, Roman BB (2010) MR imaging of pancreatic islets: tracking isolation, transplantation and function. Curr Pharm Des 16:1582–1594

Leoni L, Serai SD, Haque ME et al (2010) Functional MRI characterization of isolated human islet activation. NMR Biomed 23:1158–1165

Li J, Chaudhary A, Chmura SJ et al (2010) A novel functional CT contrast agent for molecular imaging of cancer. Phys Med Biol 55:4389–4397

Lin YJ, Koretsky AP (1997) Manganese ion enhances T1-weighted MRI during brain activation: an approach to direct imaging of brain function. Magn Reson Med 38:378–388
Lin Y, Sun Z (2010) Current views on type 2 diabetes. J Endocrinol 204:1–11
Maahs DM, West NA, Lawrence JM et al (2010) Epidemiology of type 1 diabetes. Endocrinol Metab Clin North Am 39:481–497
Marchetti P, Bugliani M, Boggi U et al (2012) The pancreatic β cells in human type 2 diabetes. Adv Exp Med Biol 771:288–309
Mayo-Smith WW, Schima W, Saini S, Slater GJ, McFarland EG (1998) Pancreatic enhancement and pulse sequence analysis using low-dose mangafodipir trisodium. AJR Am J Roentgenol 170:649–652
Medarova Z, Castillo G, Dai G et al (2007) Noninvasive magnetic resonance imaging of microvascular changes in type 1 diabetes. Diabetes 56:2677–2682
Monici M (2005) Cell and tissue autofluorescence research and diagnostic applications. Biotechnol Annu Rev 11:227–256
Montet X, Montet-Abou K, Reynolds F et al (2006a) Nanoparticle imaging of integrins on tumor cells. Neoplasia 8:214–222
Montet X, Weissleder R, Josephson L (2006b) Imaging pancreatic cancer with a peptide-nanoparticle conjugate targeted to normal pancreas. Bioconjug Chem 17:905–911
Montet-Abou K, Viallon M, Hyacinthe JN et al (2010) The role of imaging and molecular imaging in the early detection of metabolic and cardiovascular dysfunctions. Int J Obes (Lond) 34(Suppl 2):S67–S81
Moore A, Sun PZ, Cory D et al (2002) MRI of insulitis in autoimmune diabetes. Magn Reson Med 47:751–758
Moore A, Grimm J, Han B et al (2004) Tracking the recruitment of diabetogenic $CD8^+$ T-cells to the pancreas in real time. Diabetes 53:1459–1466
Normandin MD, Petersen KF, Ding YS et al (2012) In vivo imaging of endogenous pancreatic β-cell mass in healthy and type 1 diabetic subjects using ^{18}F-fluoropropyl-dihydrotetrabenazine and PET. J Nucl Med 53:908–916
Ntziachristos V (2010) Going deeper than microscopy: the optical imaging frontier in biology. Nat Methods 7:603–614
Orban T, Sosenko JM, Cuthbertson D et al (2009) Pancreatic islet autoantibodies as predictors of type 1 diabetes in the diabetes prevention trial-type 1. Diabetes Care 32:2269–2274
Pochon S, Tardy I, Bussat P et al (2010) BR55: a lipopeptide-based VEGFR2-targeted ultrasound contrast agent for molecular imaging of angiogenesis. Invest Radiol 45:89–95
Rahier J, Guiot Y, Goebbels RM et al (2008) Pancreatic β-cell mass in European subjects with type 2 diabetes. Diabetes Obes Metab 10(Suppl 4):32–42
Reiner T, Thurber G, Gaglia J et al (2011) Accurate measurement of pancreatic islet β-cell mass using a second-generation fluorescent exendin-4 analog. Proc Natl Acad Sci USA 108:12815–12820
Reuveni T, Motiei M, Romman Z et al (2011) Targeted gold nanoparticles enable molecular CT imaging of cancer: an in vivo study. Int J Nanomedicine 6:2859–2864
Rorsman P, Berggren PO, Hellman B (1982) Manganese accumulation in pancreatic β-cells and its stimulation by glucose. Biochem J 15:435–444
Selvaraju RK, Velikyan I, Johansson L, Wu Z, Todorov I, Shively J, Kandeel F, Korsgren O, Eriksson O (2013) In vivo imaging of the glucagonlike peptide 1 receptor in the pancreas with 68Ga-labeled DO3A-Exendin-4. J Nucl Med 8:1458–1463
Simpson NR, Souza F, Witkowski P et al (2006) Visualizing pancreatic β-cell mass with [^{11}C] DTBZ. Nucl Med Biol 33:855–864
Singhal T, Ding YS, Weinzimmer D et al (2011) Pancreatic β cell mass PET imaging and quantification with [^{11}C]DTBZ and [^{18}F]FP-(+)-DTBZ in rodent models of diabetes. Mol Imaging Biol 13:973–984
Souza F, Simpson N, Raffo A et al (2006) Longitudinal noninvasive PET-based β cell mass estimates in a spontaneous diabetes rat model. J Clin Invest 116:1506–1513

Turvey SE, Swart E, Denis MC et al (2005) Noninvasive imaging of pancreatic inflammation and its reversal in type 1 diabetes. J Clin Invest 115:2454–2461

Vats D, Wang H, Esterhazy D et al (2012) Multimodal imaging of pancreatic β cells in vivo by targeting transmembrane protein 27 (TMEM27). Diabetologia 55:2407–2416

Virostko J, Chen Z, Fowler M et al (2004) Factors influencing quantification of in vivo bioluminescence imaging: application to assessment of pancreatic islet transplants. Mol Imaging 3:333–342

Virostko J, Radhika A, Poffenberger G et al (2010) Bioluminescence imaging in mouse models quantifies β cell mass in the pancreas and after islet transplantation. Mol Imaging Biol 12:42–53

Virostko J, Henske J, Vinet L, Lamprianou S, Dai C, Radhika A, Baldwin RM, Ansari MS, Hefti F, Skovronsky D, Kung HF, Herrera PL, Peterson TE, Meda P, Powers AC (2011) Multimodal image coregistration and inducible selective cell ablation to evaluate imaging ligands. Proc Natl Acad Sci USA 108:20719–20724

Virostko J, Radhika A, Poffenberger G et al (2013) Bioluminescence imaging reveals dynamics of β cell loss in the non-obese diabetic (NOD) mouse model. PLoS One 8:e57784

Wang RK (2002) Signal degradation by multiple scattering in optical coherence tomography of dense tissue: a Monte Carlo study towards optical clearing of biotissues. Phys Med Biol 47:2281–2299

Wang LV, Hu S (2012) Photoacoustic tomography: in vivo imaging from organelles to organs. Science 335:1458–1462

Weissleder R (2006) Molecular imaging in cancer. Science 312:1168–1171

Wild S, Roglic G, Green A et al (2004) Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 27:1047–1053

Willmann JK, Paulmurugan R, Chen K et al (2008) US imaging of tumor angiogenesis with microbubbles targeted to vascular endothelial growth factor receptor type 2 in mice. Radiology 246:508–518

Wu Z, Liu S, Hassink M, Nair I, Park R, Li L, Todorov I, Fox JM, Li Z, Shively JE, Conti PS, Kandeel F (2013) Development and evaluation of ^{18}F-TTCO-Cys40-Exendin-4: a PET probe for imaging transplanted islets. J Nucl Med 54:244–251

Wyss C, Schaefer SC, Juillerat-Jeanneret L et al (2009) Molecular imaging by micro-CT: specific E-selectin imaging. Eur Radiol 19:2487–2494

Yin H, Park SY, Wang XJ et al (2013) Enhancing pancreatic β-cell regeneration in vivo with pioglitazone and alogliptin. PLoS One 8:e65777

Zhang Y, Chen W (2012) Radiolabeled glucagon-like peptide-1 analogues: a new pancreatic β-cell imaging agent. Nucl Med Commun 33:223–227

Zhang B, Yang B, Zhai C et al (2013) The role of exendin-4-conjugated superparamagnetic iron oxide nanoparticles in β-cell-targeted MRI. Biomaterials 34:5843–5852

Mouse Islet Isolation

5

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_33,

Abstract

Pancreatic islets transplantation is a therapeutic option for patients affected by type 1 diabetes. While it is always desirable to perform experiments directly with human islets, the limited availability of this precious tissue makes mouse islets a feasible option for experimental research in diabetes in many laboratories worldwide. This chapter summarizes from a practical perspective the main aspects of mouse islet isolation. We will discuss the technical factors that affect its success, as well as the practical issues (quality assessment, time, and cost) to take into account when selecting an islet isolation protocol. Finally, we will provide some guidelines on the necessary logistics for setting up this kind of methodology.

Keywords

Islet isolation • Islet purification • Animal models • Quality assessment

Introduction

The success of the Edmonton Protocol (Shapiro et al. 2000) contributed to the worldwide expansion of human transplantation programs and to the improvement of access to human tissue in translational studies. Unfortunately, the success of clinical islet transplantation is influenced by numerous variables, such as the location of the implant site, and strong inflammatory and immunological reactions. Moreover, the islet isolation process disrupts the interaction between blood vessels and endocrine cells, which, once the islets are transplanted, can lead to metabolic exhaustion and cell death (Piemonti et al. 2010).

In this context, rodent, and also pig and monkey islets, are useful in overcoming the challenge of clinical islet isolation. In particular, mouse islet isolation can provide a reliable means of studying islet isolation. New protocols are always under investigation, even though the protocols currently used are relatively straightforward. It is essential to carefully design preclinical studies to obtain consistent and reliable results; in this scenario it is really important to select the correct animal model, even though this is not always simple (Cantarelli et al. 2013). Lessons learned from animal models determine improvements in the different steps of islet isolation, culture techniques, and the function and viability of the transplanted islets. Animal models are important, for example, in reducing the donor-to-recipient ratio. In fact, as observed in human islet isolation, the procurement rate of mouse islets is tremendously affected by numerous factors and is far from optimal (Nano et al. 2005). It is usual to recover far less than 50 % of the islets present in the rodent pancreas. It has been estimated that a mouse pancreas has an average of 2,000 islets, but a typical murine islet isolation yield is ~200 islets (~10 %) per mouse (Carter et al. 2009). The yield from one mouse, as observed in humans (Nano et al. 2005) depends on body weight, size of the pancreas, total islet mass, and also on genetic background, as pointed out in an interesting paper by Bock and colleagues in 2005 (Bock et al. 2005). In particular, a mouse strain-dependent

hierarchical total islet number exists: B6, DBA/2, NOD, 129S6, C3H, CBA. The average number engrafted in murine islet transplants is 300–500, resulting in a donor-to-recipient ratio of 1.5–2.5 (Biarnés et al. 2002). Also, just an average of 40 % of the murine islets engraft, the rest are lost in the early post-transplant period.

Owing to these similarities, improving the yield rate in mice could become a starting point to increase the yield of human islet transplantation. Mice are also extremely useful in studying the etiopathogenesis of type 1 diabetes, increasing the usefulness of this animal model in diabetes research. A recent study performed on rodents is particularly relevant because it has not only been shown to improve the isolation procedure, but also developed a method of establishing primary islet cell clusters (Venkatesan et al. 2012). The researcher approaching animal studies has to be aware of some of the limitations of this tool. In fact, differences in the cytoarchitecture of mouse and human islets have been described (Cabrera et al. 2006), suggesting that a prototypical mammalian islet type may not exist. While in mouse pancreatic islets there are around 77 % β cells and 18 % α cells, these percentages change to 55 % and a 38 % respectively in humans. Besides, the distribution is also different. In mice, β cells are located in the core of the islet, whereas in humans β cells are scattered through the islet, intermingled with α and δ cells. Consequently, the differences in the structure of the islets have implications for the interactions between cells and, therefore, for the islet response to glucose and different metabolites, as well their ability to survive. It is clear that the use of mouse pancreatic islets in diabetes research, specifically in the islet isolation procedure, cannot yet be replaced by non-biological or computer-generated models, but, thanks to all the recent improvements, it should be possible to optimize and decrease the number of mice needed to obtain the same amount of islets.

Therefore, a successful mouse islet isolation laboratory must approach different interrelated aspects with different challenges, which this chapter aims to do from a wide perspective. The main aspects addressed in this chapter are: the steps and key aspects of rodent islet isolation, practical issues regarding islet isolation, and factors to consider in setting up a new mouse islet isolation laboratory.

Steps in Mouse Islet Isolation and Key Aspects

Modern islet research started in 1911 with the pioneering work of Bensley on the handpicking of guinea pig islets and staining them with neutral red (Bensley 1911). Since preliminary advances in the field, including the introduction of digestion of the pancreas with collagenase in islet isolation by (Moskalewski 1965) and the distention of the pancreas via the pancreatic duct followed by incubation of chopped tissue in collagenase by Lacy in 1967 (Lacy and Kostianovsky 1967), the fields of both islet isolation and islet transplantation in animal models have evolved in parallel (Lacy 1967; Kemp et al. 1973; Scharp et al. 1975). This has paved the way for the translation of these preclinical studies to human islet isolation and clinical transplantation. A complete review of all the steps that led to the development of more advanced protocols for pancreatic islet isolation have been

well highlighted by Piemonti and Pileggi (Piemonti and Pileggi 2013). In this sense, the primary goal of isolating mouse pancreatic islets (either for in vivo transplantation or for in vitro studies) is to consistently provide viable and functional islets.

Despite islet isolation being a work of craftsmanship and each laboratory having its own recipe (O'Dowd 2009; Zmuda et al. 2011; Kelly et al. 2003; Salvalaggio et al. 2002; Szot et al. 2007; Li et al. 2009), the main steps in any rodent islet isolation procedure consist of:

1. Surgery and pancreas harvesting
2. Pancreas digestion
3. Islet purification
4. Culture of the islets

However, to obtain a successful yield and good quality islets, different key aspects must be taken into account: the type and concentration of the digestive enzyme, the method of enzyme administration, the temperature and duration of the pancreas digestion step, the method of islet purification from pancreatic acinar tissue and the culture conditions following isolation. Identifying factors influencing the efficacy of the isolation procedure of pancreatic islets in rodents mice is mandatory for the standardization of this procedure, the reduction of variability, and the harvesting of good quality islets for subsequent experiments. In this section, we compare and contrast the advantages and potential disadvantages of the most common islet isolation protocols reported in the scientific literature and provide details of a successful setting up of the procedure (Table 1). Despite rats also usually being used as models in islet isolation, there are substantial differences in the isolation procedure (Table 2). Many published islet isolation protocols specific for rat islet isolation provide the necessary details to successfully perform the complex procedures. For further details on rat isolation see (Kelly et al. 2003).

Surgery and Pancreas Harvesting

Although it is desirable to perform this step under sterilized conditions, inside a laminar flow hood, it can be performed outside, with a "clean technique" without greatly increasing the incidence of contamination. Nevertheless, all reagents and surgical instruments should be sterile in order to avoid contamination. In detail, the procedures consist of the following steps.

Animal surgery: The first aspect to take into account in order to perform an efficient surgical procedure is the choice of a euthanasia method for the donor animals. Euthanizing the donor rodent is a procedure that can be differently regulated in different countries (e.g. cervical dislocation, CO_2 mouse asphyxiation, drugs). In addition, numerous authors and our personal experience suggest that after anesthesia and just before cannulation, the best option is to exsanguinate animals. This step is fundamental in order to avoid possible blood contamination during the

Table 1 Summary of the steps of the rodent islet isolation procedure and key aspects

Step	Essential Equipments	Critical step	Cautions
Surgical procedure	• Autoclave, • Hood (optional), • Surgical instruments: Surgical forceps (straight and curved), Fine forceps and scissor, hemostatic forceps to clamp off the bile duct • Ice • Dissecting microscope • 27-gague needle and 3 ml syringe	Duct cannulation	• Plan correctly the number of the animal that you want to use as donor based on your surgical expertise, in order to dedicate 1 hours and half maximum to this step to obtain a good quality of islets. • During the pulling of pancreas for harvesting pay attention to avoid contamination breaking intestine or stomach. • Keep pancreas perfuse on 4 °C before start digestion
Pancreas digestion	• Hood for cell culture with vertical laminar flow • Water bath with temperature control • Centrifuge (with temperature control) • Filter mesh	Time of digestion in order to not overdigest or underdigest	• Shaking and centrifugation steps induce sheer stress so centrifuge at 900 rpmi for 2/3 minutes max. • Stop digestion with FBS and ice • Wash carefully the mesh to avoid loosing of material stucked on mesh
Purification step	• Reagent • 15/50 ml conical tubes • Centrifuge (with temperature control) • Ice	Choice of purification methods	• The solution for purification are toxic so keep all the solution at 4 °C in order to reduce the stress of the islet once they are in contact with the solution • Centrifuge the islet without brake in order to avoid mix of the solution • Mix carefully islets in the heaviest gradient solution, otherwise a purification will be not good
Culture of the islet	• Reagents (media, FBS, Penicillin, streptomycin) • Culture dishes (10 cm Petri Plates) • Incubator with temperature and gas composition controls • Optical microscope	Culture density	• Choose the correct media based on your experiment, in order to keep islet in good shape and no in activate metabolic state. • Culture the islet as pure as possible, because starting from day +1 post culture it became difficult distinguish endocrine tissue from exocrine tissue

Table 2 Summarizing the differences between mouse and rat pancreatic islet isolation

		Mouse	Rats
Surgery	euthaninization	cervical dislocation, CO_2 asphyxiation, drugs	Isoflurane (3-5 %) in a sealed chamber or Ketamine/Xylazine ip
	needle	30 or 27 Ga 1/2" needle	PE 50 Polyethylene tubing o Blunt needles 0.6 mm × 25.4 mm
	collection of pancreas	Each pancreas is ready for digestion	Each pancreas has to be clean from lymph node, fat
	dissecting microscope	mandatory	not necessary
Pancreas digestion	enzyme	collagenase Type XI or V	Collagenase type XI or liberase
	digestion time	12 min max	12/20 min
Purification step	number of pancreas for each 50 ml tube	up to 5	1-2
Culture	culture media	RPMi	CMRL 1066, 10 % FBS, PenStrep
	culture density	250-300 IEQ/ml	not more than 300 IEQ per ml

subsequent steps and alterations in the efficacy of collagenase activity. Exsanguination, usually performed by cutting the vena cava, allows a good-quality batch of islets to be obtained. Next after euthanization, the mouse should be placed in a supine position; the abdomen cleaned with 70 % ethanol and opened with a V-incision from the pubic region up to the diaphragm, in order to expose the abdominal cavity.

Pancreas perfusion and harvesting instructions: Position the lobes of the liver against the diaphragm. Find the hepatic artery, portal vein, and bile duct bundle leading into the liver by gripping the duodenum with curved forceps. Once the liver is exposed, grip the duodenum again with curved forceps following the bile duct and clamp at the level of the ampulla, in order to spread the collagenase solution only in the pancreas and not in the intestine. If the clamp is too high or too low the enzyme will not perfuse the pancreas properly. The ampulla is a triangular white area located at the duodenum surface, where there is a confluence between the bile duct and the duodenum with bile draining from the gall bladder and enzymes from the pancreas coming together before entering the intestines. Optimal needle placement is important to prevent backflow into the liver and to be sure it drains the splenic tail of the pancreas, an islet-rich area. Particular care should be taken to avoid penetration of the fascia tissue, which would result in perfusing only the surrounding connective tissue. Once the common bile duct is clamped, it can be cannulated using different techniques. The standard procedure consists of direct swelling via injection of collagenase solution into the common bile duct (Fig. 1). In this method, the needle must be inserted into the Y-shaped junction of the cystic duct and the hepatic duct.

Contrary to this classic approach, a suggested method in the literature involves catheterization without a microscope through the duodenum end of the

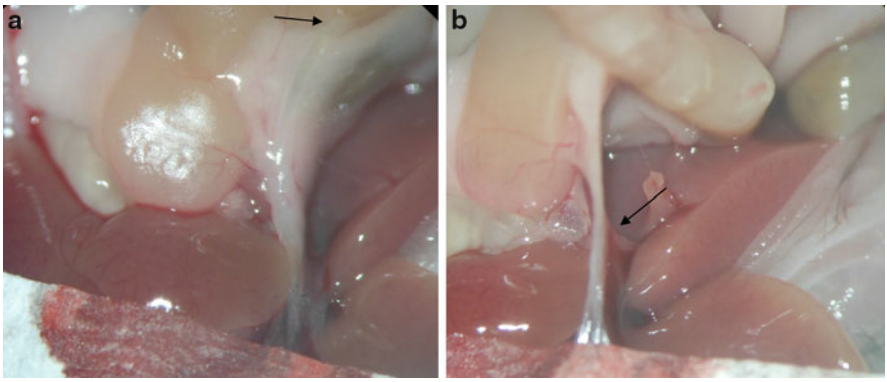

Fig. 1 Anatomy of the mouse upper intraperitoneal cavity. The mouse lying on its back with head toward the surgeon. Panel A. The arrow indicates the site of ampulla. Panel B. Injection site and common bile duct

common bile duct, after occlusion of the junction site of the hepatic and cystic ducts. At first glance this method could seem easier, but the perfusion from the intestine to the liver could drag bacteria into the pancreas, causing contamination. The pancreas is then excised and digested at 37 °C (Gotoh et al. 1985). It is important to remove fat tissue because it may affect digestion and reduce the yield (Li et al. 2009).

General Considerations The choice of the methods for cannulation depends on the skills and expertise of the operator. However, one consideration must be taken into account: perfusing the pancreas by common bile duct cannulation allows the collagenase to spread inside the pancreas using the anatomical structure and allows a 50 % higher islet yield to be obtained, because collagenase interacts closely with connective tissue, and is more cost effective (Shapiro et al. 1996). A pioneering approach in the scientific literature suggests avoiding clamping the duct, directly excising the pancreas, and cutting it into 1- to 2-mm pieces, followed by digestion of the pieces in a collagenase solution stirring or shaking directly as detailed below (Lacy and Kostianovsky 1967; O'Dowd 2009). However, this method is less efficient and can only be considered when pancreas perfusion is not possible.

Pancreas Digestion

The basis of islet isolation is the intraductal injection of collagenase in order to digest the tissue. Once the pancreas is harvested, the next step is its digestion with collagenase. The digestion can be static and/or dynamic. The collagenase used in the procedure is not a pure preparation; the ability of different batches of enzyme to yield a successful isolation is still one of the greatest obstacles to improving this procedure. The key points to take into consideration during this procedure are described below.

The choice of the enzyme: The standard digestion protocol for mouse isolation uses a crude collagenase type XI or V. Unfortunately, variations in the composition of preparations that are commercially available requires each lot to be individually tested and optimized prior to general use. The same considerations that must be taken into account when the enzyme blend is chosen for human isolation should also be borne in mind for rodent isolation. In fact it has already been reported that thermolysin concentration, the ratio of collagenase I to II, and the purity of the enzyme blends are three of the most significant parameters for achieving optimal islet isolation (Wolters et al. 1992, 1995). For human islet isolation, collagenase I is considered essential (Barnett et al. 2005); on the contrary, collagenase II is reported to play a major role in murine pancreatic islet isolation (Wolters et al. 1995; Vos-Scheperkeuter et al. 1997). With all these considerations, a highly purified preparation eliminates the intensive process of lot testing normally required for crude or enriched collagenase products, and ensures homogeneity. Recently, numerous publications have reported how using a more purified collagenase mixture can be more effective. In 2009, Yesil et al. showed that an increase in enzyme purity correlates with an increase in islet yield (Yesil et al. 2009). Stull et al. reported in 2012 that using a purified protease mixture increased the islet yield in different mice strains: up to 260 islet/mouse for C57Bl/6, 200–300 islets/mouse for CD1, 200–300 islets/mouse for 129/B6, 120–200 islets/mouse for BLKS-db/db, 100–150 islets/mouse for NOD (10 weeks old), and 100–150 islets/mouse for NOD-SCID (Stull et al. 2012). Those studies lead to more productive use of resources and improved flexibility in experimental design. Moreover, collagenase formulations have been identified as containing endotoxins in varying amounts. As observed in humans, the level of endotoxin should be considered when choosing collagenase mixtures, because it usually correlates with pro-inflammatory cytokines in models of transplantation (Jahr et al. 1999). Some interesting experiments performed in 2001 demonstrated that using endotoxin-free reagents during islet isolation (not only the enzyme, but also the wash and culture media) is a key factor for successful islet transplantation (Berney et al. 2001).

Control of digestion: The digestion step is really critical and must be taken seriously when choosing between different methods. Digestion time, digestion temperature, and collagenase administration are key factors in obtaining a good islet yield. Regarding the digestion time, this step varies among the different protocols because it is strictly dependent on the choice of cannulation methods. In fact the cannulation of the bile duct allows collagenase to spread intact pancreas and to digest the connective tissue more closely, which could result in shortening of the duration of digestion. Usually, the digestion time should take between 8 and 11 min. A good suggestion is to perform a static digestion for 6–8 min and perform an additional mechanical digestion at 37 ºC for 2 min. The mechanical digestion, by shaking (manually or automatically), is necessary because the islet yield could be low if the tissue is not well broken. The strain and the age of the rodent can also influence the time of digestion. This is because the connective tissue is different between animals. Once the correct point of the digestion has

been decided, the action of the enzyme must be terminated. Stopping the reaction is usually achieved by a combination of a cooling procedure and the removal of the enzyme. The decrease in the temperature to 4 °C and the washing could be achieved by adding Hank's solution supplemented with 10 % FBS. Some studies suggest adding some protease inhibitor such as BSA to the stop solution to prevent cell lysis and the release of proteolytic enzymes (Wolters et al. 1990; Perdrizet et al. 1995). In fact, endogenous proteolytic activity released during the dissociation process exerts a deleterious effect, causing rupture of the cells and release of DNA.

General Considerations Incubation time with the enzyme and the termination technique vary among different perfusion conditions and is strictly collagenase dependent. It is important to calibrate the optimal incubation time for each new enzyme batch and use frozen stock to mimic the real experimental conditions. Precise guidelines that suggest how to choose and use collagenase were reported by de Haan et al. in 2004 (De Haan et al. 2004).

Purification

The goal of the purification step is to extract the islets from the exocrine tissue contained in the digested pancreas. The purification step defines the mass, viability, and function of your isolated islets. The purity could affect the immunogenicity and safety of the islet recipient (Gotoh et al. 1986); in fact, acinar cells are able to secrete different digestive enzymes (e.g., gastrin-releasing peptide, amylase), that are really dangerous for the survival of the islets. On the contrary, preservation of ductal cells and fibroblast appears pivotal for the survival of pancreatic islet. Taking all this into account, the importance of the choice of the purification method is evident. Before purification, a filtration step through stainless steel filters is required. The tissue is poured through a 0.419-mm mesh that allows digested tissue to be separated from non-digested tissue, fat and lymph, and a pellet of tissue to be prepared for purification. Despite there has been a consensus on the importance of separating endocrine tissue from acinar tissue, there is some debate regarding the method of purification, particularly about the use of a density gradient to obtain a first clean-up of islet preparation (Carter et al. 2009). Specifically, the most common alternatives for purifying islets are the following.

Sedimentation: The easiest and least expensive method of purifying the filtrate tissue is sedimentation, although it is also the most inefficient method. In fact, islets normally show intrinsic variation in diameter (50–500 μm, which can overlap with acinar cell diameters).

Density gradient purification: The standard method for islet purification is density gradient centrifugation or isopycnic centrifugation (separation according to differences in density). Islets are centrifuged on density gradients long enough for them to reach the point of the gradient of equal density. Acinar cells and islets have high differences in density and are, therefore, easily separated by this method. However, acinar tissue density depends on the secretory status of the acinar cells

and their density may be affected by the size of the aggregates formed (Chadwick et al. 1993a, b; De Duve 1971), by the collagenase digestion of the pancreas and their swelling after the isolation procedure, leading to some overlapping (Berney et al. 2002). Albumin, sucrose, iodixanol, Histopaque, and Ficoll are the most common solutions used to separate islets from acinar tissue by this method of purification.

Among them, the solution most widely used for density gradient purification, as reported in the literature, is Ficoll, because it leads to a higher level of purification in comparison with other gradients, and provides a more physiologically osmotic environment for the islets (Lindall et al. 1969). Usually, Ficoll is layered in a discontinuous way and islets are isolated from acinar tissue, which is heavier. Ficoll is expensive, potentially toxic to islets because of its high content of glucose and has been associated with release of IL-1β, NO, and reactive oxygen species by islets in vitro (Jahr et al. 1995, 1999). To overcome some of these drawbacks, many laboratories have adopted other solutions, even though each of these solutions has a different osmolarity, which can lead to varied islet numbers, viability, purity, and functionality. Histopaque is a hypertonic solution also used in isolating other cell types. For this reason, some laboratories prefer this gradient (Zmuda et al. 2011). Iodixanol is a non-ionic, iso-osmolar solution, first used for pig isolation and since 2007 also used for rodents, with good results. Recently, McCall et al. (McCall et al. 2011) made a comparison of different protocols of purification (dextran, Ficoll, Histopaque, iodixanol) and concluded that Histopaque was as good as Ficoll.

It should be noted that Histopaque and Ficoll are both gradients widely used and accepted. As general guidelines, independently of the kind of solution used, the tissue should be suspended in the most dense gradient. The pellet should be resuspended very well in order to avoid a decrease in purification efficiency. The gradient must be built with different layers of the solution going from the most dense solution in the bottom of the tube to the lightest solution, to avoid mixing the layers. Then the gradient should be spun at a specific temperature and speed according to the nature of the solution, tissue volume, etc. At the end of centrifugation islets can be retrieved from the first and second gradient interfaces; the purity of the first layer is usually higher, because the second layer can also include embedded islets.

Other purification techniques: Some protocols also consider handpicking the islets before culture as an option to purify the suspension. The handpicking protocol includes a stereomicroscope with side illumination to identify islets suspended in a Petri dish with a black background. It should be performed in a laminar flow cabinet using a dissecting microscope to minimize the risk of islet contamination. Using a 2× objective, islets are selected from the acinar tissue and transferred to a second culture or third dish (depending on the initial purity of the preparation) containing culture media. An instrumental step that does not allow the widespread use of the handpicking technique is the fact that it is very tedious and time consuming. In fact, this step should be performed as fast as possible in order to minimize the time outside the sterile hood and incubator to limit exposure to contamination and pH changes. In 2002, Salvalaggio (Salvalaggio et al. 2002) compared pancreatic digest

purified either by Ficoll or by filtration, claiming superiority of the second method of islet purification. Recently, our group has compared Histopaque and filtration purification with handpicking as the gold standard method for islet purity (London et al. 1998). Our results show the significant advantage of islet purity using Histopaque versus the other methods, all of them with purity over 98.5 %. Once the endocrine tissue is completely separated from acinar tissue, the islets are ready to be cultured. In some protocols a second purification step by gradient, sedimentation or filtration is needed before culturing, to further increase islet purity and islet yield, particularly if the volume of packed tissue is still large.

General Considerations Even though the use of density gradients is well accepted in all the laboratories, further improvements in the purification step will not result from the production of new density gradient media, but rather from the continued modification of the biochemical composition of the solvents in which the established gradient media are dissolved in order to produce a more suitable physiological environment for the islet (London et al. 1998). The final purity of the isolated islet depends on the type of gradient, but is also influenced by the mouse strain. The total number of islets varies considerably depending on the strain and age of the rodent, the expertise of the technician as well as the method of isolation (Gotoh et al. 1985). It has been reported, for example, that lean rodents lead to a yield of higher purity than those with more fat (De Groot et al. 2004). Certain factors must be taken into account when choosing the purification method:

(a) The temperature: may affect the results of density gradient purification. Rodent islet purification is more efficient at 4 °C, since the characteristics of the solution used in this procedure could damage metabolically active cells.
(b) Osmolarity of the solution: hyperosmolarity could prevent edema of acinar tissue.
(c) Volume of tissue packed: as a general guideline the normal amount of tissue processed in 50-ml tubes for efficient purification should be 1 ml.

Culture

After performing islet isolation, proper culture conditions are imperative to ensuring that the islets can recover from the insult of collagenase digestion and isolation procedure. Appropriate islet culture conditions must be considered:

Culture medium: The common medium used for rodent islet culture is RPMI 1640, even though other media are used equally as frequently in different laboratories; some authors suggest using a medium commonly used in human islet culture, CMRL 1066. It is reported, for example, that this medium induces a decrease in alloreactivity; however, it seems that some immune cells, such as dendritic cells and endothelial cells, do not survive for a long period of time in CMRL 1066 (Benhamou et al. 1995). Another key factor to take into account in the choice of the medium is glucose concentration. Media with glucose concentrations below 11 mM can reduce islet insulin content and down regulate key genes related to glucose metabolism.

Culture density: Another important consideration is islet density in culture: it is common not to culture too many islets in the same dish, because this can induce necrosis as a consequence of cell damage and competition for nutrients (Dionne et al. 1993). Usually, 250–300 islets equivalent/ml for a 60- × 15-mm dish does not appear to stress the islets. Suspension culture dishes are preferred in order to decrease islet attachment.

Culture temperature: The standard protocol for rodent islet culture foresees placing them at 37 ºC with 5 % CO_2 infusion and humidified air. Some authors incubate islets at 24 °C for the first 48–72 h following isolation, with the aim of dampening their metabolic activity, preserving their viability, and smoothing their transition to in vitro culture (Ricordi et al. 1987). Moreover, it seems that culturing islets below 37 °C helps to almost completely remove the intra-islet lymphoid cells (Moore et al. 1967).

Effects of culture on future transplantation: Recently, thanks to new advances in technologies, new methods of culturing islets have been explored in the literature. Islet culture prior to transplantation is controversial for many reasons: death of endothelial cells with the impossibility of re-establishing islet vasculature in vitro; proliferation of fibroblasts with effects on gene expression; development of a necrotic core in the islets, etc. On the other hand, in vitro culture of pancreatic islets reduces their immunogenicity and prolongs their availability for transplantation. In fact, after a few hours of culture, the majority of acinar cells die, increasing the purity of the preparation (e.g., because endothelial cells die and these intra-islet blood vessels may be crucial if the islets are to be transplanted). Fibroblasts in culture can grow in a few days, totally changing the expression of genes such as vimentin. A necrotic core islet could also appear during culture. Recently, biomaterials (e.g., hydrogel, polyglycolic acid scaffold [PGA]) have been explored (Vaithilingam et al. 2014; Jun et al. 2013) in order to maintain islet 3D culture, and simulated microgravity (sMG) is believed to confer benefits to cell culture (Song et al. 2013).

General Considerations It is usually good practice to change media 24–48 h post-isolation to better preserve islet function and to remove dead islets and debris. Many laboratories, in order to increase islet yield for the experiment, perform more than one isolation and pull together the preparations at the end. It is important not to mix islets from different isolations, because the cells could be influenced by many factors, including the time spent in culture (Zmuda et al. 2011). Therefore, in order to reduce variability, the best option is to perform experiments on islets isolated at the same time. In addition, some cryogenic methods for long-term preservation of the islets have been explored. Warnock et al. (Warnock et al. 1987) reported a successful protocol for cryopreservation, with β-cell responsiveness in vitro and in vivo. Others suggest that is not possible to preserve a good endocrine function of frozen–thawed islets (Piemonti et al. 1999). Finally, selection of appropriate media has been shown to depend again on the animal source of the islets (Holmes et al. 1995).

Practical Issues: Quality, Time, and Cost

Islets are the primary source of either in vitro or in vivo experiments in the diabetes research field. Therefore, it is of great importance that the isolation procedure yields islets of sufficient quality. In that sense, it is also necessary that the assessment of that quality is performed according to standardized criteria. However, the establishment of standardized assessment tests has proved to be a challenge and it is under development in several laboratories. Islet preparations are difficult to characterize for many reasons (Colton et al. 2007):

1. Islets are cellular aggregates. Therefore, they have 3D structure, with an asymmetric shape. This implies some technical inconveniences, since many techniques suitable for single cells or cells in monolayers are unsuitable for islets. Besides, the wide range of sizes makes it difficult for them to be accurately quantified or proper visual estimations to be made, which are operator dependent and highly variable.
2. It is not easy to obtain a preparation that is 100 % pure. Different variables could affect the purity of the preparations obtained, including some aspects of the isolation procedure, as explained in the previous section (e.g., type of collagenase, methods of purification), or characteristics of the donors (animal age, sex or strain)
3. Islets are dynamic. Islet volume decreases with time after isolation because they are under stress from the moment the pancreas is harvested, during the islet isolation process, in culture, etc. Therefore, when the experiment starts islets are not completely representative of their original status.

In this section we will discuss some practical issues regarding quality, time, and cost of the islet preparations to be taken into account before starting any experiment (Table 3).

Morphology

When islets are inspected under a light microscope, islets appear spherical and golden-brown, and range between 50 to over 400 μm, most of them between 50 and 250 μm in diameter (Carter et al. 2009; Bertera et al. 2012). Bertera and colleagues reported that, in general, 20 % of the islets measure less than 50 μm, 30 % between 50 and 100 μm, 35 % between 100 and 250 μm, and 15 % greater than 250 μm (Bertera et al. 2012). Mouse islets are easily identified by their semi-opaque color in comparison to the relatively transparent exocrine tissue. Healthy isolated islets present a defined smooth rounded surface. Usually, islets show a well-preserved capsule after an overnight recovery. However, darker hypoxic areas in the center of larger islets or cells disrupting the defined surface of the islets can appear over time as a sign of reduced health. As explained in the previous section, an optimized ratio between the number of islets and the volume of culture media should be established to maintain islets with good morphology and good viability (Zmuda et al. 2011).

Table 3 Summary of the parameters, assays, and information to be considered in order to select a rodent islet isolation protocol

Parameter	Assay	Information Obtained
Morphology	Observation by light microscopy	Shape and aspect of the islets (under stereomicroscope), presence/absence of dark hypoxic areas (under inverted microscope)
Purity	Dithizone staining	What fraction of the preparation is endocrine and exocrine tissue
Yield	Stimulation of the number in a sample. Observation under the stereomicroscope	What number of islets have been obtained What is the number of IEQ
Validity	Membrane integrity tests. Observation by fluorescence microscopy.	Percentage of viable tissue in each islet and in the preparation
Function	Glucose Static Insulin Secretion Dynamic Perifusion	Insulin secretory capacity under glucose challenge
Time	Timing of the isolation/purification procedure	if the protocol is the most suitable according to the volume of work of the laboratory
Cost	Summing up the cost of the reagents taking into consideration staff wages	if the protocol is the most suitable according to the economy of the laboratory

Purity

Purity is a key factor in islet preparation, since it is intimately linked to quality. As explained in the previous section, contamination of the preparation by acinar cells can affect the immunogenicity of the graft for in vivo experiments and also its functionality, both in vitro and in vivo, owing to the interaction with the secretory response of the islets (Piemonti et al. 1999) The traditional method of assessing islet purity is by the zinc-specific binding dye dithizone (DTZ) staining (Latif et al. 1988), since pancreatic islets contain a high concentration of zinc because of its role in the synthesis, storage, and secretion of insulin. This dye stains the islets red, so that they can be visually distinguished from exocrine tissue under a light microscope. It is also helpful to quantify the islet yield both in human and mouse islets, although mouse islets can be more easily distinguished from exocrine cells at a glance. However, dithizone has a potentially toxic effect on islet function; thus, it is considered a non-vital stain (Conget et al. 1994).

Usually islets are examined with a phase contrast microscope. Islet mass can be quantified by islet counting and determination of islet diameter. A calibrated grid in the eyepiece could be useful for measuring the diameter of the islets (Ricordi et al. 1988). The purity of the islets could be determined by estimating the volume of the islets relative to the non-islet tissue. The purity is calculated as the percentage of the intersections that overlie islets out of the number of the intersections that overlie islet and non-islet tissues (Ricordi et al. 1990).

Yield

The yield obtained after islet isolation is an important parameter, taking into account that the number of mice that will be sacrificed for the experiment usually depends on the average yield obtained per mouse. In fact, the average yield per mouse is an important parameter to consider in setting up islet isolation. This yield, as explained in the previous section, also depends on different variables, such as the experience of the operator (e.g. surgery ability), and characteristic of the donor (age, weight, and strain) (De Haan et al. 2004; De Groot et al. 2004). On average, rats yield between 300 and 800 islets per animal, while the yield from a mouse pancreas is lower, around 100–150 increasing to 200–400 islets with an experienced technician, which means between 10 and 30 % of the islets in the typical rodent pancreas (Carter et al. 2009; Zmuda et al. 2011; Kelly et al. 2003; Gotoh et al. 1985).

One method of directly quantifying the yield in mouse islet preparations is handpicking. This is a time-consuming procedure, making this tedious process unfeasible for large-scale islet isolations. Thus, the standard practice is to take a small sample of the preparation (e.g., 100 μl), stain it with dithizone, and proceed to counting under the light microscope with an ocular micrometer.

According to the consensus report of 1990 (Ricordi et al. 1990), developed after the workshop of the 2nd Congress on Pancreas and Islet Transplantation (Minneapolis, 1989), the standard practice for expressing the total volume of islets is Islet Equivalents (IEQ). One IEQ is the number of "standard" islets of 150 μm in diameter that would have the same total volume (Buchwald et al. 2009). Therefore, when counting, islets are classified according to their diameter in ranges of 50 μm and their number of IEQs calculated by multiplying the number of islets with a conversion factor that is derived from the mean volume of islets in that range group divided by the volume of an islet with a diameter of 150 μm. In this regard, there is some controversy over the quantification of islets between laboratories. Although the use of IEQ could be more statistically representative of the total volume of the preparation, others consider using the absolute number of islets a more accurate measure of preparations with a small number of islets.

Viability

The viability of an islet preparation is an important parameter in quality assessment, since it is expected that a high degree of viability will correlate with a successful transplantation outcome. Pancreatic islets are cell aggregates, and it is always controversial and time-consuming to choose the correct test to check viability. A good suggestion is to perform more than one test in order to obtain a more precise picture. The most common tests are reported below.

Fluorescein diacetate and propidium iodide (FDA/PI) assay: The standard international method of determining viability is double staining with fluorescein diacetate and propidium iodide (FDA/PI) assay. This is an assay based on the simultaneous discrimination of live versus dead cells by membrane integrity stains.

FDA functions as an inclusion dye, while PI is an exclusion dye. FDA is a colorless, non-polar ester that passes through the plasma membrane and becomes hydrolyzed by non-specific cellular esterases to produce fluorescein, resulting in a strong green fluorescence. On the other hand, PI is polar and only enters dead cells, as intact membranes remain impermeable to it. Once inside the cell, it fluoresces orange/red when bound to nucleic acids. After the islet sample is stained with both dyes, it is examined under a fluorescence microscope and the approximate volume fraction of cells stained of each color is visually assessed (Colton et al. 2007).

The advantages of using FDA/PI are that it is a quick, easy, and cheap assay and therefore very convenient, apparently. However, it has some limitations (Colton et al. 2007; Barnett et al. 2004; Boyd et al. 2008):

- It does not distinguish between islets and non-islets.
- It is a subjective method, operator-dependent.
- It is not accurate. Islets are tridimensional structures and with fluorescence microscopy only the surface of the islets can be seen; therefore, information from the inside of the aggregate is lacking.
- It only differentiates between dead and non-dead cells; it does not distinguish apoptotic cells.
- The assay has to be technically optimized. The final concentration, incubation times, type of solvent, and storage conditions influence the final scoring of viability.
- There is a lack of correlation between membrane integrity stains in general and other viability assays, including mitochondrial function assays, such as MTT and ATP.
- It does not correlate with the transplantation outcome, neither in animals nor in humans.
- It overrates the percentage of live cells and the FDA can also cause background fluorescence.

Regarding the last point, a comparison study between different dyes (Barnett et al. 2004) reported that FDA/PI produced intense staining that obscured the PI signal in the core of the islets. The viability was higher than that obtained with SYTO/EB (Syto-13 ethidium bromide), and there was also a higher degree of background fluorescence, owing to extracellular, nonspecific esterase activity. However, in a more recent study (Boyd et al. 2008) a wider variety of stains were compared, and the results were found to be questionable for all dyes.

Alternative methods: Effort is are therefore being invested in the exploration of alternative methods that are currently under investigation, such as stains that are responsive to metabolic activity (e.g. tetrazolium salts, ATP, ADP/ATP, oxygen consumption rate) and/or mitochondrial membrane potential (Janjic and Wollheim 1992; Barbu and Welsh 2004; Goto et al. 2006; Papas et al. 2007). In fact, Colton et al. (2007) suggest mitochondrial function assays as an interesting alternative to membrane integrity tests, since they are able to identify cells that are damaged, but do not reach the membrane permeabilization step. Therefore, this kind of assay would provide a more accurate measurement of the viability of an islet preparation.

Functionality

In the assessment of the mouse islet preparations it is fundamental to characterize islet function. Pancreatic β cells are very sensitive to changes in extracellular glucose and the ability of the islets to regulate insulin release defines its functionality (Carter et al. 2009). Islets show a dose-dependent pattern of insulin release when incubated with different glucose concentrations. The time course of insulin secretion in response to glucose stimulation is biphasic. There is a first phase with a rapid spike in insulin secretion followed by a decrease to a prolonged second phase plateau of insulin that continues with the duration of the stimulus. Islet functionality can be assessed in vitro or in vivo.

In vitro functional test: The standard methods used to measure islet function are glucose standard insulin secretion (GSIS) and dynamic perifusion. While the first method can be useful for establishing dose–response curves in response to insulin secretagogues, questions about the kinetics of insulin secretion in response to glucose are addressed with regard to islet perifusion experiments (Nolan and O'Dowd 2009).

The GSIS assays consist of cultivating the islets first at a "low," "basal" glucose concentration in order to synchronize the cells before starting the real assay. Next, the islets are incubated at a "low" glucose concentration, then at a "high" glucose concentration, and then again with "low" glucose. When islets are exposed to "low" glucose (usually 2.8 mM) they are under "basal" or "unstimulated" conditions. On the other hand, when islets are stimulated at the "high" glucose concentrations, the concentration of insulin can be raised to different concentrations, depending on the laboratory, to 11.1 mM, which is half maximal, or over 28 mM, which is maximal. The purpose of alternating "basal" or "unstimulated" conditions with stimulated ones is to check that the islets in the preparation are able to respond properly, increasing and also decreasing insulin secretion according to the changes in glucose concentration. The decrease in the insulin secretion with the second "basal" also discards the possibility of sustained high values of insulin secretion after stimulating conditions due to cell death processes. The stimulation index (SI) can be calculated as the ratio of stimulated-to-basal insulin secretion. Healthy islets can have an SI of 2–20 depending on different factors such as strain, age, and body weight (Carter et al. 2009). A more physiological assessment of the detection of insulin release in vitro is dynamic perifusion. This technique is an adaptation of the principle of the flow respirometer developed by Carlson and colleagues (Olsson and Carlsson 2005) for the measurement of the uptake of oxygen by amphibian nerve. Even though this method is time-consuming and expensive, the technique provides accurate quantitative data on the dynamic responses to biologically active compounds and adds information on the dynamics of the hormone in terms of secretion and clearance. This technique allows the detection of insulin release in response to elevated glucose concentration and secretagogues. Islets in a perifusion media (RPMI-1640, or more often a specific medium, depending on the experimental aim) are sandwiched between two layers of sterilized material in a microchamber. Gassing, flow rates, the addition of secretagogues and fraction

collection are controlled by a programmable perfusion/perifusion apparatus. Fractions are collected every 10/30 min over 3 h. Recently, new tools (e.g., glucose nanosensor) reported in the literature have allowed the widespread use of this quality assessment technique.

Besides, it is important to take into account the purity of the preparation when any of these functional studies are performed, since the presence of exocrine tissue can interact with the secretory response of the islets. It is also advisable to culture the islets between 12 and 18 h prior to functional experiments to allow the replacement of receptors, which may have been proteolyzed during collagenase treatment (Nolan and O'Dowd 2009). However, some reports have shown improved islet function in rodents with fresh islets (King et al. 2005; Olsson and Carlsson 2005). Deciding to perform functional testing on the day of isolation or after recovery correlates with the aim of the experiments, whether the goal is to perform in vitro experiments or preclinical studies. In the latter case, it is better to perform the functional test on the day of the transplant in order to better mimic the situation of the islets on the day of engraftment.

Insulin and C-peptide content could be another measure of islet functionality. Briefly, both peptides can be extracted from the isolated islets by acidified ethanol and sonication, and can be quantified on supernatants through immunoassay tests (Rabinovitch et al. 1982).

In vivo functional test: Moreover, islet cell potency can be assessed in vivo by diabetes reversal after islet transplantation into chemically diabetic mice. Diabetes is induced in mice by streptozotocin or alloxan injection. The differences between the two methods are well explained by Cantarelli et al. (2013). Nonfasting glycemia and metabolic testing, such as the intraperitoneal glucose tolerance test or the intravenous glucose tolerance test can be performed in transplanted mice, in order to test the functionality of the islets in the graft (Juang et al. 2008; Morini et al. 2007). Histological examination of the graft can provide useful information on the morphology and cellular composition of the transplanted islets.

Time and Cost

Although obtaining good quality islets is instrumental in any experimental study, time and cost are also side issues that are gaining relevance in the daily routine of mouse islet laboratories.

As McCall and collaborators suggest (McCall et al. 2011), a mouse islet isolation protocol must be chosen when the time and costs invested are worth the benefits in terms of quality, and vice versa. In this sense, we have recently compared the quality, time, and cost of a standard rodent islet purification protocol using Histopaque with a filtration-based method, an alternative that is not mouse so well established in laboratories, taking handpicking as the gold standard method of islet purification (Ramirez-Dominguez and Castaño 2014).

Our study suggests that, while both protocols yield good-quality islets, purification of islets by filtration with 100-μm cell strainers saves almost 90 % of the time spent in purification using Histopaque density gradient. If staff time and wages are also taken into account, an important economic saving for the laboratory could be made. Therefore, it would be particularly convenient for large-scale islet isolations and/or laboratories with limited staff. The drawback of this filtration protocol is that one third of the islets are lost compared with the yield obtained by handpicking, and therefore, more animals are needed.

Thus, this study points out the importance of time and cost investments in addition to the quality of the islets in the selection of the mouse islet isolation protocol, and their impact on the management of the laboratory routine.

Factors to Consider in Setting Up a New Rodent Islet Isolation Laboratory

Performing studies on isolated human islets are unquestionably crucial for the improvement and widespread use of this clinical procedure. Unfortunately, the availability of human donors can be a hindrance, and for this reason mouse islets serve as a useful surrogate for human islets. Animal use in scientific studies is regulated by OLAW (Office of Laboratory Animal Welfare) guidelines and IACUC (Institutional Animal Care and Use Committees) committees when planning the protocol for any experiment, although the use of animals in research is always controversial.

Basic elements to consider when setting up a new laboratory for mouse islet isolation include adequate space to grow, well-trained staff, and sufficient funding to support the research.

Infrastructure

Facilities: Provision of adequate space is usually one of the general problems in any institution. In order to have an optimal supply of mouse pancreas it is mandatory that the institution where the laboratory is going to be set up has a fully working animal facility that facilitates the provision of mice of the required strain, sex, and age. Taking into account the fact that the surgery can be performed either under sterile conditions or using an aseptic technique it is not compulsory to carry it out at the animal facility; it can be done in the laboratory. The laboratory should have an area for performing the purification and quality assessment assays. In addition, there should be a separate cell culture unit where the islets will be cultured after isolation and also where the in vitro experiments will be carried out.

Equipment: A mouse islet isolation laboratory is composed mainly of the general equipment of a cell biology laboratory plus some specific materials for performing

Table 4 Essential equipment for the setting up of a mouse islet isolation program

Facility	Laboratory	Cell culture unit
Equipment	• Microcentrifuge • Magnetic stirrer with heating • Scale • Vortex • Water Bath • pHmeter • Fridge with a −20°C freezer • −80°C freezer • Planetary Shaker • Plate Reader	• Laminar Flow Hood • Fluorescence Inverted Microscope • Stereomicroscope • Centrifuge • Incubator • Water Bath

surgery (Table 4). It is important to establish a routine for the cleaning and correct maintenance of the equipment since the isolations must be carried out using an aseptic technique and the islets will be used in other experiments (either in vitro or in vivo) requiring sterility.

Staff

The most important factor in the success of a new laboratory is the members of staff who will be part of the team. The workers recruited must meet the necessary skills, knowledge, and motivation requirements to set up the new laboratory efficiently. Since mouse islet isolation requires a significant degree of expertise, it is highly desirable to hire personnel with previous expertise in the field. However, if the candidates lack the necessary scientific background, they must be adaptable and smart and capable of learning new techniques. The number and composition of the members of the team is dependent on size of the laboratory, the dynamics of the institution, and the budget. In order to have a constant source of islets it is desirable to have at least two technicians devoted to the isolation procedure who master all the techniques in the laboratory, and who can help and/or complement each other.

Budget

The final goal of the establishment of a mouse islet isolation laboratory is the translation of the studies performed with those islets to the clinic with pre-clinical assays. Therefore, the institution can receive funding from government sources at different levels, institutions, industry or private research sources. These organizations can provide either funding or the development of different research lines. Therefore, the initial investment in setting up the laboratory from the recipient institution is recovered once the laboratory is fully working.

Conclusions

Mouse islets are a constant and reliable raw material for any in vitro or in vivo study in the diabetes field. Thus, despite differences between human tissue and animal models, improvements in the mouse isolation procedure could be somehow translated to the optimization of human isolation and therefore, to better transplantation strategies.

Mouse islet isolation is a work of craftsmanship, since many variables, as well as operator expertise, influence the outcome, therefore requiring the acquisition of specific technical skills and know-how. Once the protocol is defined and islets are isolated, their quality can be assessed by different parameters using standard methods. However, time and costs invested in the selected protocol are also issues to be considered, with a direct impact on the management of the laboratory.

The set-up of a mouse islet isolation laboratory is not only an economic investment, but also a human and institutional one. It is the foundation stone for pre-clinical studies. Therefore, it brings not only scientific benefits by translating the knowledge gained from the studies of mouse islets to the clinics, but also a very valuable logistical experience when undertaking to establish a human islet isolation laboratory.

Acknowledgements This work was supported by a grant of the Basque Government for Groups of Excellence (IT-472-07) and a grant of the Department of Industry of the Basque Government (SAIO09-PE09BF01).

Cross-References

For major details on Clinical Islet Isolation/transplantation refer to the chapters:
► Advances in Clinical Islet Isolation
► Islet Isolation from Pancreatitis Pancreas for Islet Autotransplantation

References

Barbu A, Welsh N (2004) The use of tetrazolium salt-based methods for determination of islet cell viability in response to cytokines: a cautionary note. Diabetologia 47:2042–2043

Barnett MJ, McGhee-Wilson D, Shapiro AM, Lakey JR (2004) Variation in human islet viability based on different membrane integrity stains. Cell Transplant 13:481–488

Barnett MJ, Zhai X, LeGatt DF, Cheng SB, Shapiro AM, Lakey JR (2005) Quantitative assessment of collagenase blends for human islet isolation. Transplantation 80:723–728

Benhamou PY, Stein E, Hober C, Miyamoto M, Watanabe Y, Nomura Y, Watt PC, Kenmochi T, Brunicardi FC, Mullen Y (1995) Ultraviolet light irradiation reduces human islet immunogenicity without altering islet function. Horm Metab Res 27:113–120

Bensley RR (1911) Studies of the pancreas with the guinea pig. Am J Anat 12:297–388

Berney T, Molano RD, Cattan P, Pileggi A, Vizzardelli C, Oliver R, Ricordi C, Inverardi L (2001) Endotoxin-mediated delayed islet graft function is associated with increased intra-islet cytokine production and islet cell apoptosis. Transplantation 71:125–132

Berney T, Pileggi A, Molano RD, Ricordi C (2002) Epithelial cell culture: pancreatic islets. In: Atala A, Lanza RP (eds) Methods of tissue engineering. Academic, San Diego

Bertera S, Balamurugan AN, Bottino R, He J, Trucco M (2012) Increased yield and improved transplantation outcome of mouse islets with bovine serum albumin. J Transplant 2012:856386

Bhonde RR, Parab PB, Sheorin VS (1999) An in vitro model for screening oral hypoglycemics. In Vitro Cell Dev Biol Anim 35:366–368

Biarnés M, Montolio M, Nacher V, Raurell M, Soler J, Montanya E (2002) β-cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. Diabetes 51:66–72

Bock T, Pakkenberg B, Buschard K (2005) Genetic background determines the size and structure of the endocrine pancreas. Diabetes 54:133–137

Boyd V, Cholewa OM, Papas KK (2008) Limitations in the use of Fluorescein Diacetate/Propidium Iodide (FDA/PI) and cell permeable nucleic acid stains for viability measurements of isolated islets of Langerhans. Curr Trends Biotechnol Pharm 2:66–84

Buchwald P, Wang X, Khan A, Bernal A, Fraker C, Inverardi L, Ricordi C (2009) Quantitative assessment of islet cell products: estimating the accuracy of the existing protocol and accounting for islet size distribution. Cell Transplant 18:1223–1235

Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A (2006) The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proc Natl Acad Sci U S A 103:2334–2339

Cantarelli E, Citro A, Marzorati S, Melzi R, Scavini M, Piemonti L (2013) Murine animal models for preclinical islet transplantation: no model fits all (research purposes). Islets 5:79–86

Carlson FD, Brink F Jr, Bronk DW (1950) A continuous flow respirometer utilizing the oxygen cathode. Rev Sci Instrum 21:923–932

Carter JD, Dula SB, Corbin KL, Wu R, Nunemaker CS (2009) A practical guide to rodent islet isolation and assessment. Biol Proced Online 11:3–31

Chadwick DR, Robertson GS, Rose S, Contractor H, James RF, Bell PR, London NJ (1993a) Storage of porcine pancreatic digest prior to islet purification. The benefits of UW solution and the roles of its individual components. Transplantation 56:288–293

Chadwick DR, Robertson GS, Toomey P, Contractor H, Rose S, James RF, Bell PR, London NJ (1993b) Pancreatic islet purification using bovine serum albumin: the importance of density gradient temperature and osmolality. Cell Transplant 2:355–361

Colton CK, Papas KK, Pisania A, Rappel MJ, Powers DE, O'Neil JJ, Omer A, Weir G, Bonner-Weir S (2007) Characterization of islet preparations. In: Halberstadt CR (ed) Cellular transplantation: from laboratory to clinic. Elsevier, Oxford, UK, pp 85–133

Conget JI, Sarri Y, González-Clemente JM, Casamitjana R, Vives M, Gomis R (1994) Deleterious effect of dithizone-DMSO staining on insulin secretion in rat and human pancreatic islets. Pancreas 9:157–160

De Duve C (1971) Tissue fraction-past and present. J Cell Biol 50:20

De Groot M, de Haan BJ, Keizer PP, Schuurs TA, van Schilfgaarde R, Leuvenink HG (2004) Rat islet isolation yield and function are donor strain dependent. Lab Anim 38:200–206

De Haan BJ, Faas MM, Spijker H, van Willigen JW, de Haan A, de Vos P (2004) Factors influencing isolation of functional pancreatic rat islets. Pancreas 29:e15–e22

Dionne KE, Colton CK, Yarmush ML (1993) Effect of hypoxia on insulin secretion by isolated rat and canine islets of Langerhans. Diabetes 42:12–21

Goto M, Holgersson J, Kumagai-Braesch M, Korsgren O (2006) The ADP/ATP ratio: a novel predictive assay for quality assessment of isolated pancreatic islets. Am J Transplant 6:2483–2487

Gotoh M, Maki T, Kiyoizumi T, Satomi S, Monaco AP (1985) An improved method for isolation of mouse pancreatic islets. Transplantation 40:437–438

Gotoh M, Maki T, Satomi S, Porter J, Monaco AP (1986) Immunological characteristics of purified pancreatic islet grafts. Transplantation 42:387–390

Holmes MA, Clayton HA, Chadwick DR, Bell PR, London NJ, James RF (1995) Functional studies of rat, porcine, and human pancreatic islets cultured in ten commercially available media. Transplantation 60:854–860

Jahr H, Bretzel RG, Wacker T, Weinand S, Brandhorst H, Brandhorst D, Lau D, Hering BJ, Federlin K (1995) Toxic effects of superoxide, hydrogen peroxide, and nitric oxide on human and pig islets. Transplant Proc 27:3220–3221

Jahr H, Pfeiffer G, Hering BJ, Federlin K, Bretzel RG (1999) Endotoxin-mediated activation of cytokine production in human PBMCs by collagenase and Ficoll. J Mol Med (Berl) 77:118–120

Janjic D, Wollheim CB (1992) Islet cell metabolism is reflected by the MTT (tetrazolium) colorimetric assay. Diabetologia 35:482–485

Juang JH, Kuo CH, Wu CH, Juang C (2008) Exendin-4 treatment expands graft β-cell mass in diabetic mice transplanted with a marginal number of fresh islets. Cell Transplant 17:641–647

Jun Y, Kim MJ, Hwang YH, Jeon EA, Kang AR, Lee SH, Lee DY (2013) Microfluidics-generated pancreatic islet microfibers for enhanced immunoprotection. Biomaterials 34:8122–8130

Kelly CB, Blair LA, Corbett JA, Scarim AL (2003) Isolation of islets of Langerhans from rodent pancreas. Methods Mol Med 83:3–14

Kemp CB, Knight MJ, Scharp DW, Lacy PE, Ballinger WF (1973) Transplantation of isolated pancreatic islets into the portal vein of diabetic rats. Nature 244:447

King A, Lock J, Xu G, Bonner-Weir S, Weir GC (2005) Islet transplantation outcomes in mice are better with fresh islets and exendin-4 treatment. Diabetologia 48:2074–2079

Lacy PE (1967) The pancreatic β cell. Structure and function. N Engl J Med 276:187–195

Lacy PE, Kostianovsky M (1967) Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 16:35–39

Latif ZA, Noel J, Alejandro R (1988) A simple method of staining fresh and cultured islets. Transplantation 45:827–830

Li DS, Yuan YH, Tu HJ, Liang QL, Dai LJ (2009) A protocol for islet isolation from mouse pancreas. Nat Protoc 4:1649–1652

Lindall A, Steffes M, Sorenson R (1969) Immunoassayable insulin content of subcellular fractions of rat islets. Endocrinology 85:218–223

London NJ, Swift SM, Clayton HA (1998) Isolation, culture and functional evaluation of islets of Langerhans. Diabetes Metab 24:200–207

McCall MD, Maciver AH, Pawlick R, Edgar R, Shapiro AM (2011) Histopaque provides optimal mouse islet purification kinetics: comparison study with Ficoll, iodixanol and dextran. Islets 3:144–149

Melzi R, Battaglia M, Draghici E, Bonifacio E, Piemonti L (2007) Relevance of hyperglycemia on the timing of functional loss of allogeneic islet transplants: implication for mouse model. Transplantation 83:167–173

Moore GE, Gerner RE, Franklin HA (1967) Culture of normal human leukocytes. JAMA 199:519–524

Morini S, Brown ML, Cicalese L, Elias G, Carotti S, Gaudio E, Rastellini C (2007) Revascularization and remodelling of pancreatic islets grafted under the kidney capsule. J Anat 210:565–577

Moskalewski S (1965) Isolation and culture of the islets of Langerhans of the guinea pig. Gen Comp Endocrinol 44:342–353

Nano R, Clissi B, Melzi R, Calori G, Maffi P, Antonioli B, Marzorati S, Aldrighetti L, Freschi M, Grochowiecki T, Socci C, Secchi A, Di Carlo V, Bonifacio E, Bertuzzi F (2005) Islet isolation for allotransplantation: variables associated with successful islet yield and graft function. Diabetologia 48:906–912

Nolan AL, O'Dowd JF (2009) The measurement of insulin secretion from isolated rodent islets of Langerhans. Methods Mol Biol 560:43–51

O'Dowd JF (2009) The isolation and purification of rodent pancreatic islets of Langerhans. Methods Mol Biol 560:37–42

Olsson R, Carlsson PO (2005) Better vascular engraftment and function in pancreatic islets transplanted without prior culture. Diabetologia 48:469–476

Papas KK, Colton CK, Nelson RA, Rozak PR, Avgoustiniatos ES, Scott WE, Wildey GM, Pisania A, Weir GC, Hering BJ (2007) Human islet oxygen consumption rate and DNA measurements predict diabetes reversal in nude mice. Am J Transplant 7:707–713

Perdrizet GA, Rewinski MJ, Bartus SA, Hull D, Schweizer RT, Scharp DW (1995) Albumin improves islet isolation: specific versus nonspecific effects. Transplant Proc 27:3400–3402

Piemonti L, Pileggi A (2013) 25 years of the Ricordi automated method for islet isolation. Cell R4 1:8–22

Piemonti L, Bertuzzi F, Nano R, Leone BE, Socci C, Pozza G, Di Carlo V (1999) Effects of cryopreservation on in vitro and in vivo long-term function of human islets. Transplantation 68:655–662

Piemonti L, Guidotti LG, Battaglia M (2010) Modulation of early inflammatory reactions to promote engraftment and function of transplanted pancreatic islets in autoimmune diabetes. Adv Exp Med Biol 654:725–747

Rabinovitch A, Quigley C, Russell T, Patel Y, Mintz DH (1982) Insulin and multiplication stimulating activity (an insulin-like growth factor) stimulate islet (β-cell replication in neonatal rat pancreatic monolayer cultures. Diabetes 31:160–164

Ramirez-Dominguez M, Castaño L (2014) Filtration is a time-efficient option to Histopaque providing good quality islets in mouse islet isolation. Cytotechnology. doi:10.1007/s10616-014-9690-7

Ricordi C, Lacy PE, Sterbenz K, Davie JM (1987) Low-temperature culture of human islets or in vivo treatment with L3T4 antibody produces a marked prolongation of islet human-to-mouse xenograft survival. Proc Natl Acad Sci U S A 84:8080–8084

Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW (1988) Automated method for isolation of human pancreatic islets. Diabetes 37:413–420

Ricordi C, Gray DW, Hering BJ, Kaufman DB, Warnock GL, Kneteman NM, Lake SP, London NJ, Socci C, Alejandro R et al (1990) Islet isolation assessment in man and large animals. Acta Diabetol Lat 27:185–195

Salvalaggio PR, Deng S, Ariyan CE, Millet I, Zawalich WS, Basadonna GP, Rothstein DM (2002) Islet filtration: a simple and rapid new purification procedure that avoids ficoll and improves islet mass and function. Transplantation 74:877–879

Scharp DW, Murphy JJ, Newton WT, Ballinger WF, Lacy PE (1975) Transplantation of islets of Langerhans in diabetic rhesus monkeys. Surgery 77:100–105

Shapiro AM, Hao E, Rajotte RV, Kneteman NM (1996) High yield of rodent islets with intraductal collagenase and stationary digestion–a comparison with standard technique. Cell Transplant 5:631–638

Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 343:230–238

Song Y, Wei Z, Song C, Xie S, Feng J, Fan J, Zhang Z, Shi Y (2013) Simulated microgravity combined with polyglycolic acid scaffold culture conditions improves the function of pancreatic islets. Biomed Res Int 2013:150739

Stull ND, Breite A, McCarthy R, Tersey SA, Mirmira RG (2012) Mouse islet of Langerhans isolation using a combination of purified collagenase and neutral protease. J Vis Exp. doi:10.3791/4137

Szot GL, Koudria P, Bluestone JA (2007) Murine pancreatic islet isolation. J Vis Exp (7)255

Vaithilingam V, Kollarikova G, Qi M, Larsson R, Lacik I, Formo K, Marchese E, Oberholzer J, Guillemin GJ, Tuch BE (2014) Beneficial effects of coating alginate microcapsules with macromolecular heparin conjugates-in vitro and in vivo study. Tissue Eng Part A 20:324–334

Venkatesan V, Chalsani M, Nawaz SS, Bhonde RR, Challa SS, Nappanveettil G (2012) Optimization of condition(s) towards establishment of primary islet cell cultures from WNIN/Ob mutant rat. Cytotechnology 64:139–144

Vos-Scheperkeuter GH, van Suylichem PT, Vonk MW, Wolters GH, van Schilfgaarde R (1997) Histochemical analysis of the role of class I and class II Clostridium histolyticum collagenase in the degradation of rat pancreatic extracellular matrix for islet isolation. Cell Transplant 6:403–412

Warnock GL, Rajotte RV, Evans MG, Ellis D, DeGroot T, Dawidson I (1987) Isolation of islets of Langerhans following cold storage of human pancreas. Transplant Proc 19:3466–3468

Wolters GH, van Suylichem PT, van Deijnen JH, van Schilfgaarde R (1990) Factors influencing the isolation process of islets of Langerhans. Horm Metab Res Suppl 25:20–26

Wolters GH, Vos-Scheperkeuter GH, van Deijnen JH, van Schilfgaarde R (1992) An analysis of the role of collagenase and protease in the enzymatic dissociation of the rat pancreas for islet isolation. Diabetologia 35:735–742

Wolters GH, Vos-Scheperkeuter GH, Lin HC, van Schilfgaarde R (1995) Different roles of class I and class II Clostridium histolyticum collagenase in rat pancreatic islet isolation. Diabetes 44:227–233

Yesil P, Michel M, Chwalek K, Pedack S, Jany C, Ludwig B, Bornstein SR, Lammert E (2009) A new collagenase blend increases the number of islets isolated from mouse pancreas. Islets 1:185–190

Zmuda EJ, Powell CA, Hai T (2011) A method for murine islet isolation and subcapsular kidney transplantation. J Vis Exp. doi:10.3791/2096

Regulation of Pancreatic Islet Formation

6

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Contents

Abstract

Pancreatic islets are complex structures formed by five different hormone-expressing cells surrounded by endothelial cells, nerves, and fibroblasts. Dysfunction of insulin-producing cells (β-cells) causes diabetes. Generation of β-like cells that can compensate the loss of β-cell mass in type 1 diabetes or defects in β-cell insulin secretion in type 2 diabetes is a current challenge in biomedicine. The knowledge of the molecular basis governing pancreas development and islet formation will help us to generate in vitro or in vivo

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_36,

β-like cells to treat diabetes. Pancreas development is a highly complicated process, which is regulated by signaling pathways, transcription factors, nutrients, and other environmental factors. Collectively, these signals and factors act coordinated, in a spatial and temporal manner, throughout the embryonic pancreas. In this review we will summarize the main steps in pancreas development and will highlight the key transcription factors that have been shown to play essential roles in pancreas specification, maintenance of multipotent pancreatic progenitors, endocrine differentiation, and islet maturation. We will also discuss the role of microRNAs (miRNAs) in regulating islet cell fate.

Keywords
Endocrine progenitor cells • Transcription factors • Signaling pathways • Development • Differentiation • Gene regulatory networks

Introduction

Blood glucose homeostasis in adult mammals is maintained by the islets of Langerhans lodged within the exocrine tissue of the pancreas. Islets comprise approximately 2 % of the pancreas. Pancreatic islets contain several different cell types, including endocrine cells, endothelial cells, nerves, and fibroblasts. Rodent pancreatic islets house three main cell types, each of which produces a different endocrine product: (i) β-cells, which make up 60–70 % of the islets and release insulin; (ii) α-cells (15–20 %), which secrete glucagon; and (iii) δ-cells (5–10 %), which produce somatostatin. Minor cell types, which secrete a number of other peptides, make up about 5 % of the islets. These cells are pancreatic polypeptide-producing PP cells and ghrelin-producing cells, termed ε-cells (Cabrera et al. 2006; Steiner et al. 2010). Interestingly, cell composition and spatial organization within an islet vary among species. The prototypic islet has β-cells forming a core surrounded by other endocrine cells in the periphery and corresponds to normal rodent islets. However, in human islets, α-, β-, and δ-cells appear to be randomly distributed throughout the islet and the composition differs to that of rodents: 50 % of β-cells, 40 % of α-cells, 10 % of δ-cells, and few PP cells (Cabrera et al. 2006). Variation in islet structure between species may result from different developmental mechanisms.

Diabetes mellitus (DM), characterized by hyperglycemia, stems from defects in insulin secretion, insulin action, or both. The vast majority of cases of DM fall into two broad etiopathogenetic categories: type 1 and type 2 DM (T1DM and T2DM). In the case of T1DM, the cause is an absolute deficiency of insulin secretion due to a cellular-mediated autoimmune destruction of β-cells. Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. This form of diabetes accounts for only 5–10 % of cases. For T2DM, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. This form of DM is much

more prevalent and accounts for 90–95 % of patients with the disease. T2DM is considered a complex polygenic disorder in which common genetic variants interact with environmental factors (mainly lifestyle) to unmask the disease. Minor forms of DM include maturity-onset diabetes of the young (MODY) characterized by mutation in genes that will cause defects in insulin secretion and general β-cells dysfunction. This monogenetic form of DM is frequently characterized by an early onset of hyperglycemia generally before the age of 25 years.

To date, treatments for T2DM include insulin sensitizers and secretagogues as well as exogenous insulin therapy, while the latter is mandatory for T1DM. Pancreas/islet transplantation has been successfully used for the treatment of T1DM, but the shortage of pancreatic islets donors has motivated efforts to develop alternative renewable sources of β-cells (Soria et al. 2008). A promising approach has been the differentiation of embryonic/adult stem cells into β-cells.

Although success in generating insulin-producing cells from stem cells has been mitigated and even controversial, reports from the past decade do confirm a slow but promising progression toward producing such cells from a variety of stem cell sources such as embryonic stem cells, adult stem cells (pancreatic and non-pancreatic), induced pluripotent stem cells, and endocrine precursor or progenitor cells (Bonner-Weir et al. 2000; Soria et al. 2000; Ianus et al. 2003; Runhke et al. 2005; Kroon et al. 2008; Xu et al. 2008; Zhang et al. 2009; Thorel et al. 2010; Dave et al. 2013). Nevertheless, islets are intricate miniorgans that comprise several cell types in addition to β-cells and which most likely play an important role. In this context, it has been demonstrated that isolated pancreatic β-cells are less efficient than pancreatic islets releasing insulin indicating that a functional architecture is essential to integrate response to nutrients (Soria et al. 2010). Thus, β-cells and non-β-cells are organized in islets in close intimacy to a dense vascularization and innervation that responds not only to glucose and other nutrients but also to hormones, neurotransmitters, and paracrine factors. In order to reproduce physiological blood glucose control, tissue engineering should consider the generation of pancreatic islets, more than β-cells alone.

In order to address this important issue, one needs to better understand how pancreatic islets development and formation proceed. Tremendous progress has been achieved in our understanding of transcription factors that govern the embryonic development of the pancreas and islet cell formation. Advances have also been made in characterizing the role of environmental factors in pancreatic islet development (Dumortier et al. 2007; Guillemain et al. 2007; Heinis et al. 2010). More recently, miRNA and epigenetics have emerged as important contributors of pancreas development and cell fate decisions during endocrine cell development.

The majority of proposed models on human pancreas development are derived from animal models, mainly mice. This is based on the assumption that the molecular and cellular aspects of pancreas development are conserved, although some aspects may differ. In addition, the development of pancreatic islets and the differentiation of its five cell types are very complex and tightly regulated process. Hence, there is still much to be learned regarding the transcription factors and epigenetic mechanisms underlying islet cell differentiation. In this chapter, we will discuss the

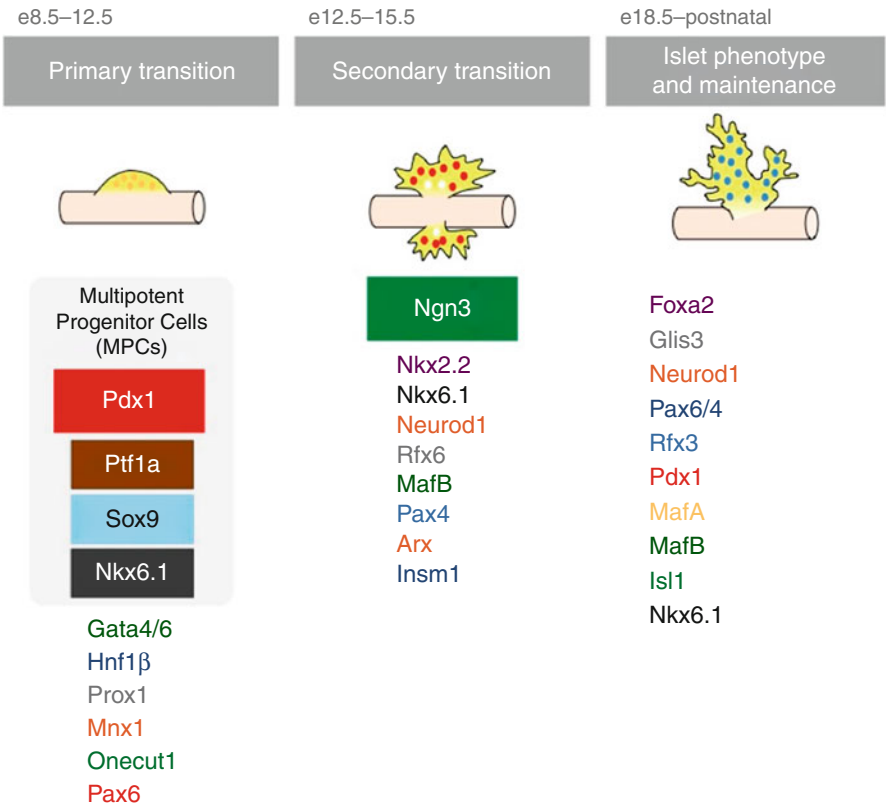

Fig. 1 Schematic representation showing some of the key transcription factors important for the mouse pancreatic islet formation

current knowledge of transcription factors that play a key role in pancreatic islet development and differentiation. The chapter will focus mainly on pancreatic islet cells and pancreas embryogenesis and organogenesis will be briefly discussed.

Overview of Pancreas Organogenesis and Pancreatic Islet Cell Differentiation

Pancreas development is a highly complex process, which is regulated by several signaling interconnected systems. The pancreas derives from definitive endoderm. Pancreatic developmental stages are classified as primary, secondary, and tertiary transitions (Pictet et al. 1972) (Fig. 1). Specification of definitive endoderm toward pancreatic fate (primary transition) occurs from embryonic day (e) 8.5–12.5 in mice. From the gut tube endoderm, two buds emerge (dorsal and ventral) and grow into the visceral mesoderm (Villaseñor et al. 2008). At e8.5, duodenal homeobox

factor-1 (Pdx1)-expressing multipotent progenitor cells appear in the ventral bud and subsequently in the dorsal pancreas, as well as in other locations (caudal stomach and proximal duodenum) (Jonsson et al. 1994). Already by e9.5 glucagon-expressing cells are detected. Insulin-positive cells, co-expressing glucagon, are seen at e10.5 (Herrera et al. 1991). In some studies, these cells are called the first wave or protodifferentiated β-cells. By e10.5 the gut tube rotates resulting in the fusion of both the dorsal and ventral buds (Slack 1995). Ghrelin-expressing cells can be detected at this time (Prado et al. 2004).

During the second transition period (e13.5 to E16.5), the pancreatic epithelium branches extend into the mesoderm forming ductal short fingerlike lobules (Villaseñor et al. 2010). This branching extends until birth forming the bulk of the pancreas. This period is critical for the formation of the exocrine and endocrine cells. Between e12.5 and E14.5, two domains of cells appear (trunk and tip). From the trunk will originate endocrine and ductal cells, while the tip domain will give rise to acinar cells (Zhou et al. 2007; Solar et al. 2009; Schaffer et al. 2010). From e13.5 onward a second wave of hormone-expressing cells including glucagon-, insulin-, somatostatin-, ghrelin-, and PP-positive cells will occur (Herrera et al. 1991; Prado et al. 2004). Thereafter, at e15.5 the endocrine precursors delaminate from the pancreatic epithelium and remaining cells within epithelium differentiate to form the exocrine compartment of the pancreas (Puri and Hebrok 2010). Migration of differentiated endocrine cells and final formation of the islets of Langerhans take place during the tertiary transition (e16.5 to birth). During this period, endocrine precursor cells migrate and coalesce into small islet-like clusters. Then, the clusters progressively receive more and more cells and proliferate into larger endocrine aggregates. It seems that islet cell proliferation extends throughout the life span, although at much lower rate, except in response to increased metabolic demands (Bonner-Weir et al. 2010).

In humans the first transition period occurs 2–3 weeks after blastocyst formation. A dorsal and a ventral outgrowth are already visible at days 25–26 of gestation (Piper et al. 2002) and a pancreas is observed at sixth week of gestational age (wGA) (Like and Orci 1972). The second transition period proceeds from 8 to 11 wGA (Polak et al. 2000). The critical window of differentiation of endocrine cells is from 9 to 23 wGA (Sarkar et al. 2008). Glucagon cells are found at 7 wGA (Assan and Biollot 1973), followed by insulin, somatostatin, and PP cells at 8–10 wGA (Stefan et al. 1983). Similarly to mice, a subpopulation of primitive endocrine cells that co-express insulin, glucagon, and somatostatin is detected at 8 wGA. This subpopulation has a low proliferation rate (Beringue et al. 2002). Endocrine differentiation in the human fetal pancreas also takes place in the islands of epithelial tissue but within a much larger volume of mesenchymal tissue (Sugiyama et al. 2007). In humans, small islet-like clusters appear at 11 wGA which become highly vascularized by 20–23 wGA (Jeon et al. 2009). The peak glucagon-positive cell proliferation occurs at 20 wGA and at 23 wGA for insulin and somatostatin cells (Sarkar et al. 2008). Intensive cell proliferation will continue after birth and during the perinatal period with the subsequent final generation of pancreatic islets. In contrast to rodents, human fetuses have functional islets able to develop nutrient-induced insulin release (Nicolini et al. 1990).

Transcription Factors Involved in Pancreas Specification and Multipotent Pancreatic Progenitors

Most of our understanding about pancreas development has arisen from studies in animal models where several transcription factors have been genetically manipulated. Here we will review some of the key transcription factors that have been shown to play crucial roles in determining the pancreatic fate and generating pancreatic progenitors.

Within the primitive gut tube, the pancreatic domain is defined by the overlapping expression of Pdx1 and pancreas-specific transcription factor 1a (Ptf1a) (Chiang and Melton 2003; Kawaguchi et al. 2002). Pdx1 expression is first observed at e8.5 in the prepancreatic endoderm and its expression becomes restricted to pancreatic endocrine cells just before birth. Ptf1a is first expressed at e9.5 and its expression becomes restricted to acinar precursor cells. Pdx1 and Ptf1a play multiple roles at different stages of embryonic pancreas and in the adult pancreas. Inactivation of either Pdx1 or Ptf1a in mice leads to pancreas agenesis. Similarly, mutations in Pdx1 or Ptf1a genes cause severe pancreatic hypoplasia in humans (Kawaguchi et al. 2002; Ahlgren et al. 1996; Offield et al. 1996). Based on the early expression of these two transcription factors and the dramatic phenotype of Pdx1 and Ptf1a knockout mice, it has been proposed that the combination of both transcription factors determines the pancreatic fate within the foregut endoderm. However, recent studies of Pdx1 and Ptf1a double knockout mice have shown that in the absence of these two transcription factors, the pancreas is specified and a pancreatic rudiment is formed (Burlison et al. 2008), suggesting that other transcription factors also might contribute to determine the pancreatic fate.

Recent studies have shown that two members of the GATA zinc finger transcription factor family, GATA4 and GATA6, are crucial for pancreas development in mice (Carrasco et al. 2012; Xuan et al. 2012). GATA4 and GATA6 are expressed in the foregut endoderm prior to Pdx1 and Ptf1a expression. Given the early expression of GATA factors in the prepancreatic endoderm, it has been suggested that they might be potential candidates for pancreas specification. Indeed, GATA4 and GATA6 might even play redundant roles in pancreas formation in mice. Analysis of double GATA4/GATA6 knockout mice revealed pancreatic agenesis and a dramatic decrease in the expression levels of Pdx1 and Ptf1a. Furthermore, transgenic mice analysis and ChIP analysis have shown that GATA sites in the Pdx1 area III conserved region are required for Pdx1 transcriptional activity, indicating a direct regulation of Pdx1 by GATA factors (Carrasco et al. 2012; Xuan et al. 2012). However, initial pancreas formation also occurs in the absence of GATA4 and GATA6, indicating that these two transcription factors are also dispensable for pancreas specification (Xuan et al. 2012). Other transcription factors that are expressed in the prepancreatic endoderm, such as motor and pancreas homeobox 1 (Mnx1), hematopoietically expressed homeobox (Hhex), hepatocyte nuclear factor-1-β (Hnf1b), and the SRY-box containing HMG transcription factor Sox17, might play a role in pancreas specification. However, it is plausible that organ specification is not achieved by a single transcription factor, but by a combination of transcription factors.

Following pancreas specification, a massive proliferation of undifferentiated cells, known as multipotent pancreatic progenitor cells (MPCs), forms the pancreatic bud. The MPCs within the pancreatic epithelium expand and branch to form a ductal tree. These morphological changes in the pancreatic epithelial occur between e9.5 and e12.5 in mice. Lineage tracing analyses have shown that MPCs retain the potential to give rise to all pancreatic lineages (acinar, ducts, and endocrine) until e12.5 (Solar et al. 2009; Kawaguchi et al. 2002; Zhou et al. 2007; Kopinke and Murtaugh 2010; Kopinke et al. 2011). The transcription factors that have been shown to be critical for the formation and maintenance of MPCs include Pdx1, Ptf1a, SRY (sex-determining region Y)-box 9 (Sox9), prospero homeobox 1(Prox1), Mnx1, onecut homeobox 1 (Onecut1), Hnf1b, and GATA4/GATA6. Many of these transcription factors regulate each other and their own expression to form a specific cross-regulatory network. Thus Pdx1 is directly activated by forkhead box A2 (Foxa2), GATA4, GATA6, hepatocyte nuclear factor 6 (Hnf6), and Ptf1a. Sox9 regulates the expression of other MPC genes like Hnf1b, Hnf6, and FoxA2. Several MPC genes, like Nk homeobox protein 6.1 (Nkx6.1), Hnf6, and Mnx1, have been described as direct targets of Ptf1A. This cross-regulatory network during early formation ensures the proliferation, expansion, and identity of the MPCs that are required for the normal progression of pancreas development (Fig. 1).

Endocrine Commitment and Islet Differentiation

Between e12.5 and e15.5 in mouse pancreas development, a period known as secondary transition is characterized by a massive wave of endocrine and exocrine cell differentiation (Pictet and Rutter 1972). During these pancreatic developmental stages, the pancreatic epithelium is well defined into two domains, tip and trunk, to generate acinar progenitor cells and endocrine/ductal progenitor cells, respectively (Zhou et al. 2007; Solar et al. 2009; Kopp et al. 2011). These two domains are also delimitated by the expression of specific transcription factors. Thus, Ptf1a is specifically expressed in the tip cells, whereas Sox9, Hnf1b, and Nkx6.1 are expressed in the trunk domain (Kopp et al. 2011; Schaffer et al. 2010; Solar et al. 2009; Zhou et al. 2007; Hald et al. 2008). The mechanism that controls this lineage allocation is not clear yet, but studies have shown that Ptf1a and Nkx6 factors (Nkx6.1 and Nkx6.2) mutually antagonize each other to specify either the exocrine or ductal/endocrine fate, likely via repression of Ptf1a expression by Nkx6 proteins (Schaffer et al. 2010). In the secondary transition, scattered cells within the trunk transiently express the master regulator of endocrine commitment neurogenin 3 (Ngn3) (Fig. 1).

Ngn3 expression is first observed at e9.5. Its expression peaks during the secondary transition and decreases at later stages of pancreas development (Schwitzgebel et al. 2000). Inactivation of Ngn3 in mice causes loss of all endocrine cell types, but the exocrine pancreas is properly formed (Gradwhol et al. 2000). A complex cross-regulatory transcription factor network directly regulates Ngn3 activation. This transcription factor network includes Foxa2, GLI-similar zinc finger protein 3 (Glis3), Hnf1b, Pdx1, and Sox9 (Arda et al. 2013; Lynn et al. 2007a;

Oliver-Krasinski et al. 2009; Ejarque et al. 2013; Lee et al. 2001; Yang et al. 2011). Lineage tracing studies of Ngn3-positive cells have determined that they are allocated to a single endocrine cell lineage. Therefore, Ngn3-positive cells are unipotent endocrine precursors (Desgraz and Herrera 2009). Still it is not clear how Ngn3-expressing precursors are instructed to specific islet cell fate, but it has been suggested that the timing of Ngn3 activation in precursor cells can determine the endocrine cell type formed (Johansson et al. 2007). Thus, Ngn3 expression induced in early pancreas development promotes the formation of α-glucagon-producing cells. The induction of Ngn3 expression from e11.5 onward promotes the generation of β- and PP cells. From e14.5 onward, Ngn3-expressing cells become competent to generate δ-cells, whereas the competence to form α-cells markedly diminishes (Fig. 1).

A number of transcription factors downstream of Ngn3 have been shown to play important roles in endocrine cell type differentiation, like neuronal differentiation 1 (Neurod1), paired box gene 4 (Pax4), insulinoma-associated 1 (Insm1), Nk homeobox protein 2.2 (Nkx2.2), and myelin transcription factor 1 (Myt1) (Smith et al. 2003; Mellitzer et al. 2006; Huang et al. 2000; Watada et al. 2003; Gasa et al. 2004; Wang et al. 2008; Smith et al. 2010; Arda et al. 2013), although in some cases the direct activation of their promoter by Ngn3 has not yet been established. Defects in islet formation have recently been described in mice lacking the transcription factor regulatory X-box binding 6 (Rfx6). Rfx6 is broadly expressed in the gut endoderm including the nascent pancreatic bud at e9.5. From e10.5, Rfx6 is only found in endocrine cells and its expression persists in adult islet cells (Smith et al. 2010; Soyer et al. 2010). Rfx6 mutant mice display a dramatic reduction in all endocrine precursor cell types except PP cells. Other transcription factors whose inactivation produces differential loss of endocrine cell types are Pax4, aristaless-related homeobox (Arx), and Nkx-homeodomain factors.

Three Nkx genes are expressed from early stages of pancreas development; however, their function in multipotent pancreatic progenitors is not clear yet as they seem to be dispensable for early pancreas formation. Nkx6.1 and Nkx6.2 play partial redundant roles in endocrine formation (Sussel et al. 1998; Sander et al. 2000; Henseleit et al. 2005). Nkx6.1/Nkx6.2 double mutants have reduced the number of α- and β-cells. However, in Nkx6.1 only β-cell formation is affected. Nkx6.2 null mice have no obvious defects in pancreas formation and islet differentiation (Henseleit et al. 2005). Inactivation of Nkx2.2 causes total loss of β-cells and a reduced number of α- and PP cells (Sussel et al. 1998). A recent study has shown a genetic interaction between Nkx2.2 and Neurod1 in the specification of endocrine cell lineages. Activation of Neurod1 by Nkx2.2 is required for β-cell formation, while Nkx2.2 repress Neurod1 in order to properly allocate α-cells (Mastracci et al. 2013).

The opposing activities of Pax4, an important β-cell differentiation transcription factor, and Arx, a key factor in α-cell specification, are another example of regulation of fate choice between α- and β-cells (Sosa-Pineda et al. 1997; Collombat et al. 2003). During endocrine differentiation, Pax4 and Arx expression becomes restricted to β- and α-cells, respectively. Pax4 inactivation causes loss of β- and δ-cells (Collombat et al. 2003). Ectopic expression of Pax4 and Arx in

endocrine progenitor cells induces β- and α-cell formation (Collombat et al. 2003, 2007, 2009). Thus, Pax4 promotes β- and δ-cell fate, while Arx promotes α-cell fate at the expense of β- and δ-cell fate. The antagonist interaction between these two transcription factors might be mediated by reciprocal repression at the transcriptional level (Collombat et al. 2003, 2007). Arx also has an antagonist relationship with Nkx6.1 in determining endocrine fate choice. Misexpression of Nkx6.1 in endocrine progenitor cells promotes β-cell formation at the expense of the α-cell lineage (Schaffer et al. 2013), while ectopic expression of Arx, as discussed above, results in the opposite alteration. It is important to note that maintenance of endocrine cell identity requires not only the activation of specific genes for a particular cell lineage but also the repression of other genes. This statement is well illustrated in the study in which inactivation of Nkx2.2 specifically in β-cells causes β- to α-cell transdifferentiation due to derepression of Arx (Papizan et al. 2011).

Pdx1 might also play an important role in the commitment of β-cell fate choice. Enforced expression of Pdx1 in endocrine progenitors induces β-cell formation and decreases the number of α-cells (Yang et al. 2011). In agreement with the notion of Pdx1 as regulator of β-cell fate, the inactivation of Pdx1 in embryonic β-cell produces an increase in α-cells (Gannon et al. 2008).

In summary, the studies described above illustrate the plasticity of different endocrine cell types as a result of forced expression of lineage-specific transcription factors. These results might be relevant in reprogramming strategies to obtain new sources of β-cells for diabetes therapies.

Maintenance of Islet Cell Identity and Function

From e16.5 onward, the endocrine cells coalesce into clusters of different cells to generate the pancreatic islets. A significant number of transcription factors have been shown to be required for the terminal cell differentiation and maintenance of islet function, including Foxa2, Glis3, Neurod1, paired box gene 6 (Pax6), transcription factor regulatory X-box binding 3 (Rfx3), Pdx1, v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA), v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MafB), and ISL LIM homeobox 1 (Isl1) as shown by inactivation analyses of these genes in differentiated islets. Thus, ablation of Isl1 prior to secondary transition results in the reduction of mature endocrine cell number (Du et al. 2009). Inactivation of Pdx1 in adult islet cells causes loss of β-cell mass, downregulation of amylin/IAPP, loss of insulin expression, and downregulation of Glut2 (Ahlgren et al. 1998; Lottmann et al. 2001; Thomas et al. 2001; Holland et al. 2005). Ablation of Neurod1 after differentiation resulted in glucose intolerance, decreased insulin release, and reduced insulin 1 (Ins1) expression (Gu et al. 2010) (Fig. 1).

MafA and MafB transcription factors determine the degree of β-cell maturation, as MafB is required during β-cell development and MafA is crucial for mature β-cell function (Nishimura et al. 2006). MafB is expressed at e12.5 in both insulin- and glucagon-positive cells and its expression becomes restricted to α-cells at postnatal stages (Artner et al. 2006). MafB ablation produces a decrease in the

number of insulin- and glucagon-positive cells and the appearance of insulin-positive cells is delayed (Artner et al. 2007). Loss of MafB is associated with downregulation of transcription factors necessary for β-cell maturation and function such as Pdx1, MafA, Nkx6.1, and Glut2 at late stages of development. However, the expression levels of these genes are still normal until e15.5 (Artner et al. 2007; Nishimura et al. 2008). Several studies have shown that MafB is essential for insulin and glucagon transcriptional activation and it is an important regulator of β-cell maturation genes, such as Slc2a2 (glucose sensing), Slc30a8 (vesicle maturation), Camk2b (Ca^{2+} signaling), and Nnat (insulin secretion) (Artner et al. 2010).

MafA is a β-cell-specific transcription factor that interacts with Pdx1 and Neurod1 to activate β-cell genes, including insulin (Aramata et al. 2007; Wang et al. 2007). MafA is expressed during pancreas development and its expression restricts to insulin-positive cells from e13.5 onward (Matsuoka et al. 2004). MafA seems to be dispensable for pancreas organogenesis, as $MafA^{-/-}$ mice and pancreas-specific MafA mutant mice do not have any obvious defect in pancreas formation. However, MafA null mice display aberrant adult islet architecture and defects in β-cell function (Zhang et al. 2005; Artner et al. 2010), indicating that MafA activity might be important exclusively in adult β-cells. It has been hypothesized that MafA expression levels could be a sensitive indicator of the functionality of β-cells, as changes in glucose levels regulate MafA activity (Raum et al. 2006).

miRNA Expression During Islet Cell Development and Epigenomic Phenomena

In recent years miRNAs have emerged as novel regulators of β-cell development and function. miRNAs are single-stranded RNA molecules ranging in size from 18 to 22 nucleotides. The mammalian genome encodes for several hundred miRNAs that fine-tune gene expression through modulation of target mRNAs (Ambros 2004). miRNAs play a fundamental role in regulating gene expression in key biological events such as cell proliferation, differentiation, death, and malignant transformation (Bartel 2004). In addition, miRNAs seem to have a major role during embryonic development (Tang et al. 2007). The role of miRNAs during embryogenesis is particularly apparent in knockout mice lacking one of several key miRNA-processing genes such as Dicer, DiGeorge syndrome critical region gene 8 (Dgcr8), Drosha, or argonaute RISC catalytic component 2 (Ago2). Indeed, these knockout mice die during early gestation with severe developmental defects (Bernstein et al. 2003; Morita et al. 2007).

miRNAs appear to be critical for pancreas development. To date, 125 miRNAs have been shown to be involved in pancreatic development by regulating ductal, exocrine, and endocrine pancreatic pathways (Lynn et al. 2007b; Table 1). Specific deletion of Dicer in pancreatic progenitors produces defects in all pancreatic lineages and has a major impact in endocrine β-cells. The endocrine defect was associated with an increase in the notch-signaling target hairy and enhancer of split 1 (Hes1) and a reduction in the formation of endocrine cell progenitors expressing

Table 1 miRNAs involved in pancreas development. Partial list of miRNAs proved to be necessary for pancreas development, together with their corresponding targeted transcription factors, in a temporal fashion

miRNAs	Pancreatic organogenesis	Transcription factors
miR-124a, miR-23b	Primary transition	Hnf3b, Hlxb9, Pdx1, Hes1, Isl1
miR-15a, miR-15b, miR-195, miR-16, miR-503, miR-541, miR-214	Secondary transition	Hnf6, Ngn3, NeuroD
–	Tertiary transition	–
miR-9, miR-375	Maturation and maintenance	Ptf1a, insulin release

the Hes1 target gene Ngn3. However, when Dicer was disrupted specifically in differentiated β-cells using the RIP-Cre mouse line, only small effects on pancreatic islet cell morphology and no apparent changes in β-cell mass and function were found (Kalis et al. 2011). Another miRNA involved in islet cell development is miRNA124α. It has been shown that miRNA124α regulates Foxa2 gene expression and that of its targets Pdx1, Kir6.2, and Sur1 (Baroukh et al. 2007). The last three genes also have important roles in glucose metabolism and insulin secretion. miRNA23b has been proposed to be involved in Hes1 regulation (Kimura et al. 2004) which tightly controls the number of Ngn3-producing cells. miR-15a, miR-15b, miR-16, and miR-195 also have important roles in regulating translation of Ngn3 in adult mice. These miRNAs are expressed at least 200-fold higher in the regenerating mouse pancreas as compared to E10.5 or E16.5 developing mouse pancreas. Moreover, overexpression of the mentioned miRNAs shows reduction in the number of hormone-producing cells (Joglekar et al. 2007).

An important miRNA for islet development is miR-375. Morpholino blockage of miRNA375 causes defects in the morphology of the pancreatic islet, in zebra fish. In this animal model, miR-375 is essential for formation of pancreatic islet and its knockdown results in dispersed pancreatic islets in later stages of embryonic development. Of note miRNA375 is conserved between zebra fish and mammals (Kloosterman et al. 2007). In addition, during pancreas organogenesis, miR-375 exhibits increased expression occurring together with augmented insulin transcript expression and β-cell proliferation (Jogeklar et al. 2009). Moreover, it has been found that pancreatic islet-specific expression of miR-375 is regulated, in part, at the transcriptional level, because a region in the promoter of miR-375 contains consensus-binding sequences for Hnf6 and Insm1 (Avnit-Sagi et al. 2009). Finally, chromatin immunoprecipitation experiments have shown that NeuroD1 interacts with conserved sequences both upstream and downstream of the miR-375 gene and Pdx1 also interacted with the upstream region of the miR-375 gene (Keller et al. 2007). All these findings indicate that miR-375 gene is a target for key pancreatic transcription factors. Finally, miRNAs have also been involved in human pancreatic islet development (Van de Bunt et al. 2013). In this regard, four different islet-specific miRNAs (miR-7, miR-9, miR-375, and miR-376) have been found expressed at high levels during human pancreatic islet development (Correa-Medina et al. 2009; Joglekar et al. 2009).

The knowledge gain on the functional role of miRNA375 in pancreatic islet development has resulted in the design of a protocol capable to generate islet cells from human embryonic stem cells (hESCs) into islet cells by overexpressing miR-375, in the absence of any extrinsic factors. The authors transduced hESCs with lentiviral vectors containing human miR-375 precursor and aggregated to form human embryoid bodies for up to 21 days. The differentiated cells obtained expressed Foxa2, HNF4α, Pdx1, Pax6, Nkx6.1, Glut2, and insulin. Insulin-positive cells were observed by immunohistochemistry. Moreover, they were able to detect insulin release upon glucose stimulation (Lahmy et al. 2013). Liao et al. (2013) have found that when hESCs are differentiated to insulin-producing cells, using a specific differentiation protocol, cells possessed distinct miRNA signatures during early and late stages of the differentiation process. They validate the functional roles for miR-200a in regulating definitive endoderm specification during early stages of differentiation. Moreover, they verified that miR-30d and let-7e regulate the expression of Rfx6. Finally, they identify critical miRNA–mRNA interactions occurring during the differentiation process. Another study followed the dynamic expression of miRNAs during the differentiation of hESCS into insulin-producing cells. This expression was compared with that in the development of human pancreatic islets. It was found that the dynamic expression patterns of miR-375 and miR-7 were similar to those seen in the development of human fetal pancreas, whereas the dynamic expression of miR-146a and miR-34a showed specific patterns during the differentiation. Furthermore, the expression of Hnf1β and Pax6, the predicted target genes of miR-375 and miR-7, was reciprocal to that of miR-375 and miR-7 (Wei et al. 2013).

Thus, there exist abundant data indicating that miRNAs are important in regulating ductal, exocrine, and endocrine development. Furthermore, some authors suggest that miRNA could mediate silencing of Ngn3 thereby favoring β-cell regeneration (Joglekar et al. 2007). The identification of miRNA targets and understanding the miRNA–mRNA interactions are key for elucidating the mechanisms of miRNA function in pancreas development.

The cascade of transcription factors that directs the differentiation of the foregut endoderm into the mature pancreatic islets has slowly emerged in the last decade. More recent studies have established a role of epigenetic mechanisms in cell fate decisions during endocrine pancreas development. Epigenetic events refer to modification of DNA which cause changes to the function and/or regulation of DNA. Epigenetic marks control the expression of genes that function in embryonic development, and other epigenetic programming events can happen. Recently, it has been shown that epigenetic processes contribute to the control of the transcriptional hierarchy that regulates gene expression during development, involving both histone modifications and DNA methylation and leading to facilitate or prevent recruitment of effector protein complexes (Avrahami and Kaestner 2012).

Genome-wide epigenetic studies of human pancreatic islets, using chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-Seq analysis),

has found the presence of some bivalent marks in developmental regulatory genes of adult human islets (Barski et al. 2007; Heintzman et al. 2009). Transcription regulation involves an "open chromatin" structure. One way to study the involvement of chromatin structure in gene regulation is to define these open and closes regions and identity active DNA regulatory regions using Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE) technology. With this technology coupled to high-throughput sequencing, two research groups have identified sites of open chromatin, in human pancreatic islets, at islet-specific genes involved in islet cell development, such as Pdx1, NeuroD, Nkx6.1, Arx, and Isl1 (Gunton et al. 2005; Gaulton et al. 2010). In this regard, a recent study (Papizan et al. 2011) has shown that in β-cells, Nkx2.2 is part of a repression complex, together with DNMT3a, a de novo DNA methyltransferase important for establishing methylation patterns during development, the groucho-related repressor Grg3, and the histone deacetylase HDAC1. Analysis of the methylation profiles of endocrine cell fate determination genes identified CpG-rich areas in the regulatory region of Arx. Bisulfite-sequencing analysis of FACS-purified α- and β-cells revealed that one of the CpG-rich areas in the Arx promoter is hypermethylated in β-cells, but hypomethylated in α-cells (Collombat et al. 2005, 2007). Finally, using β-cell-specific ablation of the DNMT1 gene, a DNA methyltransferase that restores CpG methylation pattern after DNA replication in S-phase of the cell cycle suggests a possible role for DNA methylation in regulating β-cell identity (Dhawan et al. 2011). Thus, it is likely that epigenomic phenomena are involved in fine-tuning development and function of pancreatic cell types.

Concluding Remarks

To date, the generation of fully functional islet cells from embryonic and adult stem cells or progenitor cells has yet to be achieved. This suggests that our knowledge of the transcription factors, microRNAs, and epigenetic marks coordinating in islet cell development is far from complete. Further dissection of the transcriptional network orchestrating pancreatic islet development and how miRNAs and epigenetic alterations influence this network is the next challenge in order to develop more robust in vitro differentiation protocols.

Acknowledgments We thank members of the Stem Cell and Cell Therapy and Regenerative Medicine Departments from CABIMER for stimulating discussions on diabetes cell therapy and pancreas development. A. R. is supported by a grant from ISCIII co-funded by Fondos FEDER (PI11/01125). M. C. is supported by a predoctoral fellowship from Spanish Ministry of Education. I. D. is supported by a contract from Consejería de Salud (Junta de Andalucía, PI00-0008 to A. R.). B. R. G. is supported by grants from the Consejeria de Salud, Fundacion Publica Andaluza Progreso y Salud, Junta de Andalucia (PI-0727-2010), Instituto de Salud Carlos III co-funded by Fondos FEDER (PI10/00871) and by the Juvenile Diabetes Research Foundation (17-2013-372). FM is supported by grants from Junta de Andalucía (BIO-311). We apologize to colleagues whose work could not be cited because of space constraints.

Cross-References

- (Dys)Regulation of Insulin Secretion by Macronutrients
- Electrical, Calcium, and Metabolic Oscillations in Pancreatic Islets
- Human Islet Autotransplantation
- Immunology of β-Cell Destruction
- Pancreatic β Cells in Metabolic Syndrome
- Stem Cells in Pancreatic Islets
- The comparative Anatomy of Islets
- Wnt Signaling in Pancreatic Islets

References

Ahlgren U, Jonsson J, Edlund H (1996) The morphogenesis of the pancreatic mesenchyme is uncoupled from that of the pancreatic epithelium in IPF1/PDX1-deficient mice. Development 122:1409–1416

Ahlgren U, Jonsson J, Jonsson L, Simu K, Edlund H (1998) β-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the β-cell phenotype and maturity onset diabetes. Genes Dev 12:1763–1768

Ambros V (2004) The function of animals micro RNAs. Nature 431:350–355

Aramata S, Hang SI, Kataoka K (2007) Roles and regulation of transcription factor MafA in islet β-cell. Endocr J 54:659–666

Arda HE, Benitez CM, Kim SK (2013) Gene regulatory networks governing pancreas development. Dev Cell 25(1):5–13

Artner I, Le Lay J, Hang Y, Elghazi L, Schisler JC, Henderson E, Sosa-Pineda B, Stein R (2006) MafB: an activator of the glucagon gene expressed in developing islet α- and β-cells. Diabetes 55:297–304

Artner I, Blanchi B, Raum JC, Guo M, Kaneko T, Cordes S, Sieweke M, Stein R (2007) MafB is required for islet β cell maturation. Proc Natl Acad Sci USA 104:3853–3858

Artner I, Hang Y, Mazur M, Yamamoto T, Guo M, Lindner J, Magnuson MA, Stein R (2010) MafA and MafB regulate genes critical to β-cells in a unique temporal manner. Diabetes 59:2530–2539

Assan R, Biollot J (1973) Pancreatic glucagon and glucagon-like material in tissues and plasma from human fetuses 6-26 weeks old. Pathol Biol (Paris) 21:149–155

Avnit-Sagi T, Kantorovich L, Kredo-Russo S, Hornstein E, Walker MD (2009) The promoter of the pri-miR-375 gene directs expression selectively to the endocrine pancreas. PLoS One 4:e5033

Avrahami D, Kaestner KH (2012) Epigenetic regulation of pancreas development and function. Semin Cell Dev Biol 23:693–700

Baroukh N, Ravier MA, Loder MK, Hill EV, Bounacer A, Scharfmann R, Rutter GA, Van Obberghen E (2007) MicroRNA-124a regulates Foxa2 expression and intracellular signaling in pancreatic β-cell lines. J Biol Chem 282:19575–19588

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K (2007) High-resolution profiling of histone methylations in the human genome. Cell 129:823–837

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297

Beringue F, Blondeau B, Castellotti MC, Breant B, Czernichow P, Polak M (2002) Endocrine pancreas development in growth-retarded human fetuses. Diabetes 51:385–391

Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, Mills AA, Elledge SJ, Anderson KV, Hannon GJ (2003) Dicer is essential for mouse development. Nat Genet 35:215–217

Bonner-Weir S, Taneja M, Weir GC, Tatarkiewicz K, Song KH, Sharma A, O'Neil JJ (2000) In vitro cultivation of human islets from expanded ductal tissue. Proc Natl Acad Sci USA 97:7999–8004

Bonner-Weir S, Li WC, Ouziel-Yahalom L, Guo L, Weir GC, Sharma A (2010) β-cell growth and regeneration: replication is only part of the story. Diabetes 59:2340–2348

Burlison JS, Long Q, Fujitani Y, Wright CV, Magnuson MA (2008) Pdx-1 and Ptf1a concurrently determine fate specification of pancreatic multipotent progenitor cells. Dev Biol 316(1):74–86

Cabrera O, Berman M, Kenyon NS, Ricordi C, Berggren P, Caicedo A (2006) The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proc Natl Acad Sci USA 103:2334–2339

Carrasco M, Delgado I, Soria B, Martín F, Rojas A (2012) GATA4 and GATA6 control mouse pancreas organogenesis. J Clin Invest 122:3504–3515

Chiang MK, Melton DA (2003) Single-cell transcript analysis of pancreas development. Dev Cell 4(3):383–393

Collombat P, Mansouri A, Hecksher-Sorensen J, Serup P, Krull J, Gradwohl G, Gruss P (2003) Opposing actions of Arx and Pax4 in endocrine pancreas development. Genes Dev 17:2591–2603

Collombat P, Hecksher-Sørensen J, Broccoli V, Krull J, Ponte I, Mundiger T, Smith J, Gruss P, Serup P, Mansouri A (2005) The simultaneous loss of Arx and Pax4 genes promotes a somatostatin-producing cell fate specification at the expense of the α- and β-cell lineages in the mouse endocrine pancreas. Development 132:2969–2980

Collombat P, Hecksher-Sorensen J, Krull J, Berger J, Riedel D, Herrera PL, Serup P, Mansouri A (2007) Embryonic endocrine pancreas and mature β cells acquire α and PP cell phenotypes upon Arx misexpression. J Clin Invest 117(4):961–970

Collombat P, Xu X, Ravassard P, Sosa-Pineda B, Dussaud S, Billestrup N, Madsen OD, Serup P, Heimberg H, Mansouri A (2009) The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into a and subsequently b cells. Cell 138:449–462

Correa-Medina M, Bravo-Egana V, Rosero S, Ricordi C, Edlund H, Diez J, Pastori RL (2009) MicroRNA miR-7 is preferentially expressed in endocrine cells of the developing and adult human pancreas. Gene Expr Patterns 9:193–199

Dave SD, Vanikar AV, Trivedi HL (2013) In-vitro generation of human adipose tissue derived insulin secreting cells: up-regulation of Pax-6, Ipf-1 and Isl-1. Cytotechnology 65:299–307

Desgraz R, Herrera PL (2009) Pancreatic neurogenin 3-expressing cells are unipotent islet precursors. Development 136:3567–3574

Dhawan S, Georgia S, Tschen SI, Fan G, Bhushan A (2011) Pancreatic β cell identity is maintained by DNA methylation-mediated repression of Arx. Dev Cell 20:419–429

Du A, Hunter CS, Murray J, Noble D, Cai CL, Evans SM, Stein R, May CL (2009) Islet-1 is required for the maturation, proliferation, and survival of the endocrine pancreas. Diabetes 58:2059–2069

Dumortier O, Blondeau B, Duvillie B, Reusens B, Breant B, Remacle C (2007) Different mechanisms operating during different critical time-windows reduce rat fetal β-cell mass due to a maternal low-protein or low energy diet. Diabetologia 50:2495–2503

Ejarque M, Cervantes S, Pujadas G, Tutusaus A, Sanchez L, Gasa R (2013) Neurogenin3 cooperates with Foxa2 to autoactivate its own expression. J Biol Chem 26:11705–11717

Gannon M, Ables ET, Crawford L, Lowe D, Offield MF, Magnuson MA, Wright CV (2008) pdx-1 function is specifically required in embryonic β cells to generate appropriate numbers of endocrine cell types and maintain glucose homeostasis. Dev Biol 314(2):406–417

Gasa R, Mrejen C, Leachman N, Otten M, Barnes M, Wang J, Chakrabarti S, Mirmira R, German M (2004) Proendocrine genes coordinate the pancreatic islet differentiation program in vitro. Proc Natl Acad Sci USA 101(36):13245–13250

Gaulton KJ, Nammo T, Pasquali L, Simon JM, Giresi PG, Fogarty MP, Panhuis TM, Mieczkowski P, Secchi A, Bosco D, Berney T, Montanya E, Mohlke KL, Lieb JD, Ferrer J (2010) A map of open chromatin in human pancreatic islets. Nat Genet 42:255–259

Gradwohl G, Dierich A, LeMeur M, Guillemot F (2000) Neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. Proc Natl Acad Sci USA 97:1607–1611

Gu C, Stein GH, Pan N, Goebbels S, Hörnberg H, Nave KA, Herrera P, White P, Kaestner KH, Sussel L, Lee JE (2010) Pancreatic β cells require NeuroD to achieve and maintain functional maturity. Cell Metab 11:298–310

Guillemain G, Filhoulaud G, Da Silva-Xavier G, Rutter GA, Scharfmann R (2007) Glucose is necessary for embryonic pancreatic endocrine cell differentiation. J Biol Chem 282:15228–15237

Gunton JE, Kulkarni RN, Yim S, Okada T, Hawthorne WJ, Tseng YH, Roberson RS, Ricordi C, O'Connell PJ, Gonzalez FJ, Kahn CR (2005) Loss of ARNT/HIF1β mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. Cell 122:337–349

Hald J, Sprinkel AE, Ray M, Serup P, Wright C, Madsen OD (2008) Generation and characterization of Ptf1a antiserum and localization of Ptf1a in relation to Nkx6.1 and Pdx1 during the earliest stages of mouse pancreas development. J Histochem Cytochem 56(6):587–595

Heinis M, Simon MT, Ilc K, Mazure NM, Pouyssegur J, Scharfmann R, Duvillie B (2010) Oxygen tension regulates pancreatic β-cell differentiation through hypoxia-inducible factor 1α. Diabetes 59:662–669

Heintzman ND, Hon GC, Hawkins RD, Kheradpour P, Stark A, Harp LF, Ye Z, Lee LK, Stuart RK, Ching CW, Ching KA, Antosiewicz-Bourget JE, Liu H, Zhang X, Green RD, Lobanenkov VV, Stewart R, Thomson JA, Crawford GE, Kellis M, Ren B (2009) Histone modifications at human enhancers reflect global cell-type-specific gene expression. Nature 459:108–112

Henseleit KD, Nelson SB, Kuhlbrodt K, Hennings JC, Ericson J, Sander M (2005) NKX6 transcription factor activity is required for α- and β-cell development in the pancreas. Development 132:3139–3149

Herrera PL, Huarte J, Sanvito F, Meda P, Orci L, Vasalli JD (1991) Embryogenesis of the murine endocrine pancreas; early expression of pancreatic polypeptide gene. Development 113:1257–1265

Holland AM, Góñez LJ, Naselli G, Macdonald RJ, Harrison LC (2005) Conditional expression demonstrates the role of the homeodomain transcription factor Pdx1 in maintenance and regeneration of β-cells in the adult pancreas. Diabetes 54:2586–2595

Huang HP, Liu M, El-Hodiri HM, Chu K, Jamrich M, Tsai MJ (2000) Regulation of the pancreatic islet-specific gene β2 (neuroD) by neurogenin 3. Mol Cell Biol 20:3292–3307

Ianus A, Holz GG, Theise ND, Hussain MA (2003) In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J Clin Invest 111:843–850

Jeon J, Correa-Medina M, Ricordi C, Edlund H, Diez JA (2009) Endocrine cell clustering during human pancreas development. J Histochem Cytochem 57:383–393

Jogeklar MV, Parek VS, Hardikar AA (2007) New pancreas from old: microregulators of pancreas regeneration. Trends Endocrinol Metab 18:393–400

Joglekar MV, Joglekar VM, Hardikar AA (2009) Expression of islet-specific microRNAs during human pancreatic development. Gene Expr Patterns 9:109–113

Johansson KA, Dursun U, Jordan N, Gu G, Beermann F, Gradwohl G, Grapin-Botton A (2007) Temporal control of neurogenin3 activity in pancreas progenitors reveals competence windows for the generation of different endocrine cell types. Dev Cell 12:457–465

Jonsson J, Carlsson L, Edlund T, Edlund H (1994) Insulin-promoter-factor 1 is required for pancreas development. Nature 371:606–609

Kalis M, Bolmeson C, Esguerra JL, Gupta S, Edlund A, Tormo-Badia N, Speidel D, Holmberg D, Mayans S, Khoo NK, Wendt A, Eliasson L, Cilio CM (2011) β cell specific deletion of Dicer1 leads to defective insulin secretion and diabetes mellitus. PLoS One 6:e29166

Kawaguchi Y, Cooper B, Gannon M, Ray M, MacDonald RJ, Wright CV (2002) The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. Nat Genet 32(1):128–134

Keller DM, McWeeney S, Arsenlis A, Drouin J, Wright CV, Wang H, Wollheim CB, White P, Kaestner KH, Goodman RH (2007) Characterization of pancreatic transcription factor Pdx-1 binding sites using promoter microarray and serial analysis of chromatin occupancy. J Biol Chem 282:32084–32092

Kimura H, Kawasaki H, Taira K (2004) Mouse microRNA-23b regulates expression of Hes1 in P19 cells. Nucleic Acids Symp Ser 48:213–214

Kloosterman WP, Lagendijk AK, Ketting RF, Moulton JD, Plasterk RH (2007) Targeted inhibition of miRNA maturation with morpholinos reveals a role for miR-375 in pancreatic islet development. PLoS Biol 5:e203

Kopinke D, Murtaugh LC (2010) Exocrine-to-endocrine differentiation is detectable only prior to birth in the uninjured mouse pancreas. BMC Dev Biol 10:38

Kopinke D, Brailsford M, Shea JE, Leavitt R, Scaife CL, Murtaugh LC (2011) Lineage tracing reveals the dynamic contribution of $Hes1^+$ cells to the developing and adult pancreas. Development 138(3):431–441

Kopp JL, Dubois CL, Schaffer AE, Hao E, Shih HP, Seymour PA, Ma J, Sander M (2011) $Sox9^+$ ductal cells are multipotent progenitors throughout development but do not produce new endocrine cells in the normal or injured adult pancreas. Development 138(4):653–665

Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE (2008) Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 26:443–452

Lahmy R, Soleimani M, Sanati MH, Behmanesh M, Kouhkan F, Mobarra N (2013) Pancreatic islet differentiation of human embryonic stem cells by microRNA overexpression. J Tissue Eng Regen Med. doi: 10.1002/term.1787

Lee JC, Smith SB, Watada H, Lin J, Scheel D, Wang J, Mirmira RG, German MS (2001) Regulation of the pancreatic pro-endocrine gene neurogenin3. Diabetes 50(5):928–936

Liao X, Xue H, Wang YC, Nazor KL, Guo S, Trivedi N, Peterson SE, Liu Y, Loring JF, Laurent LC (2013) Matched miRNA and mRNA signatures from an hESC-based in vitro model of pancreatic differentiation reveal novel regulatory interactions. J Cell Sci 126:3848–3861

Like AA, Orci L (1972) Embryogenesis of the human pancreatic islets: a light and electron microscopic study. Diabetes 21:511–534

Lottmann H, Vanselow J, Hessabi B, Walther R (2001) The Tet-On system in transgenic mice: inhibition of the mouse pdx-1 gene activity by antisense RNA expression in pancreatic β-cells. J Mol Med (Berl) 79:321–328

Lynn FC, Skewes-Cox P, Kosaka Y, Mcmanus MT, Harfe BD, German MS (2007a) Micro RNA expression is required for pancreatic islet cell genesis in the mouse. Diabetes 56: 2938–2945

Lynn FC, Smith SB, Wilson ME, Yang KY, Nekrep N, German MS (2007b) Sox9 coordinates a transcriptional network in pancreatic progenitor cells. Proc Natl Acad Sci USA 104(25):10500–10505. 0704054104 [pii]

Mastracci TL, Anderson KR, Papizan JB, Sussel L (2013) Regulation of Neurod1 contributes to the lineage potential of $Neurogenin3^+$ endocrine precursor cells in the pancreas. PLoS Genet 9(2):e1003278

Matsuoka TA, Artner I, Henderson E, Means A, Sander M, Stein R (2004) The MafA transcription factor appears to be responsible for tissue-specific expression of insulin. Proc Natl Acad Sci USA 101:2930–2933

Mellitzer G, Bonné S, Luco RF, Van De Casteele M, Lenne-Samuel N, Collombat P, Mansouri A, Lee J, Lan M, Pipeleers D, Nielsen FC, Ferrer J, Gradwohl G, Heimberg H (2006) IA1 is NGN3-dependent and essential for differentiation of the endocrine pancreas. EMBO J 25:1344–1352

Morita S, Horii T, Kimura M, Goto Y, Ochiya T, Hatada I (2007) One Argonaute family member, Eif2c2 (Ago2), is essential for development and appears not to be involved in DNA methylation. Genomics 89:687–696

Nicolini U, Hubinont C, Santolaya J, Fisk NM, Rodeck CH (1990) Effects of fetal intravenous glucose challenge in normal and growth retarded fetuses. Horm Metab Res 22:426–430

Nishimura W, Kondo T, Salameh T, El Khattabi I, Dodge R, Bonner-Weir S, Sharma A (2006) A switch from MafB to MafA expression accompanies differentiation to pancreatic β-cells. Dev Biol 293:526–539

Nishimura W, Rowan S, Salameh T, Mass RL, Bonner-Weir S, Sell SM, Sharma A (2008) Preferential reduction of β cells derived form Pax6-MafB pathway MafB deficient mice. Dev Biol 314:443–456

Offield MF, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, Hogan BL, Wright CV (1996) Pdx1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development 122:983–995

Oliver-Krasinski JM, Kasner MT, Yang J, Crutchlow MF, Rustgi AK, Kaestner KH, Stoffers DA (2009) The diabetes gene Pdx1 regulates the transcriptional network of pancreatic endocrine progenitor cells in mice. J Clin Invest 119(7):1888–1898. 37028 [pii]

Papizan JB, Singer RA, Tschen SI, Dhawan S, Friel JM, Hipkens SB, Magnuson MA, Bhushan A, Sussel L (2011) Nkx2.2 repressor complex regulates islet β-cell specification and prevents β-to-α-cell reprogramming. Genes Dev 25:2291–2305

Pictet R, Rutter WJ (1972) Development of the embryonic endocrine pancreas. In: Greep RO, Astwood EB, Steiner DF, Freinkel N, Geiger SR (eds) Handbook of physiology, vol I. American Physiological Society, Washington, DC, pp 25–76

Pictet RL, Clark WR, Williams RH, Rutter WJ (1972) An ultrastructural analysis of the developing embryonic pancreas. Dev Biol 29:436–467

Piper K, Ball SG, Turnpenny LW, Brickwood S, Wilson DI, Hanley NA (2002) β-cell differentiation during human development does not rely on nestin-positive precursors: implications for stem cell-derived replacement therapy. Diabetologia 45:1045–1047

Polak M, Bouchareb-Banaei L, Scharfmann R, Czernichow P (2000) Early pattern of differentiation in the human pancreas. Diabetes 49:225–232

Prado CL, Pugh-Bernard AE, Elghazi L, Sosa-Pîneda B, Sussel L (2004) Ghrelin cells replace insulin-producing β cells in two mouse models of páncreas development. Proc Natl Acad Sci USA 101:2924–2929

Puri S, Hebrok M (2010) Cellular plasticity within the pancreas: lessons learned from development. Dev Cell 18:342–356

Raum JC, Gerrish K, Artner I, Henderson E, Guo M, Sussel L, Schisler JC, Newgard CB, Stein R (2006) FoxA2, Nkx2.2, and PDX-1 regulate islet β-cell-specific MafA expression through conserved sequences located between base pairs -8118 and -7750 upstream from the transcription start site. Mol Cell Biol 26:5735–5743

Runhke M, Ungefroren H, Nussler A, Martin F, Brulport M, Schormann W, Hengstler JG, Klapper W, Ulrichs K, Hutchinson JA, Soria B, Parwaresch RM, Heeckt P, Kremer B, Fändrich F (2005) Differentiation of in vitro-modified human peripheral blood monocytes into hepatocyte-like and pancreatic islet-like cells. Gastroenterology 128:1774–1786

Sander M, Sussel L, Conners J, Scheel D, Kalamaras J, Dela Cruz F, Schwitzgebel V, Hayes-Jordan A, German M (2000) Homeobox gene Nkx6.1 lies downstream of Nkx2.2 in the major pathway of β-cell formation in the pancreas. Development 127:5533–5540

Sarkar SA, Kobberup S, Wong R, Lopez AD, Quayum N, Still T, Kutchma A, Jensen JN, Gianani R, Beattie GM, Jensen J, Hayek A, Hutton JC (2008) Global gene expression profiling and histochemical analysis of the developing human fetal pancreas. Diabetologia 51:285–297

Schaffer AE, Freude KK, Nelson SB, Sander M (2010) Nkx6 transcription factors and Ptf1a function as antagonistic lineage determinants in multipotent pancreatic progenitors. Dev Cell 18(6):1022–1029

Schaffer AE, Taylor BL, Benthuysen JR, Liu J, Thorel F, Yuan W, Jiao Y, Kaestner KH, Herrera PL, Magnuson MA, May CL, Sander M (2013) Nkx6.1 controls a gene regulatory network required for establishing and maintaining pancreatic β cell identity. PLoS Genet 9(1):e1003274

Schwitzgebel VM, Scheel DW, Conners JR, Kalamaras J, Lee JE, Anderson DJ, Sussel L, Johnson JD, German MS (2000) Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. Development 127(16):3533–3542

Slack JM (1995) Developmental biology of the pancreas. Science 26:1203–1205

Smith SB, Gasa R, Watada H, Wang J, Griffen SC, German MS (2003) Neurogenin3 and hepatic nuclear factor 1 cooperate in activating pancreatic expression of Pax4. J Biol Chem 278:38254–38259

Smith SB, Qu HQ, Taleb N, Kishimoto NY, Scheel DW, Lu Y, Patch AM, Grabs R, Wang J, Lynn FC, Miyatsuka T, Mitchell J, Seerke R, Désir J, Vanden Eijnden S, Abramowicz M, Kacet N, Weill J, Renard ME, Gentile M, Hansen I, Dewar K, Hattersley AT, Wang R, Wilson ME, Johnson JD, Polychronakos C, German MS (2010) Rfx6 directs islet formation and insulin production in mice and human. Nature 463:775–780

Solar M, Cardalda C, Houbracken I, Martin M, Maestro MA, De Medts N, Xu X, Grau V, Heimberg H, Bouwens L, Ferrer J (2009) Pancreatic exocrine duct cells give rise to insulin-producing β cells during embryogenesis but not after birth. Dev Cell 17:849–860

Soria B, Roche E, Berná G, León-Quinto T, Reig JA, Martin F (2000) Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. Diabetes 49:157–162

Soria B, Bedoya FJ, Tejedo JR, Hmadcha A, Ruiz-Salmerón R, Lim S, Martin F (2008) Cell therapy for diabetes mellitus: an opportunity for stem cells? Cells Tissues Organs 188:70–77

Soria B, Tudurí E, González A, Hmadcha A, Martin F, Nadal A, Quesada I (2010) Pancreatic islet cells: a model for calcium-dependent peptide release. HSFP J 4:52–60

Sosa-Pineda B, Chowdhury K, Torres M, Oliver G, Gruss P (1997) The Pax4 gene is essential for differentiation of insulin-producing β cells in the mammalian pancreas. Nature 386:399–402

Soyer J, Flasse L, Raffelsberger W, Beucher A, Orvain C, Peers B, Ravassard P, Vermot J, Voz ML, Mellitzer G, Gradwohl G (2010) Rfx6 is an Ngn3-dependent winged helix transcription factor required for pancreatic islet cell development. Development 137:203–212

Stefan Y, Grasso S, Perrelet A, Orci L (1983) A quantitative immunofluorescence study of the endocrine cell populations in the developing human pancreas. Diabetes 32:293–301

Steiner DJ, Kim A, Miller K, Hara M (2010) Pancreatic islet plasticity: interspecies comparison of islet architecture and composition. Islets 2:135–145

Sugiyama T, Rodriguez RT, McLean GW, Kim SK (2007) Conserved markers of fetal pancreatic epithelium permit prospective isolation of islet progenitor cells by FACS. Proc Natl Acad Sci USA 104:175–180

Sussel L, Kalamaras J, Hartigan-O'Connor DJ, Meneses JJ, Pedersen RA, Rubenstein JL, German MS (1998) Mice lacking the homeodomain transcription factor Nkx2.2 have diabetes due to arrested differentiation of pancreatic β cells. Development 125:2213–2221

Tang F, Kaneda M, O'Carroll D, Hajkova P, Barton SC, Sun YA, Lee C, Tarakhovsky A, Lao K, Surani MA (2007) Maternal microRNAs are essential for mouse zygote development. Genes Dev 21:644–648

Thomas MK, Devon ON, Lee JH, Peter A, Schlosser DA, Tenser MS, Habener JF (2001) Development of diabetes mellitus in aging transgenic mice following suppression of pancreatic homeoprotein IDX-1. J Clin Invest 108:319–329

Thorel F, Népote V, Avril I, Kohno K, Desgraz R, Chera S, Herrera PL (2010) Conversion of adult pancreatic α-cells to β-cells after extreme β-cell loss. Nature 464:1149–1154

Van de Bunt M, Gaulton KJ, Parts L, Moran I, Johnson PR, Lindgren CM, Ferrer J, Gloyn AL, McCarthy MI (2013) The miRNA profile of human pancreatic islets and β-cells and relationship to type 2 diabetes pathogenesis. PLoS One 8(1):e55272

Villaseñor A, Chong DC, Cleaver O (2008) Biphasic Ngn3 expression in the developing pancreas. Dev Dyn 237:3270–3279

Villaseñor A, Chong DC, Henkemeyer M, Cleaver O (2010) Epithelial dynamics of pancreatic branching morphogenesis. Development 137:4295–4305

Wang H, Brun T, Kataoka K, Sharma AJ, Wollheim CB (2007) MafA controls genes implicated in biosynthesis and secretion. Diabetologia 50:348–358

Wang S, Hecksher-Sorensen J, Xu Y, Zhao A, Dor Y, Rosenberg L, Serup P, Gu G (2008) Myt1 and Ngn3 form a feed-forward expression loop to promote endocrine islet cell differentiation. Dev Biol 317:531–540

Watada H, Scheel DW, Leung J, German MS (2003) Distinct gene expression programs function in progenitor and mature islet cells. J Biol Chem 278:17130–17140

Wei R, Yang J, Liu GQ, Gao MJ, Hou WF, Zhang L, Gao HW, Liu Y, Chen GA, Hong TP (2013) Dynamic expression of microRNAs during the differentiation of human embryonic stem cells into insulin-producing cells. Gene 518:246–255

Xu X, D'Hoker J, Stangé G, Bonné S, De Leu N, Xiao X, Van de Casteele M, Mellitzer G, Ling Z, Pipeleers D, Bouwens L, Scharfmann R, Gradwohl G, Heimberg H (2008) β cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 132:197–207

Xuan S, Borok MJ, Decker KJ, Battle MA, Duncan SA, Hale MA, Macdonald RJ, Sussel L (2012) Pancreas-specific deletion of mouse Gata4 and Gata6 causes pancreatic agenesis. J Clin Invest 122:3516–3528

Yang YP, Thorel F, Boyer DF, Herrera PL, Wright CV (2011) Context-specific α- to-β-cell reprogramming by forced Pdx1 expression. Genes Dev 25(16):1680–1685

Zhang C, Moriguchi T, Kajihara M, Esaki R, Harada A, Shimohata H, Oishi H, Hamada M, Morito N, Hasegawa K, Kudo T, Engel JD, Yamamoto M, Takahashi S (2005) MafA is a key regulator of glucose-stimulated insulin secretion. Mol Cell Biol 25:4969–4976

Zhang D, Jiang W, Liu M, Sui X, Yin X, Chen S, Shi Y, Deng H (2009) Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. Cell Res 19:429–438

Zhou Q, Law AC, Rajagopal J, Anderson WJ, Gray PA, Melton DA (2007) A multipotent progenitor domain guides pancreatic organogenesis. Dev Cell 13:103–114

(Dys)Regulation of Insulin Secretion by Macronutrients

7

Philip Newsholme, Kevin Keane, Celine Gaudel, and Neville McClenaghan

Contents

Abstract

Pancreatic β-cells are often referred to as "fuel sensors" as they continually monitor and respond to dietary nutrients, under the modulation of additional neurohormonal signals, in order to secrete insulin to best meet the needs of the organism. β-Cell nutrient sensing requires metabolic activation, resulting in production of stimulus-secretion coupling signals that promote insulin biosynthesis

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_4,

and release. The primary stimulus for insulin secretion is glucose, and islet β-cells are particularly responsive to this important nutrient secretagogue. It is important to consider individual effects of different classes of nutrient or other physiological or pharmacological agents on metabolism and insulin secretion. However, given that β-cells are continually exposed to a complex milieu of nutrients and other circulating factors, it is important to also acknowledge and examine the interplay between glucose metabolism and that of the two other primary nutrient classes, the amino acids and fatty acids. It is the mixed nutrient sensing and outputs of glucose, amino and fatty acid metabolism that generate the metabolic coupling factors (MCFs) involved in signaling for insulin exocytosis. Primary MCFs in the β-cell include ATP, NADPH, glutamate, long chain acyl-CoA and diacylglycerol and are discussed in detail in this article.

Keywords

Pancreatic β-cells • Insulin secretion • Nutrient metabolism • Incretins • Signal transduction • Stimulus-secretion coupling • Gene expression • Desensitization

Abbreviations

ACC	Acetyl-CoA carboxylase
CPT-1	Carnitine Palmitoyl Transferase 1
DAG	Diacylglycerol
FFA	Free fatty acid
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
Gly3P	Glycerol-3-phosphate
GSIS	Glucose-stimulated insulin secretion
LC-acyl	CoA long-chain acyl-CoA
MCF	Metabolic coupling factors
PI3K	Phosphatidylinositide-3-kinases
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C

Overview of β-Cell Stimulus-Secretion Coupling

Pancreatic β-cells are often referred to as "fuel sensors," continually monitoring and responding to circulating nutrient levels, under the modulation of additional neurohormonal signals, in order to secrete insulin to best meet the needs of the organism. β-cell nutrient sensing involves notable metabolic activation, resulting in production of coupling signals that promote insulin biosynthesis and secretion. The primary stimulus of insulin secretion is glucose, and islet β-cells are particularly responsive to this important nutrient secretagogue, coupling metabolic and other stimuli with the insulin-secretory machinery. In writing this chapter we are

fully aware that most of the studies cited have utilized rat-, mouse- or hamster-derived insulinoma β-cell lines to study function in vitro. This is due to the inherent difficulty in maintaining primary rodent islet β-cell mass and function for more than a few days in vitro and of course the scarcity of human islets for research purposes. The generation of functional and stable human β-cell lines has also proved difficult. Recently, three novel insulin-secreting human β-cell lines have been generated and deposited at European Collection of Cell Cultures (*ECACC*) although their full characterization is still in its infancy (McCluskey et al. 2011; Guo-Parke et al. 2012). Nevertheless, the major rodent β-cell lines have provided substantial data and insights into cell function in normal or pathogenic situations. The most widely used cell lines include INS 1, MIN 6, RINm5F and BRIN-BD11. It is important to state that in vivo intact islet structures (comprising α, β and δ-cells, which secrete glucagon, insulin and somatostatin, respectively) are required to maintain appropriate and pulsatile hormone secretion in response to nutrient stimuli.

Elevation in blood glucose concentrations results in rapid rises in intracellular glucose levels as glucose is transported across the β-cell plasma membrane. Glucose uptake and metabolism are two essential steps in the so-called "glucose-stimulated insulin secretion" (GSIS) pathway. GSIS represents the increase in insulin secretion over basal release in response to increased extracellular, and ultimately intracellular, glucose. As illustrated in Fig. 1, glucose rapidly enters β-cells, through specific glucose transporters (GLUT-1 in humans; GLUT-2 in rodents), after which it is swiftly phosphorylated by the enzyme glucokinase, which has a high Km for glucose. These primary steps, particularly glucokinase, determine the rate of glucose utilization by the β-cell over a range of physiological glucose levels (3–20 mM) and the combination of transport and phosphorylation determines metabolic flux through glycolysis.

Increased β-cell glycolytic flux results in a rapid increase in production of reducing equivalents, an increased activity of shuttle mechanisms (responsible for transferring electrons to the mitochondrial matrix), and TCA cycle activity, leading to increased ATP production in mitochondria. The outcome is an enhanced cytoplasmic ATP to ADP ratio, which prompts closure of ATP-sensitive K^+ (K_{ATP}) channels in the plasma membrane evoking membrane depolarization, and subsequent opening of voltage-gated Ca^{2+} channels (Fig. 1). This culminates in an increase in cellular Ca^{2+} influx – a primary driver of the GSIS mechanism (Straub and Sharp 2002). Ca^{2+} and vesicle docking and fusion events can also be modulated by agents acting through phospholipase C (PLC)/protein kinase C (PKC) or adenylate cyclase (AC)/protein kinase A (PKA) pathways as shown in Fig. 1.

Importantly, nutrients (including amino acids and lipids), insulinotropic drugs (including the sulphonylureas), or neurohormonal signals (including incretin hormones and autonomic innervation) can markedly affect glucose-stimulated insulin secretion (Fig. 2). Much interest has revolved around acute enhancement of β-cell function by the two incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), currently being hailed

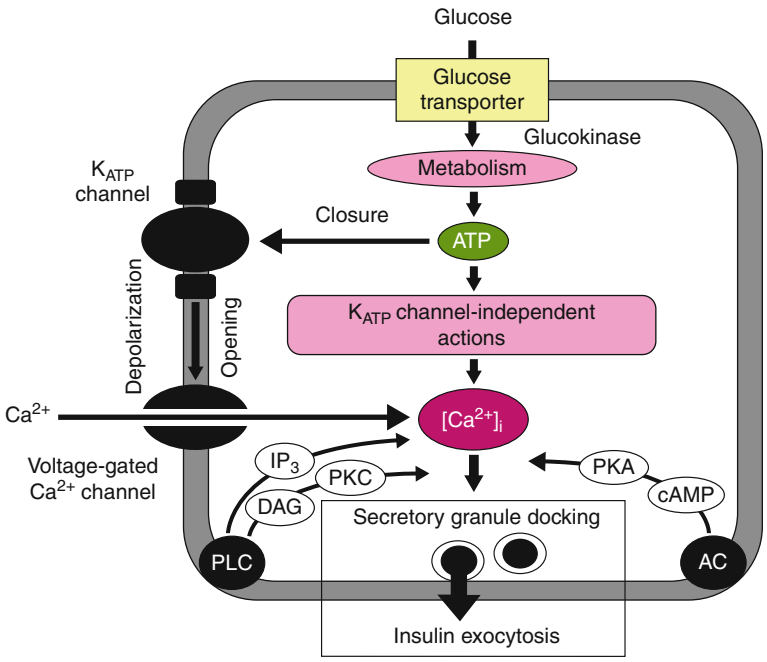

Fig. 1 Metabolic stimulus-secretion coupling in the β-cell. Glucose metabolism results in an enhanced cytoplasmic ATP/ADP ratio, which prompts closure of ATP-sensitive K^+ (K_{ATP}) channels in the plasma membrane evoking membrane depolarization, and subsequent opening of voltage-gated Ca^{2+} channels. This culminates in an increase in cellular Ca^{2+} influx – a primary driver of the GSIS mechanism. Ca^{2+} and vesicle docking and fusion events can also be modulated by agents acting through the phospholipase C (*PLC*)/protein kinase C (*PKC*) or adenylate cyclase (*AC*)/protein kinase A (*PKA*) pathways

as important new therapeutics for type 2 diabetes (for review (Green and Flatt 2007)). These two new classes of therapeutic agent, along with their receptor agonists (e.g. exenatide, liraglutide and GIP fatty acid derivatives), could offer considerable advantages over sulphonylureas and other insulinotropic drugs, as their insulin-secretory action is glucose- and/or nutrient-dependent (Green and Flatt 2007; Peterson 2012). The incretin mimetics may also play a role in maintaining β-cell mass in the hostile type 2 diabetes environment (Green and Flatt 2007). Moreover, there is a parallel strategy to overcome the rapid local degradation and short plasma half-lives of GLP-1 and GIP through development of therapeutic dipeptidyl peptidase-4 (DPP IV) inhibitors that prevent DPP IV-mediated cleavage of GLP-1 and GIP. However, the latter strategy relies on the endogenous production of GLP-1 and GIP to elicit the insulinotropic response, which is perhaps less elegant than the use of stable engineered incretin mimetics. Also, GLP-1 agonists would appear to stimulate a greater reduction in postprandial glucose, body weight and glycated haemoglobin (A1C) than DPP IV

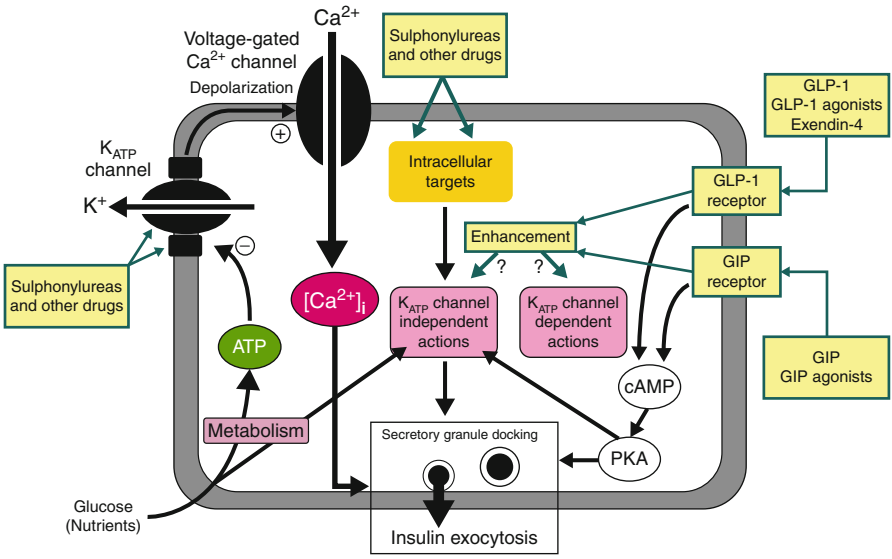

Fig. 2 Modulation of insulin secretion by insulinotropic drugs and incretins. In the presence of glucose, insulinotropic drugs (including the sulphonylureas), or neurohormonal signals (including the two incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) and autonomic innervation) can markedly affect insulin secretion via modulation of signal transduction and/or ion channel activity. Sulphonylurea drugs mainly act via promoting closure of the K_{ATP} channel and thus membrane depolarization. GLP-1 and GIP mediate their effects through G-protein-coupled receptors and associated signal transduction pathways which include serine/threonine kinase activation and phosphorylation of proteins associated with the molecular mechanism of exocytosis

inhibitors alone, and modulation of their structure with lipophilic chains extends plasma half-life by allowing interaction with serum albumin (Peterson 2012; Reid 2012). So, while GLP-1, GIP and related-receptor agonists possess great potential as a new generation of anti-diabetic agents, as yet they are not considered primary therapies for maintenance of glycaemic control in the majority of diabetic individuals (Reid 2012).

It is convenient to also consider individual effects of different classes of nutrient or other physiological or pharmacological agents on metabolism and insulin secretion. However, given that β-cells are continually exposed to a complex milieu of nutrients and other circulating factors, it is important to also acknowledge and examine the interplay between glucose metabolism and that of the two other primary nutrient classes, the amino acids and fatty acids. Cumulatively, it is the mixed nutrient sensing and outputs of glucose, amino and fatty acid metabolism that generate the metabolic coupling factors (MCFs) involved in signaling for insulin exocytosis (Charles and Henquin 1983; Newsholme et al. 2007a). Primary MCFs in the β-cell include ATP, NADPH, glutamate, long-chain acyl-CoA and diacylglycerol (DAG) and are discussed further below.

Phases and Pulsatility of Insulin Secretion

Tight regulation of insulin secretion is necessary for glucose homeostasis, where disturbances are associated with glucose intolerance and diabetes. However, glucose stimulated insulin secretion is under stimulatory and inhibitory control by hormones and neurotransmitters and regular oscillations of circulating insulin in normal subjects can even occur without accompanying changes in plasma glucose – a response related to the so-called "pacemaker" function of the pancreas (for review (Tengholm and Gylfe 2009)). Pulsatile insulin secretion from individual islets appears to follow a dominating pancreatic frequency, where a rhythmic variation in islet secretion is synchronized with oscillations in β-cell cytoplasmic Ca^{2+}. In clusters of β-cells exposed to intermediate stimulatory concentrations of glucose, synchronized oscillations can spread to silent cells as the glucose concentration is increased (Tengholm and Gylfe 2009). This demonstrates β-cell recruitment and intracellular coupling in glucose regulation of insulin secretion.

While foetal islets demonstrate a monophasic (first phase) secretory response, in mature adult islets, insulin secretion occurs very rapidly after glucose administration, and is reported to occur with precise and biphasic kinetics (Fig. 3). Over the years there has been debate as to the underlying mechanisms regulating this biphasic pattern of insulin secretion, which has been proposed to involve at least two signaling pathways, the so-called K_{ATP} channel-dependent pathway, noted above, and another K_{ATP} channel-independent pathway. While both phases would appear to be critically dependent on Ca^{2+} influx, and can be modulated by various agents (including sulphonylureas and incretin hormones; Fig. 2), they affect different pools of insulin-secretory granules. It is understood that whereas the K_{ATP}-dependent pathway prompts exocytosis of an "immediately releasable pool" of granules that elicits and represents the first phase response, the K_{ATP} channel-independent pathway, working in synergy with the K_{ATP}-dependent pathway plays an important role the second phase response (see review (Straub and Sharp 2002)).

Primary Metabolic Factors Regulating Glucose-Stimulated Insulin Secretion

Clearly glucose metabolism plays a central role in the regulation of β-cell function, and glucose-derived carbons are understood to be metabolized following three main pathways generating MCFs for activation of insulin exocytosis: (i) glycolysis followed by TCA cycle-dependent glucose oxidation; (ii) anaplerosis; (iii) provision of glycerol-3-phosphate (Gly3P) for glycerolipid/fatty acid (GL/FA) cycling (see review (Nolan and Prentki 2008)). While the first two of these pathways are linked to GSIS, there is less clarity regarding the latter, though Gly3P is believed to be incorporated into GL and GL/FA cycling which could produce lipid-signaling MCFs for insulin secretion.

Products of glucose metabolism can activate isoforms of PLC, promoting the generation of 1,4,5 inositol-triphosphate (IP_3) and a plasma membrane associated

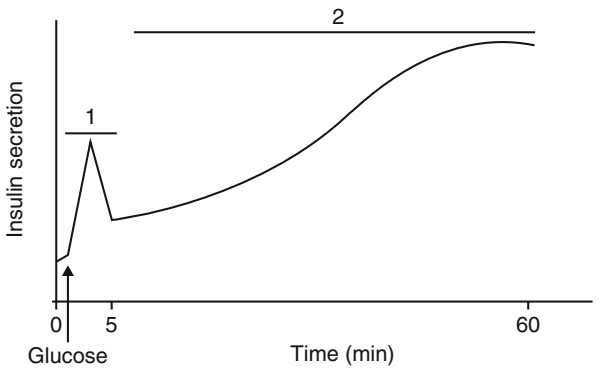

Fig. 3 Biphasic insulin secretion. Insulin secretion from islet β-cells occurs very rapidly after glucose administration, and is reported to occur with precise and biphasic kinetics. (i) First phase insulin secretion which is dependent on ATP generation and a rise in intracellular Ca^{2+}. (ii) Second phase insulin secretion which is dependent on mitochondrial metabolism and a rise in intracellular Ca^{2+}

pool of DAG, a potent activator of specific isoforms of protein kinase C (Newsholme et al. 2007a; Nolan and Prentki 2008), which can help mediate insulin-secretory granule trafficking and exocytosis (Newsholme et al. 2007a; Nolan and Prentki 2008) (Fig. 1). Inositol-triphosphate (IP_3) stimulates Ca^{2+} efflux from the endoplasmic reticulum and increases Ca^{2+} concentration in the cytosol, also favouring activation of the secretory mechanism. Other glucose-derived MCFs such as ATP, LC-acylCoA and DAG can also amplify insulin secretion, and Gly3P metabolism through GL/FA cycling can produce nutrient-derived MCFs not dependent on mitochondrial metabolism. It has been suggested that extracellular externalization of ATP within insulin vesicles stimulates localized plasma membrane accumulation of DAG that is spatially restricted (Wuttke et al. 2013). Since DAG interacts with protein kinase C as outlined above, external ATP levels generated by stimulated β-cells may lead to sustained release of insulin, via a feedback mechanism involving association with the purinoreceptor $P2Y_1$, and this may occur in either a paracrine, or possibly autocrine fashion (Wuttke et al. 2013). In addition, various amino acids and their metabolic products may also impact on GSIS by a combination of enhancement of glucose oxidation, anaplerosis, and direct plasma membrane depolarization effects (Brennan et al. 2002; Smith et al. 1997; Dixon et al. 2003; Sener and Malaisse 1980).

Pancreatic β-cell glucose metabolism also increases arachidonic acid (AA) production, mainly by activation of phospholipase A_2 (Keane and Newsholme 2008). The AA metabolites, prostaglandins (PGs) and leukotrienes, would appear to provide respective positive and negative modulation of glucose-stimulated insulin secretion (Keane and Newsholme 2008). While it would appear that free fatty acids do not stimulate insulin secretion in the absence of glucose, there is a substantial body of evidence to indicate that they are essential for GSIS (Salehi et al. 2005). Recent reports utilizing human islets suggest that non-esterified

AA is critical for normal pancreatic β-cell function. Inhibition of the release of endogenous AA by inhibiting PLA_2 activity resulted in a significant reduction of GSIS perhaps acting via G-protein-coupled receptor(s) (Persaud et al. 2007). Furthermore, AA demonstrated a regulatory and protective role in the BRIN-BD11 β-cell line (Keane et al. 2011). It increased expression of genes involved in cell proliferation and fatty acid metabolism (e.g. cyclo-oxygenase I and II), while reducing the expression of pro-inflammatory factors induced by the saturated fatty acid palmitate. These included iNOS (inducible NO synthase), NF-kB (nuclear factor k B) and NOX (NADPH oxidase), suggesting that that AA may prove to be beneficial in a lipotoxic environment such as is observed in diabetic patients.

Increases and oscillations in the intracellular Ca^{2+} concentration associated with the mechanism of GSIS can stimulate mitochondrial generation of ROS (via electron transport chain activity), whereas Ca^{2+}, via PKC activation and subsequent phosphorylation/translocation of the cytosolic regulatory subunit $P47^{phox}$, may enhance β-cell NOX-dependent generation of ROS (Kruman et al. 1998; Yu et al. 2006; Morgan et al. 2007). The O_2^- and H_2O_2 so produced, acutely stimulate (Morgan et al. 2009), but chronically induce, inhibitory effects on β-cell metabolic pathways, and can promote K_{ATP} channel opening, with resulting inhibitory effects on insulin secretion (Nakazaki et al. 1995).

Metabolically-active pancreatic β-cells have inherently relatively low levels of free radical detoxifying and redox-regulating enzymes, such as, glutathione reductase, glutathione peroxidase, catalase and thioredoxin, rendering them vulnerable to damage and destruction. The consequence of limited scavenging systems is that upon Ca^{2+} stimulation of mitochondrial and NADPH oxidase systems, ROS concentrations in β-cells may increase rapidly, and to high levels. Given this, the β-cell, while utilizing necessary and positive aspects of ROS production for insulin production and release (Morgan et al. 2009; Newsholme et al. 2012), is susceptible to the damaging effects of unregulated ROS generation and accumulation. The following sections give an overview of both positive actions of a range of important nutrient regulators of β-cells together with insights into mechanisms underlying nutrient-induced desensitization and toxicity associated with prolonged exposure.

Investigating Nutrient Regulation of β-Cell Metabolism and Insulin Secretion

Many insights into the mechanisms regulating insulin production and secretion have been gleaned from studies of freshly isolated islets, constituent β-cells and increasingly bioengineered β-cell lines. While early insulin-secreting cell lines, such as RINm5F and HIT-T15, represented rather crude β-cell models, advances in molecular biology and emerging bioengineering technologies offer considerable opportunities to improve and establish more appropriate clonal β-cells (see (McClenaghan 2007)). Indeed, bioengineered pancreatic β-cells, such as the

popular glucose-responsive pancreatic BRIN-BD11 cells (McClenaghan et al. 1996a), have helped facilitate studies of the mechanisms of β-cell metabolism, insulin secretion, cell dysfunction and destruction. Combining the attributes of long-term functional stability of BRIN-BD11 cells with state-of-the-art NMR approaches have enabled the authors to unravel complexities, and provide novel insights into the relationships between glucose, fatty acid and amino acid handling and insulin secretion (Brennan et al. 2002, 2003; McClenaghan 2007). The following sections give a brief overview of the complex mechanisms regulating nutrient-stimulated insulin secretion and gene expression by pancreatic β-cells in response to various stimuli, utilizing isolated islet β-cells and other insulin-secreting cells.

Mechanisms Underlying β-Cell Actions of Glucose

As noted earlier, glucose is a primary physiological β-cell fuel, stimulating insulin secretion as a result of its metabolism and generation of MCFs. As illustrated in Fig. 4, after internalization through membrane-associated transporters, glucose is rapidly metabolized to pyruvate, following initial phosphorylation by glucokinase (GK) to glucose 6-phosphate, and subsequent glycolytic reactions. The third reaction in glycolysis is catalysed by phosphofructokinase (PFK), itself a key β-cell metabolic control site, and fluctuations in its activity result in oscillations in glycolytic flux (Nielsen et al. 1997, 1998; Westermark and Lansner 2003). The end product of glycolysis, pyruvate, is metabolized by either pyruvate dehydrogenase (PDH; the glucose oxidation pathway) or pyruvate carboxylase (PC; the anaplerosis/cataplerosis pathway) to acetyl-CoA or oxaloacetate, respectively, resulting in enhanced mitochondrial tricarboxycylic acid (TCA) cycle activity (Fig. 4).

Among the enzymes responsible for glucose metabolism, GK, PC and PDH appear to play particularly important regulatory roles in the insulin-secretory pathway (Fig. 4). In β-cells, PC activity is high even though the cell does not participate in gluconeogenesis (MacDonald 1995a), which suggests this enzyme exerts anaplerotic functions. Note that β-cells lack phosphoenolpyruvate carboxykinase (an essential enzyme for gluconeogenesis, converting oxaloacetate to phosphoenol pyruvate) (MacDonald 1995b). A recent study has highlighted that siRNA targeted to PC resulted in a reduction of insulin secretion from INS-1 cells (Xu et al. 2008), consistent with the observation that PC activity may be reduced in type 2 diabetes (MacDonald et al. 1996). Furthermore, these researchers showed that PC expression was elevated in the islets of mildly hyperglycaemic mice, while it was reduced in severely hyperglycaemic mice. The authors suggested that the increased levels of PC may possibly correlate to an adaptive response of β-cells to insulin resistance, with reduced PC expression corresponding to β-cell failure (Han and Liu 2010). Interestingly, overexpression of PC in INS-1 cells resulted in increased insulin release (Xu et al. 2008), again supporting an important role for this enzyme in the maintenance of GSIS. However, inhibition of PDH by

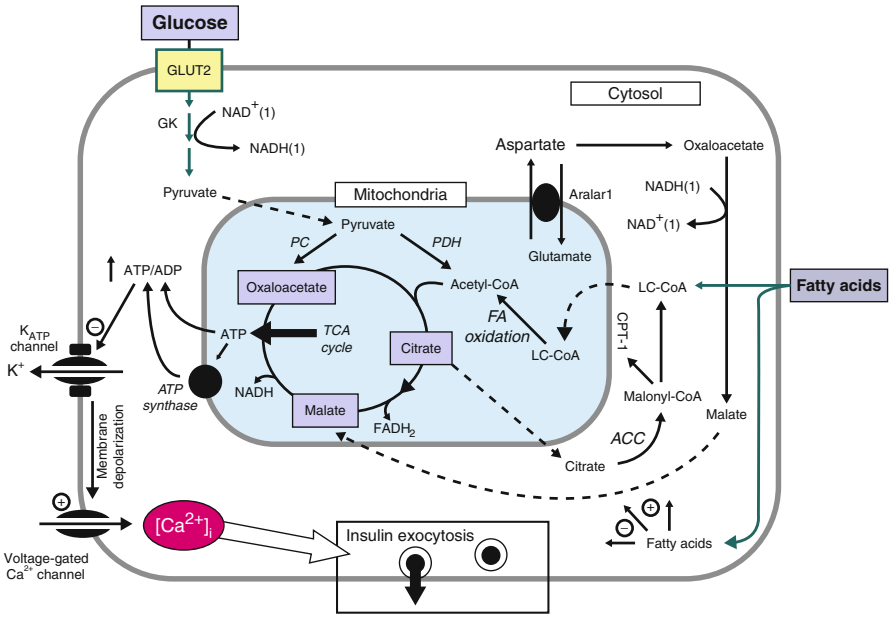

Fig. 4 Mechanisms of glucose and fatty acid enhanced mitochondrial activity and ATP generation. The end product of glycolysis – pyruvate – is metabolized by either pyruvate dehydrogenase (*PDH*; so committing glucose to the oxidation pathway) or pyruvate carboxylase (*PC*; so committing glucose to the anaplerosis/cataplerosis pathway). The products will be acetyl-CoA or oxaloacetate, respectively, which will contribute to enhanced mitochondrial tricarboxycylic acid (*TCA*) cycle activity. The malate-aspartate shuttle transfers cytosolic NADH to the mitochondrial matrix, a process which requires aspartate-glutamate exchange across the mitochondrial inner membrane by Aralar1. The generation of ATP and the increase in intracellular Ca^{2+} drives insulin secretion. Fatty acids may potentiate insulin secretion via generation of LC acyl-CoA and stimulation of signal transducing events

overexpression of PDH kinase 4 in INS-1 cells did not result in a decrease of insulin secretion (Xu et al. 2008), although it is important not to over-interpret this observation by dismissing an important regulatory role of pyruvate dehydrogenase.

Transfer of electrons from TCA cycle to the mitochondrial electron transport chain is mediated by NADH and $FADH_2$ formation, resulting in ATP generation (Fig. 4). The increase in intracellular ATP to ADP ratio leads to the characteristic closure of K_{ATP} channels (Cook and Hales 1984), membrane depolarization, opening of voltage gated Ca^{2+} channels and rapid rise in intracellular Ca^{2+} concentration, leading to mobilization and ultimately fusion of insulin-containing granules with the plasma membrane and insulin release (Fig. 4) (Tarasov et al. 2004; Wiederkehr and Wollheim 2006). The primary actions of glucose are mediated by potentiation of ATP concentration by enhanced TCA cycle substrate (oxidative and anaplerotic) supply. Generation of other additive factors derived from glucose metabolism might also be promoted by mitochondrial Ca^{2+} elevation (Maechler et al. 1997).

Pyruvate may be converted in the β-cell to both acetyl-CoA and oxaloacetate, as discussed above (Fig. 4). A number of possibilities for mitochondrial metabolism of pyruvate exist: (i) generation of CO_2 via TCA cycle activity; (ii) export from the mitochondria as glutamate (due to 2-oxoglutarate conversion to glutamate via transamination or glutamate dehydrogenase activity); (iii) export from the mitochondria as malate to be converted back to pyruvate by $NADP^+$-dependent malic enzyme; and (iv) export from the mitochondria as citrate to be acted on by ATP citrate lyase and subsequently acetyl CoA carboxylase (Carpentier et al. 2000) to form malonyl-CoA which is an inhibitor of carnitine palmitoyl transferase-1 and thus an inhibitor of fatty acid oxidation (malonyl-CoA can subsequently be used for fatty acid synthesis via the action of fatty acid synthase).

One of these pathways, the so-called pyruvate-malate cycle, predicts a role for malate in insulin secretion via generation of the stimulus–secretion coupling factor NADPH (Jensen et al. 2008). Flow of the cycle requires oxaloacetate derived from pyruvate (via PC) to be converted to malate via a reversal of the malate dehydrogenase reaction, consuming NADH and generating NAD^+ in the mitochondrial matrix. Following this, malate is exported to the cytosol, converted to pyruvate via $NADP^+$-dependent malate dehydrogenase, generating NADPH (Newsholme et al. 2007b). Glucose stimulation of β-cells or isolated rodent islet cells increases malate levels (Jensen et al. 2006; Macdonald 2003) and while the workings of the malate–pyruvate cycle (recently reviewed (Jensen et al. 2008)) are known, concerns have been raised as to the operation and impact of this cycle under physiologic conditions.

Normal TCA cycle activity ensures the malate → oxaloacetate direction of flux, thus generating NADH in the mitochondrial matrix. In addition, malate → oxaloacetate conversion forms part of the malate-aspartate shuttle, which has a high activity in the β-cell, and is essential for transfer of cytosolic NADH to the mitochondrial matrix (for review (Bender et al. 2006)). In β-cells, reducing equivalents may be transported to the mitochondrial matrix by either the glycerol-phosphate or the malate-aspartate shuttle (Eto et al. 1999). Inhibition of the malate–aspartate shuttle by amino–oxyacetate (which acts on transamination reactions and inhibits cytosolic NADH reoxidation) has been demonstrated to attenuate the secretory response to nutrients, thus highlighting the dominance of this latter shuttle in the β-cell. In addition, Aralar1 (see Fig. 4), a mitochondrial aspartate–glutamate carrier which takes part in the malate–aspartate shuttle, has been demonstrated to play an important role in glucose-induced insulin secretion, as its deletion in INS-1 cells leads to a complete loss of malate–aspartate shuttle activity in mitochondria, and to a 25 % decrease of insulin release in response to glucose (Marmol et al. 2009). Moreover, overexpression of Aralar1 in BRIN-BD11 cells, enhances GSIS and amino acid-stimulated insulin secretion, while increasing glycolytic capacity (Bender et al. 2009).

One key constituent of the malate-aspartate NADH shuttle is the mitochondrial aspartate–glutamate transporter, with its two Ca^{2+}-sensitive isoforms, Citrin and Aralar1, expressed in excitatory tissues (Rubi et al. 2004; del Arco and Satrustegui 1998). However, Aralar1 is the dominant aspartate–glutamate transporter isoform

expressed in β-cells (Rubi et al. 2004), and the function of this transporter in the malate-aspartate shuttle is illustrated in Fig. 4. Adenoviral-mediated overexpression of Aralar1 in INS-1E β-cells and rat pancreatic islets enhanced glucose-evoked NAD(P)H generation, electron transport chain activity and mitochondrial ATP formation, and Aralar1 was demonstrated to exert its effect on insulin secretion upstream of the TCA cycle (Rubi et al. 2004). Indeed, the capacity of the aspartate-glutamate transporter appeared to limit NADH shuttle activity and subsequent mitochondrial metabolism. Thus, it is highly improbable that a malate-pyruvate cycle is active and important to insulin secretion, if the malate–aspartate shuttle is indeed a key component of stimulus–secretion coupling.

An alternative pyruvate-cycling pathway has been proposed, where generation of citrate from condensation of oxaloacetate (OAA) and acetyl-CoA occurs in the TCA cycle, followed by export of citrate from the mitochondria via the citrate–isocitrate carrier, cleavage of citrate by ATP citrate lyase to OAA and acetyl-CoA, and recycling to pyruvate via a cytosolic malate dehydrogenase and $NADP^+$-dependent malic enzyme (Jensen et al. 2008). In this proposed cycle, OAA to malate formation occurs in the cytosol, similar to the malate–aspartate shuttle. Acetyl-CoA can also serve a substrate for acetyl-CoA carboxylase, leading to formation of long-chain acyl-CoA accumulation in the cytosol via malonyl-CoA and in β-cells, glucose stimulation increases malonyl-CoA levels before insulin release (Corkey et al. 1989), and addition of long-chain acyl-CoA results in a stimulation of insulin secretion (Deeney et al. 2000).

However, the evidence used to refute a role of fatty acid synthesis is not compelling. Suppression of citrate lyase mRNA levels by 92 % and citrate lyase protein levels by 75 % by adenovirus-mediated siRNA delivery did not affect GSIS in 832/13 β-cells compared with cells treated with a control adenovirus (Joseph et al. 2007). Also, citrate lyase suppression in primary islet preparations using recombinant adenovirus technology to suppress citrate lyase expression by 65 % reported no impact on GSIS (Joseph et al. 2007). It is possible to reinterpret these findings if we consider that citrate lyase is expressed at very high levels in β-cells (Roche et al. 1998), so suppression of protein expression, even by 65–75 %, would not be expected to be sufficient to alter the synthesis of key LC-acyl-CoA species.

Mechanisms Underlying β-Cell Actions of Lipids

Fatty acids appear to freely diffuse into pancreatic β-cells through the plasma membrane (Hamilton and Kamp 1999). As illustrated in Fig. 4, inside β-cells, fatty acids are transformed in long-chain acyl-CoA, by acyl-CoA synthase (ACS), and enter the mitochondria via Carnitine Palmitoyl Transferase 1 (CPT-1), so β-oxidation can occur when glucose levels are low. The resulting acetyl-CoA is subsequently oxidized in the TCA cycle and under these conditions, ATP generation is sufficient for β-cell survival, and to maintain basal levels of insulin

secretion (Fig. 4). When the extracellular glucose concentration is increased, fatty acid oxidation is inhibited, due to formation of malonyl-CoA by acetyl-CoA carboxylase (Carpentier et al. 2000). Malonyl-CoA under glucose stimulatory conditions is derived from glucose carbon, via formation of citrate. Malonyl-CoA inhibits CPT-1, thus blocking transport of long-chain acyl-CoA into the mitochondria (Prentki et al. 2002) (Fig. 4). Accumulation of long-chain acyl-CoA in the cytosol leads to an increase of intracellular Ca^{2+} levels and to changes in acylation state of proteins involved both in regulation of ion channel activity and exocytosis (Keane and Newsholme 2008; Yaney and Corkey 2003; Haber et al. 2006). In addition, long-chain acyl-CoA can also enhance fusion of insulin-secretory vesicles with plasma membrane and insulin release (Deeney et al. 2000).

However, effects of fatty acids on glucose-induced insulin secretion are directly correlated with chain length and the degree of unsaturation, where long-chain fatty acids (such as palmitate or linoleate) *acutely* improve, but *chronically* reduce insulin release in response to glucose stimulation (Newsholme et al. 2007a). It is possible that chronic elevated synthesis of triacylglycerol species such as tripalmitin is detrimental to β-cell function due to adverse morphological changes (Moffitt et al. 2005) but it is more likely that apoptosis is triggered by lipid-specific signaling pathways and/or endoplasmic reticulum stress-activated pathways, so resulting in β-cell failure and death (reviewed in (Newsholme et al. 2007a)). A study by the authors demonstrated that 24 h culture of BRIN-BD11 cells with the polyunsaturated fatty acid, arachidonic acid (AA), increased insulin secretion in response to the amino acid L-alanine. On the other hand, 24 h exposure of BRIN-BD11 cells to saturated fatty acid palmitic acid in culture inhibited L-alanine-induced insulin secretion (Dixon et al. 2004). Interestingly, AA exhibited a protective function in the BRIN-BD11 β-cell line by preventing the detrimental effects of palmitic acid (Keane et al. 2011).

A recent advance in the understanding of the mechanism(s) by which non-esterified fatty acids (NEFAs) modulate insulin secretion in vivo was the discovery of high levels of expression of the membrane-bound G-protein-coupled receptor GPR40, a putative NEFA receptor in human and animal islet β-cell preparations (Tomita et al. 2006). GPR40 mRNA levels positively correlated with the insulinogenic index (Tomita et al. 2006). Furthermore, it has also been demonstrated that omega-3 fatty acids can interact with the GPR120 receptor, and mediate insulin-sensitisation and anti-inflammatory effects in obese mice models (Oh et al. 2010). Other G-protein-coupled receptors that may be important in islet physiology are GPR41 and GPR119 (Oh and Lagakos 2011; Nguyen et al. 2012). However, while the potential signaling mechanism (s) by which G-protein-coupled receptors regulates insulin secretion are still under investigation, it appears likely that they involve changes in intracellular Ca^{2+} mobilization (Salehi et al. 2005; Itoh and Hinuma 2005; Shapiro et al. 2005; Newsholme and Krause 2012).

Mechanisms Underlying β-Cell Actions of Amino Acids

Under appropriate conditions, amino acids enhance insulin secretion from primary islet cells and β-cell lines (Charles and Henquin 1983; Brennan et al. 2002; Smith et al. 1997; Dixon et al. 2003; Sener and Malaisse 1980). In vivo, L-glutamine and L-alanine are quantitatively the most abundant amino acids in blood and extracellular fluids, closely followed by the branched chain amino acids (Blau et al. 2003). However, individual amino acids do not evoke insulin-secretory responses in vitro when added at physiological concentrations, rather, combinations of physiological concentrations of amino acids or high concentrations of individual amino acids are much more effective. In vivo, amino acids derived from dietary proteins and those released from intestinal epithelial cells, in combination with glucose, stimulate insulin secretion, thereby leading to protein synthesis and amino acid transport in target tissues such as skeletal muscle (Keane and Newsholme 2008).

While amino acids can potentially affect a number of aspects of β-cell function, a relatively small number of amino acids promote or synergistically enhance insulin release from pancreatic β-cells (Fajans et al. 1967; McClenaghan et al. 1996b). As illustrated in Fig. 5, the mechanisms by which amino acids enhance insulin secretion are understood to primarily rely on: (i) direct depolarization of the plasma membrane (e.g., cationic amino acid, L-arginine); (ii) metabolism (e.g., glutamine, leucine); and (iii) co-transport with Na^+ and cell membrane depolarization (e.g., L-alanine). Notably, partial oxidation, e.g., L-alanine (Brennan et al. 2002) may also initially increase the cellular content of ATP impacting on K_{ATP} channel closure prompting membrane depolarization, Ca^{2+} influx and insulin exocytosis.

Additional mitochondrial signals that affect insulin secretion may also be generated (Fig. 6) (Dukes et al. 1994; Malaisse-Lagae et al. 1982; Maechler 2002), and in β-cells, the mTOR-signaling pathway acts in synergy with growth factor/insulin signaling to stimulate mitochondrial function and insulin secretion (Kwon et al. 2004). At present, the mechanism by which amino acids activate the mTOR complex has not been fully elucidated, and recent publications have suggested that amino acids can activate both mTOR1 and mTOR2 complexes (Tato et al. 2011). Furthermore, recent data suggests that amino acids regulate mTOR signaling via class I PI3K enzymes, in addition to the already established class III PI3K enzyme (hVps34). This novel signaling mechanism may impact on a variety of conditions that display differences in nutrient processing such as that observed in diabetes and cancer (Tato et al. 2011). However, given that amino acid nutrients may play a role in the pathophysiological of many disorders, it is interesting to speculate involvement of kinase stimulation or inhibition of a phosphatase utilizing mTOR as a substrate (Kwon et al. 2004; McDaniel et al. 2002; Briaud et al. 2003).

Arginine: This amino acid stimulates insulin release through electrogenic transport into the β-cell via the mCAT2A amino acid transporter (Fig. 6), thereby increasing membrane depolarization, rise in intracellular Ca^{2+} through opening of voltage-gated Ca^{2+} channels and insulin secretion (Sener et al. 2000). However, in

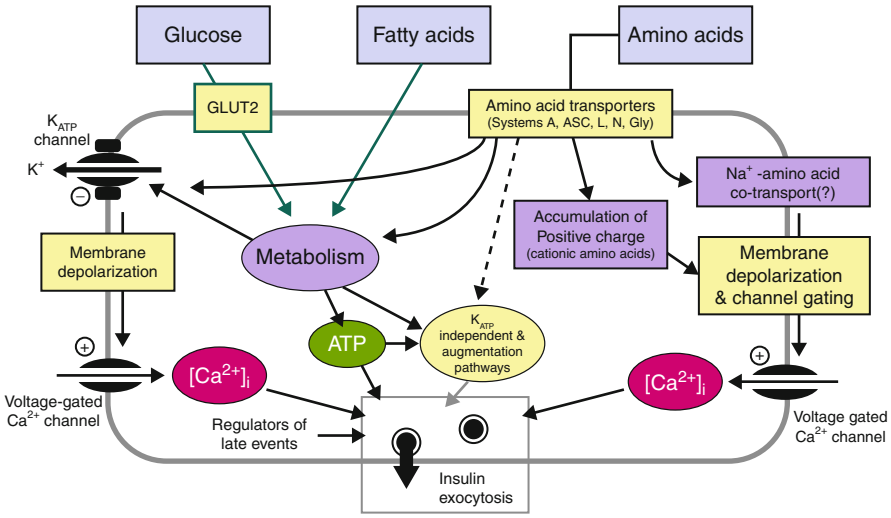

Fig. 5 Common mechanisms of nutrient-stimulated insulin secretion. Glucose metabolism is essential for stimulation of insulin secretion. The mechanisms by which amino acids enhance insulin secretion are understood to primarily rely on (i) direct depolarization of the plasma membrane (e.g., cationic amino acid, L-arginine); (ii) metabolism (e.g., alanine, glutamine, leucine); and (iii) co-transport with Na^+ and cell membrane depolarization (e.g., alanine). Notably, rapid partial oxidation may also initially increase both the cellular content of ATP (impacting on K_{ATP} channel closure prompting membrane depolarization) and other stimulus–secretion coupling factors. In the absence of glucose, fatty acids may be metabolized to generate ATP and maintain basal levels of insulin secretion

some situations, arginine principally through its metabolism is understood to exert a negative effect on β-cell insulin release.

The potentially detrimental effect of arginine metabolism hinges on arginine-derived nitric oxide (NO) through the action of inducible nitric oxide synthase (iNOS) (McClenaghan et al. 2009). High levels of NO are known to interfere with β-cell mitochondrial function and generation of key stimulus–secretion coupling factors, which could lead to a reduction in cellular insulin output (Newsholme and Krause 2012; McClenaghan et al. 2009).

Glutamine: Among the amino acids, glutamine is considered one of the most important, playing an essential role in promotion and maintenance of functionality of various organs and cells, including pancreatic β-cells (Curi et al. 2005). Both rat islets and BRIN-BD11 cells consume glutamine at high rates (Dixon et al. 2003), but notably while glutamine can potentiate GSIS and interact with other nutrient secretagogues, it does not initiate an insulin-secretory response (McClenaghan et al. 1996b). In rat islets, glutamine is converted to y-amino butyric acid (GABA) and aspartate (Fig. 6), and in the presence of leucine oxidative metabolism is increased. Previously, it was shown that the potential glutamine synthetase inhibitor – methionine sulfoximide – completely abolished GSIS in normal mouse islets (Li et al. 2004), a phenomenon reversed by addition of glutamine or

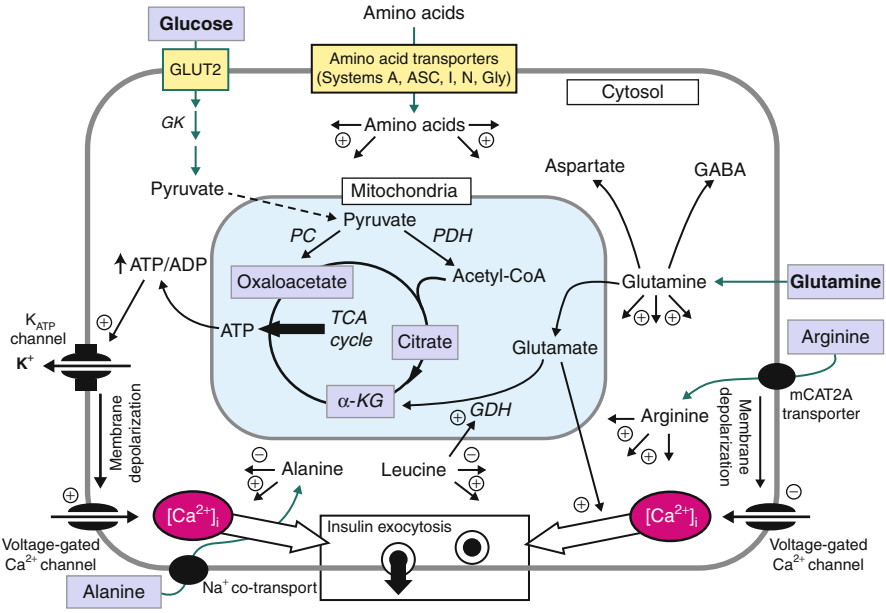

Fig. 6 Glucose, alanine, glutamine, leucine and arginine are the major nutrient drivers of insulin secretion. Metabolism of glucose, alanine and glutamine result in enhanced TCA cycle activity and generation of metabolic secretion coupling factors including ATP, Ca^{2+} and glutamate. Leucine may enhance glutamine oxidation via activation of glutamate dehydrogenase (*GDH*). Arginine may depolarize the plasma membrane by net import of positive charge thus causing opening of voltage-gated Ca^{2+} channels. The key sites of metabolic control in the β-cell are indicated; glucokinase (*GK*), pyruvate dehydrogenase (*PDH*), pyruvate carboxylase (*PC*), glutamate dehydrogenase (*GDH*)

a non-metabolizable analogue. However, it is important to note that this inhibitor may block a number of glutamate-utilizing enzymes and so the outcome cannot be interpreted to arise as a result of a specific action on glutamine synthetase.

Glutamate: The ability of glutamate to stimulate insulin secretion and its actions in β-cells has been hotly debated. Intracellular generation of L-glutamate has been proposed to participate in nutrient-induced stimulus-secretion coupling as an additive factor in the amplifying pathway of GSIS (Maechler and Wollheim 1999). During glucose stimulation, total cellular glutamate levels have been demonstrated to increase in human, mouse and rat islets, as well as clonal β-cells (Brennan et al. 2002; Dixon et al. 2003; Maechler and Wollheim 1999; Broca et al. 2003), whereas other studies have reported no change (Danielsson et al. 1970; MacDonald and Fahien 2000). The observation that mitochondrial activation in permeabilized β-cells directly stimulates insulin exocytosis (Maechler et al. 1997) pioneered the identification of glutamate as a putative intracellular messenger (Maechler and Wollheim 1999; Hoy et al. 2002). However, in recent years, the role of L-glutamate in direct actions on insulin secretion has been challenged (MacDonald and Fahien 2000; Bertrand et al. 2002).

For example, stimulatory (16.7 mM) glucose did not increase intracellular L-glutamate concentrations in rat islets in one study (MacDonald and Fahien 2000), and while L-glutamine (10 mM) increased the L-glutamate concentration tenfold, this was not accompanied by a stimulation of insulin release. In a separate study, incubation with glucose resulted in a significant increase in L-glutamate concentration in depolarized mouse and rat islets, but L-glutamine while increasing L-glutamate content did not alter insulin secretion (Bertrand et al. 2002). Additionally, in this latter study, BCH-induced activation of GDH lowered L-glutamate levels, but increased insulin secretion. However, it is probable that experimental conditions in which L-glutamine is used as L-glutamate precursor may lead to saturating concentrations of L-glutamate without necessarily activating the K_{ATP}-dependent pathway and associated increase in insulin secretion (Broca et al. 2003). It is likely that during enhanced glucose metabolism, the concentration of the key TCA cycle intermediate α-ketoglutarate (2-oxoglutarate) is elevated and a proportion of this metabolite is subsequently transaminated to glutamate (Brennan et al. 2003). It is the opinion of the authors that the glutamate so formed may indirectly stimulate insulin secretion through additive actions on the malate-aspartate shuttle (as glutamate is a substrate for the mitochondrial membrane aspartate/glutamate carrier 1, thus may increase the capacity of the shuttle, see Fig. 4) or by contribution to glutathione synthesis (as glutamate is one of the three amino acids required for glutathione synthesis) and subsequent positive effects on cellular redox state and mitochondrial function (for further detail see (Brennan et al. 2003)). As glutamate is not readily taken up into β-cells it is difficult to design robust experiments considering intracellular actions of this metabolizable nutrient. Indeed, glutamate release from β-cells has recently been reported (Kiely et al. 2007), adding complexity to this story and offering the intriguing possibility of other β-cell actions. Some of the latest data has shown that glutamate can be transported into insulin-containing vesicle while inside the cell (Gammelsaeter et al. 2011). This may enhance Ca^{2+}-dependent insulin secretion through glutamate receptors. Currently, this and other aspects of β-cell glutamate signaling and actions are under investigation by the authors.

Leucine: Prolonged exposure of rat islets to leucine increases ATP, cytosolic Ca^{2+}, and potentiates glucose-stimulated insulin secretion. In addition, chronic exposure to leucine leads to an increase in both ATP synthase and glucokinase, which can sensitize pancreatic β-cells to glucose-induced insulin secretion (Yang et al. 2006). Leucine-induced insulin secretion involves allosteric activation of glutamate dehydrogenase (GDH) leading to an increase in glutamine → glutamate → 2-oxoglutarate flux, elevated mitochondrial metabolism and an increase in ATP production leading to a membrane depolarization (Fig. 6). Additionally transamination of leucine to α-ketoisocaproate (KIC) and entry into TCA cycle via acetyl-CoA can contribute to ATP generation by increasing the oxidation rate of the amino acid and thus stimulation of insulin secretion. Moreover, it has been reported that α-keto acids (including KIC) can directly block K_{ATP} channel activity and exert additional K_{ATP} channel-independent effects thereby inducing insulin secretion (Heissig et al. 2005; McClenaghan and Flatt 2000). Notably, a recent study reported patients with mutations in the regulatory (GTP binding) site of GDH had increased

β-cell responsiveness to leucine, presenting with hypoglycaemia after a protein rich meal (Heissig et al. 2005; Hsu et al. 2001). In addition, mice harbouring a β-cell-specific GDH deletion exhibit a marked decrease (37 %) in glucose-induced insulin secretion, supporting an essential role of GDH in insulin release (Carobbio et al. 2009).

Alanine: Effects of L-alanine have been studied in BRIN-BD11 cells and primary rat islet cells, which consume high rates of this amino acid (Dixon et al. 2003). Moreover, L-alanine is known to potentiate GSIS by enhancing glucose utilization and metabolism (Brennan et al. 2002), and numerous studies have highlighted L-alanine as a potent initiator of insulin release. The authors have utilized BRIN-BD11 cells to study the actions of L-alanine on β-cells demonstrating an influence on GSIS by electrogenic Na^+ transport, and exploited ^{13}C nuclear magnetic resonance technologies to trace L-alanine metabolism, demonstrating generation of glutamate, aspartate and lactate. Interestingly, the authors have also developed an integrated mathematical model to determine the effect of L-alanine metabolism and L-alanine-mediated Ca^{2+}-handling on GSIS and amino acid-stimulated insulin secretion (Salvucci et al. 2013). Here, using BRIN-BD11 cells to validate the model in vitro, the authors found that elevated intracellular ATP and Ca^{2+} levels were required for complete insulin secretory responses. Furthermore, this model confirmed that L-alanine-associated Na^+ co-transport acted in synergy with membrane depolarization leading to K^+_{ATP}-independent Ca^{2+} influx and insulin secretion (Salvucci et al. 2013). In addition, L-alanine metabolism to pyruvate followed by oxidation via the TCA cycle could increase ATP levels and thus promote insulin secretion via the K^+_{ATP}-dependent mechanism, and could also generate putative stimulus-secretion factors such as intracellular L-glutamate or citrate that may result in increased insulin secretion (Salvucci et al. 2013). Other studies using the respiratory poison oligomycin have also illustrated the importance of metabolism and oxidation of alanine for its ability to stimulate insulin secretion (Brennan et al. 2002).

Overview of Nutrient Regulation of β-Cell Gene Expression

Glucose can impact on insulin secretion and pancreatic β-cell function by regulating gene expression, enabling mammals to adapt metabolic activity to changes in nutrient supply. In pancreatic β-cells, in addition to a fundamental role in the regulation of insulin secretion and pancreatic β-cell function, glucose serves as a principal physiological regulator of insulin gene expression (Poitout et al. 2006). Glucose is known to control transcription factor recruitment, level of transcription, alternative splicing and stability of insulin mRNA (Bensellam et al. 2009). To cover all aspects of the diverse actions of glucose and other key nutrients on β-cell gene expression is certainly outside the scope of this chapter, but the following gives an overview of some notable aspects of this complex area of study.

In β-cells, three transcriptional factors bind to insulin promoter to regulate insulin gene expression: pancreatic and duodenal homeobox 1 (Pdx-1), neurogenic differentiation 1 (NeuroD1) and V-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA), acting in synergy and stimulating insulin gene expression in response to increasing plasma glucose (Andrali et al. 2008). However, consistent with detrimental β-cell actions of prolonged exposure to high glucose concentrations, impairments of Pdx-1 and MafA binding to the insulin promoter have been noted, in turn leading to decreased insulin biosynthesis, content and capacity for secretion. Similarly, prolonged exposure to high fatty acid levels can impair insulin gene expression, this time accompanied by an accumulation of triglycerides in β-cells – particularly palmitate – where the negative effect may be attributable to ceramide formation (Kelpe et al. 2003). Moreover, palmitate is known to induce a decrease in binding activity of transcriptional factors on the insulin promoter, where both Pdx-1 translocation to the nucleus and MafA expression are affected (Hagman et al. 2005).

An important role of amino acids on gene expression has recently been highlighted (Newsholme et al. 2006). In an Affymetrix microarray study utilizing BRIN-BD11 cells, prolonged (24 h) exposure to alanine and glutamine upregulated β-cell gene expression, particularly genes involved in metabolism, signal transduction and oxidative stress (Cunningham et al. 2005; Corless et al. 2006). This upregulation could be due to alanine metabolism, provision of amino acid stimulus-secretion coupling factors and lipid metabolites (such as long-chain acyl-CoAs), and leading to an alteration of cellular redox state (Brennan et al. 2002; Dixon et al. 2003). Interestingly, 24 h exposure of BRIN-BD11 cells to glutamine strongly increased calcineurin catalytic and regulatory subunit mRNA expression (Corless et al. 2006) and this Ca^{2+}-binding protein has been reported to play a role in the somatostatin induced inhibition of exocytosis in mouse pancreatic β-cells (Renstrom et al. 1996). Glutamine can also increase Pdx-1 and acetyl-CoA carboxylase mRNA expression. Of the amino acids, alanine and glutamine appear to play particularly important roles in the regulation of gene expression (Newsholme et al. 2006; Corless et al. 2006) and further study of the precise mechanisms underlying these actions should help understanding of β-cell responses to nutrient supply, metabolism and secretory and functional integrity.

Nutrient-Induced β-Cell Desensitization, Dysfunction, and Toxicity

Persistently elevated fuel supply such as glucose, amino acids, fatty acids (or a mixture) is known to exert detrimental effects on a number of cells, and can induce insulin resistance in muscle – perhaps as a first line protective adaptation to fuel overload (Tremblay et al. 2007). The β-cell does not protect itself by blocking uptake of excess nutrients and thus is vulnerable to potential excess activation of mitochondrial metabolism, ROS production, elevated intracellular Ca^{2+} and cell injury (Nolan and Prentki 2008; Morgan et al. 2007; Newsholme et al. 2007c). While expansion of β-cell mass can offer part of a compensatory response,

desensitization may also help reduce the burden on β-cells. Desensitization is commonly observed in eukaryotic cells, is believed to have an underlying role in cell protection (McClenaghan 2007), and may be defined as a readily induced and reversible state of cellular refractoriness attributed to repeated or prolonged exposure to high concentrations of a stimulus.

While acute exposure to glucose generally promotes increased metabolism and generation of MCFs, as well as changes in insulin gene transcription and translation, chronic exposure to high levels of this sugar has been associated with β-cell deterioration, with glucose desensitization in the first instance progressing to glucotoxicity likely arising from oxidative stress. Likewise, while the acute β-cell actions of fatty acids are usually positive, chronic exposure can exert substantive changes to nutrient metabolism and so-called lipotoxicity, and both the hyperglycaemia and hyperlipidemia of diabetes can alter insulin secretion and β-cell function. However, while experimental glucotoxicity and lipotoxicity can be independently demonstrated, it is clear that these two are interrelated adverse forces on the β-cell (Prentki et al. 2002). Some characteristics of this so-called "glucolipotoxicity" (Prentki et al. 2002) are: (i) impaired glucose oxidation, resulting in ACC inhibition (due to an increase in cellular AMP levels as ATP generation decreases, subsequent activation of AMP kinase, and phosphorylation of ACC, so inhibiting generation of malonyl-CoA and LC acyl-CoA), (ii) promotion of fatty acid oxidation due to relief of CPT-1 inhibition and (iii) enhanced FFA esterification and lipid accumulation with respect to the excess FFA that are not oxidized. These combined effects lead to a decrease in glucose-induced insulin secretion, impaired insulin gene expression and an increase in β-cell failure and even cell death (Newsholme et al. 2007a).

Although mechanisms by which chronic exposure to high levels of glucose and/or lipids damage β-cells have been the subject to intense clinical and experimental investigation, much less attention has been directed to other diet-derived factors, including the other major nutrient class, the amino acids. As noted earlier, prolonged exposure to amino acids such as alanine or glutamine may (at least in the first instance) upregulate gene expression of certain metabolic and signal transduction elements, and can also offer enhanced protection against cytokine-induced apoptosis (Cunningham et al. 2005). However, these primary observations also indicated an alteration in β-cell responsiveness, later studied by the authors in more detail (McClenaghan et al. 2009). These latter studies demonstrate for the first time that the desensitization phenomenon previously reported with other pharmacological and physiological agents (see review (McClenaghan 2007)) may extend to the amino acids, where 18 h exposure to L-alanine resulted in reversible alterations in metabolic flux (a reduction in flux), Ca^{2+} handling (reduced level of intracellular Ca^{2+}) and insulin secretion (reduction in insulin secretion).

More intriguing evidence for detrimental β-cell actions of amino acids relate to the reported effects of acute and chronic exposure to homocysteine (Patterson et al. 2006, 2007). Interestingly, elevated circulating homocysteine and hyperhomocysteinemia have emerged as important risk factors for cardiovascular disease and other diseases of the metabolic syndrome, including type 2 diabetes. Studies of prolonged effects of

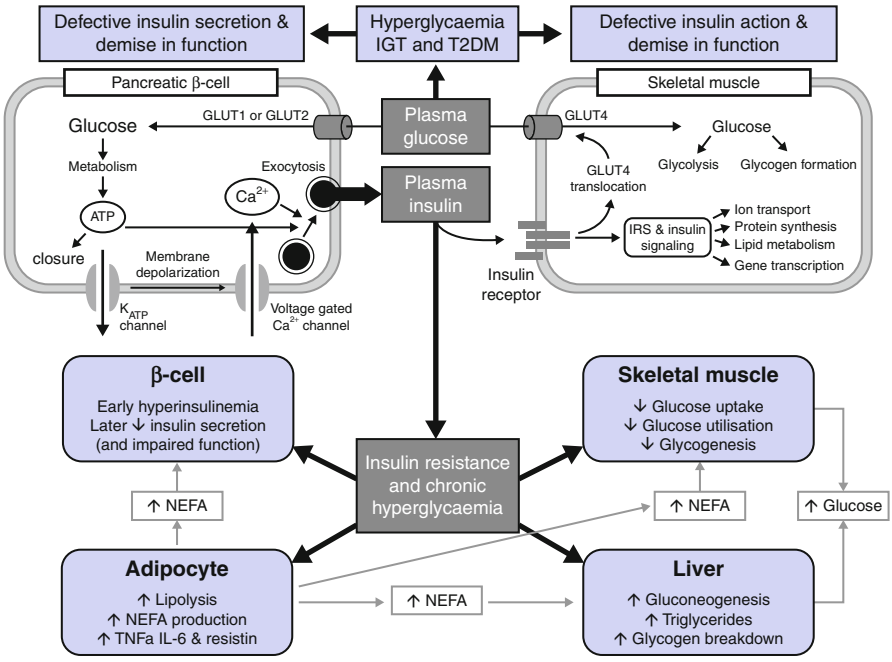

Fig. 7 Interplay between β-cells and insulin-sensitive tissues in the pathogenesis of type 2 diabetes. Key interplay between nutrient handling by insulin secreting β-cells and insulin-sensitive cells such as skeletal muscle, adipocytes and liver regulates whole body nutrient homeostasis. Defective insulin secretion (due to excessive nutrient-induced desensitization of the β-cell, see main text) will result in high plasma levels of glucose. Insulin resistance in muscle and adipose tissue will result in reduced glucose uptake. Insulin resistance in the liver will result in enhanced glucose release into the blood, compounding hyperglycaemia. Insulin resistance in the adipose tissue will result in elevated fatty acid release and pro-inflammatory factor release, contributing to insulin resistance due to impairment of insulin-signaling pathways and also reduced insulin secretion from the β-cell due to impairment of regulation of nutrient metabolism

alanine and homocysteine in the authors' laboratories represent compelling evidence for the existence of β-cell amino acid desensitization. While these data prompt further study, it is interesting to speculate that nutrient-induced desensitization may be a first line compensatory mechanism to over-nutrition. However, if observations on the "toxic" effects of glucose/lipids also extend to amino acids, this would support the view that prolonged over-nutrition generally results in adverse β-cell events which may contribute to the pathogenesis of diabetes.

Conclusion

Pancreatic β-cells are well equipped to respond as metabolic fuel sensors, and additionally possess inherent mechanisms to adapt to nutrient overconsumption in order to preserve glucose homeostasis. Glucose signaling is of primary

importance in the β-cell, and as discussed both fatty acids and amino acids can interface with central signaling pathways to help regulate insulin secretion. Inherently, the metabolic sensing ability of the β-cell comes at the expense of its protection and islet β-cells are more vulnerable than other cells in the body to excess fuel supply. However, as illustrated in Fig. 7, β-cells play a key role in countering nutrient over-consumption through hyperinsulinemia and β-cell expansion as initial attempts to curb the characteristic hyperglycaemia of impaired glucose tolerance (IGT) and type 2 diabetes. Ultimately it is the interplay between nutrient handling by β-cells and other insulin-sensitive cells such as skeletal muscle, adipocytes and liver that dictates whole body nutrient homeostasis (Fig. 7). It would seem that β-cell failure due to excess nutrients is dominant in the pathogenesis of type 2 diabetes with a significant underlying genetic or environmental susceptibility defect, contributing to the process (Nolan and Prentki 2008). However, the alarming epidemic rise in diabesity only serves to highlight how precious and important β-cells are to the maintenance of whole body metabolism. This also prompts further efforts to understand the complexities of β-cell function, demise and destruction, and indeed novel targets and treatments for diabetes, obesity and the metabolic syndrome.

Cross-References

- ▶ Electrical, Calcium, and Metabolic Oscillations in Pancreatic Islets
- ▶ High Fat Programming of β-Cell Dysfunction
- ▶ Role of Mitochondria in β-Cell Function and Dysfunction
- ▶ Role of NADPH Oxidase in β Cell Dysfunction
- ▶ The β-Cell in Human Type 2 Diabetes

References

Andrali SS, Sampley ML, Vanderford NL, Ozcan S (2008) Glucose regulation of insulin gene expression in pancreatic β-cells. Biochem J 415:1–10

Bender K, Newsholme P, Brennan L, Maechler P (2006) The importance of redox shuttles to pancreatic β-cell energy metabolism and function. Biochem Soc Trans 34:811–814

Bender K, Maechler P, McClenaghan NH, Flatt PR, Newsholme P (2009) Overexpression of the malate-aspartate NADH shuttle member Aralar1 in the clonal β-cell line BRIN-BD11 enhances amino-acid-stimulated insulin secretion and cell metabolism. Clin Sci (Lond) 117:321–330

Bensellam M, Van Lommel L, Overbergh L, Schuit FC, Jonas JC (2009) Cluster analysis of rat pancreatic islet gene mRNA levels after culture in low-, intermediate- and high-glucose concentrations. Diabetologia 52:463–476

Bertrand G, Ishiyama N, Nenquin M, Ravier MA, Henquin JC (2002) The elevation of glutamate content and the amplification of insulin secretion in glucose-stimulated pancreatic islets are not causally related. J Biol Chem 277:32883–32891

Blau N, Duran M, Blaskovics M, Gibson K (2003) Amino acid analysis. Physician's guide to the laboratory diagnosis of metabolic diseases, 2nd edn. Springer, New York, pp 11–26

Brennan L, Shine A, Hewage C, Malthouse JP, Brindle KM, McClenaghan N, Flatt PR, Newsholme P (2002) A nuclear magnetic resonance-based demonstration of substantial oxidative L-alanine metabolism and L-alanine-enhanced glucose metabolism in a clonal pancreatic β-cell line: metabolism of L-alanine is important to the regulation of insulin secretion. Diabetes 51:1714–1721

Brennan L, Corless M, Hewage C, Malthouse JP, McClenaghan NH, Flatt PR, Newsholme P (2003) ^{13}C NMR analysis reveals a link between L-glutamine metabolism, D-glucose metabolism and gamma-glutamyl cycle activity in a clonal pancreatic β-cell line. Diabetologia 46:1512–1521

Briaud I, Lingohr MK, Dickson LM, Wrede CE, Rhodes CJ (2003) Differential activation mechanisms of Erk-1/2 and p70(S6K) by glucose in pancreatic β-cells. Diabetes 52:974–983

Broca C, Brennan L, Petit P, Newsholme P, Maechler P (2003) Mitochondria-derived glutamate at the interplay between branched-chain amino acid and glucose-induced insulin secretion. FEBS Lett 545:167–172

Carobbio S, Frigerio F, Rubi B, Vetterli L, Bloksgaard M, Gjinovci A, Pournourmohammadi S, Herrera PL, Reith W, Mandrup S, Maechler P (2009) Deletion of glutamate dehydrogenase in β cells abolishes part of the insulin secretory response not required for glucose homeostasis. J Biol Chem 284:921–929

Carpentier A, Mittelman SD, Bergman RN, Giacca A, Lewis GF (2000) Prolonged elevation of plasma free fatty acids impairs pancreatic β-cell function in obese non-diabetic humans but not in individuals with type 2 diabetes. Diabetes 49:399–408

Charles S, Henquin JC (1983) Distinct effects of various amino acids on $^{45}Ca^{2+}$ fluxes in rat pancreatic islets. Biochem J 214:899–907

Cook DL, Hales CN (1984) Intracellular ATP directly blocks K^+ channels in pancreatic β-cells. Nature 311:271–273

Corkey BE, Glennon MC, Chen KS, Deeney JT, Matschinsky FM, Prentki M (1989) A role for malonyl-CoA in glucose-stimulated insulin secretion from clonal pancreatic β-cells. J Biol Chem 264:21608–21612

Corless M, Kiely A, McClenaghan NH, Flatt PR, Newsholme P (2006) Glutamine regulates expression of key transcription factor, signal transduction, metabolic gene, and protein expression in a clonal pancreatic β-cell line. J Endocrinol 190:719–727

Cunningham GA, McClenaghan NH, Flatt PR, Newsholme P (2005) L-Alanine induces changes in metabolic and signal transduction gene expression in a clonal rat pancreatic β-cell line and protects from pro-inflammatory cytokine-induced apoptosis. Clin Sci (Lond) 109:447–455

Curi R, Lagranha CJ, Doi SQ, Sellitti DF, Procopio J, Pithon-Curi TC, Corless M, Newsholme P (2005) Molecular mechanisms of glutamine action. J Cell Physiol 204:392–401

Danielsson A, Hellman B, Idahl LA (1970) Levels of α-ketoglutarate and glutamate in stimulated pancreatic β-cells. Horm Metab Res 2:28–31

Deeney JT, Gromada J, Hoy M, Olsen HL, Rhodes CJ, Prentki M, Berggren PO, Corkey BE (2000) Acute stimulation with long chain acyl-CoA enhances exocytosis in insulin-secreting cells (HIT T-15 and NMRI β-cells). J Biol Chem 275:9363–9368

del Arco A, Satrustegui J (1998) Molecular cloning of Aralar, a new member of the mitochondrial carrier superfamily that binds calcium and is present in human muscle and brain. J Biol Chem 273:23327–23334

Dixon G, Nolan J, McClenaghan N, Flatt PR, Newsholme P (2003) A comparative study of amino acid consumption by rat islet cells and the clonal β-cell line BRIN-BD11 – the functional significance of L-alanine. J Endocrinol 179:447–454

Dixon G, Nolan J, McClenaghan NH, Flatt PR, Newsholme P (2004) Arachidonic acid, palmitic acid and glucose are important for the modulation of clonal pancreatic β-cell insulin secretion, growth and functional integrity. Clin Sci (Lond) 106:191–199

Dukes ID, McIntyre MS, Mertz RJ, Philipson LH, Roe MW, Spencer B, Worley JF 3rd (1994) Dependence on NADH produced during glycolysis for β-cell glucose signaling. J Biol Chem 269:10979–10982

Eto K, Tsubamoto Y, Terauchi Y, Sugiyama T, Kishimoto T, Takahashi N, Yamauchi N, Kubota N, Murayama S, Aizawa T, Akanuma Y, Aizawa S, Kasai H, Yazaki Y, Kadowaki T (1999) Role of NADH shuttle system in glucose-induced activation of mitochondrial metabolism and insulin secretion. Science 283:981–985

Fajans SS, Floyd JC Jr, Knopf RF, Conn FW (1967) Effect of amino acids and proteins on insulin secretion in man. Recent Prog Horm Res 23:617–662

Gammelsaeter R, Coppola T, Marcaggi P, Storm-Mathisen J, Chaudhry FA, Attwell D, Regazzi R, Gundersen V (2011) A role for glutamate transporters in the regulation of insulin secretion. PLoS One 6:e22960

Green BD, Flatt PR (2007) Incretin hormone mimetics and analogues in diabetes therapeutics. Best Pract Res Clin Endocrinol Metab 21:497–516

Guo-Parke H, McCluskey JT, Kelly C, Hamid M, McClenaghan NH, Flatt PR (2012) Configuration of electrofusion-derived human insulin-secreting cell line as pseudoislets enhances functionality and therapeutic utility. J Endocrinol 214:257–265

Haber EP, Procopio J, Carvalho CR, Carpinelli AR, Newsholme P, Curi R (2006) New insights into fatty acid modulation of pancreatic β-cell function. Int Rev Cytol 248:1–41

Hagman DK, Hays LB, Parazzoli SD, Poitout V (2005) Palmitate inhibits insulin gene expression by altering PDX-1 nuclear localization and reducing MafA expression in isolated rat islets of Langerhans. J Biol Chem 280:32413–32418

Hamilton JA, Kamp F (1999) How are free fatty acids transported in membranes? Is it by proteins or by free diffusion through the lipids? Diabetes 48:2255–2269

Han J, Liu YQ (2010) Reduction of islet pyruvate carboxylase activity might be related to the development of type 2 diabetes mellitus in Agouti-K mice. J Endocrinol 204:143–152

Heissig H, Urban KA, Hastedt K, Zunkler BJ, Panten U (2005) Mechanism of the insulin-releasing action of α-ketoisocaproate and related α-keto acid anions. Mol Pharmacol 68:1097–1105

Hoy M, Maechler P, Efanov AM, Wollheim CB, Berggren PO, Gromada J (2002) Increase in cellular glutamate levels stimulates exocytosis in pancreatic β-cells. FEBS Lett 531:199–203

Hsu BY, Kelly A, Thornton PS, Greenberg CR, Dilling LA, Stanley CA (2001) Protein-sensitive and fasting hypoglycaemia in children with the hyperinsulinism/hyperammonemia syndrome. J Pediatr 138:383–389

Itoh Y, Hinuma S (2005) GPR40, a free fatty acid receptor on pancreatic β cells, regulates insulin secretion. Hepatol Res 33:171–173

Jensen MV, Joseph JW, Ilkayeva O, Burgess S, Lu D, Ronnebaum SM, Odegaard M, Becker TC, Sherry AD, Newgard CB (2006) Compensatory responses to pyruvate carboxylase suppression in islet β-cells. Preservation of glucose-stimulated insulin secretion. J Biol Chem 281:22342–22351

Jensen MV, Joseph JW, Ronnebaum SM, Burgess SC, Sherry AD, Newgard CB (2008) Metabolic cycling in control of glucose-stimulated insulin secretion. Am J Physiol Endocrinol Metab 295: E1287–E1297

Joseph JW, Odegaard ML, Ronnebaum SM, Burgess SC, Muehlbauer J, Sherry AD, Newgard CB (2007) Normal flux through ATP-citrate lyase or fatty acid synthase is not required for glucose-stimulated insulin secretion. J Biol Chem 282:31592–31600

Keane D, Newsholme P (2008) Saturated and unsaturated (including arachidonic acid) non-esterified fatty acid modulation of insulin secretion from pancreatic β-cells. Biochem Soc Trans 36:955–958

Keane DC, Takahashi HK, Dhayal S, Morgan NG (2011) Curi, Newsholme P. Arachidonic acid actions on functional integrity and attenuation of the negative effects of palmitic acid in a clonal pancreatic β-cell line. Clin Sci (Lond) 120:195–206

Kelpe CL, Moore PC, Parazzoli SD, Wicksteed B, Rhodes CJ, Poitout V (2003) Palmitate inhibition of insulin gene expression is mediated at the transcriptional level via ceramide synthesis. J Biol Chem 278:30015–30021

Kiely A, McClenaghan NH, Flatt PR, Newsholme P (2007) Pro-inflammatory cytokines increase glucose, alanine and triacylglycerol utilization but inhibit insulin secretion in a clonal pancreatic β-cell line. J Endocrinol 195:113–123

Kruman I, Guo Q, Mattson MP (1998) Calcium and reactive oxygen species mediate staurosporine-induced mitochondrial dysfunction and apoptosis in PC12 cells. J Neurosci Res 51:293–308

Kwon G, Marshall CA, Pappan KL, Remedi MS, McDaniel ML (2004) Signaling elements involved in the metabolic regulation of mTOR by nutrients, incretins, and growth factors in islets. Diabetes 53(Suppl 3):S225–S232

Li C, Buettger C, Kwagh J, Matter A, Daikhin Y, Nissim IB, Collins HW, Yudkoff M, Stanley CA, Matschinsky FM (2004) A signaling role of glutamine in insulin secretion. J Biol Chem 279:13393–13401

MacDonald MJ (1995a) Influence of glucose on pyruvate carboxylase expression in pancreatic islets. Arch Biochem Biophys 319:128–132

MacDonald MJ (1995b) Feasibility of a mitochondrial pyruvate malate shuttle in pancreatic islets. Further implication of cytosolic NADPH in insulin secretion. J Biol Chem 270:20051–20058

Macdonald MJ (2003) Export of metabolites from pancreatic islet mitochondria as a means to study anaplerosis in insulin secretion. Metabolism 52:993–998

MacDonald MJ, Fahien LA (2000) Glutamate is not a messenger in insulin secretion. J Biol Chem 275:34025–34027

MacDonald MJ, Tang J, Polonsky KS (1996) Low mitochondrial glycerol phosphate dehydrogenase and pyruvate carboxylase in pancreatic islets of Zucker diabetic fatty rats. Diabetes 45:1626–1630

Maechler P (2002) Mitochondria as the conductor of metabolic signals for insulin exocytosis in pancreatic β-cells. Cell Mol Life Sci 59:1803–1818

Maechler P, Wollheim CB (1999) Mitochondrial glutamate acts as a messenger in glucose-induced insulin exocytosis. Nature 402:685–689

Maechler P, Kennedy ED, Pozzan T, Wollheim CB (1997) Mitochondrial activation directly triggers the exocytosis of insulin in permeabilized pancreatic β-cells. Embo J 16:3833–3841

Malaisse-Lagae F, Sener A, Garcia-Morales P, Valverde I, Malaisse WJ (1982) The stimulus-secretion coupling of amino acid-induced insulin release. Influence of a non-metabolized analogue of leucine on the metabolism of glutamine in pancreatic islets. J Biol Chem 257:3754–3758

Marmol P, Pardo B, Wiederkehr A, del Arco A, Wollheim CB, Satrustegui J (2009) Requirement for Aralar and its Ca^{2+}-binding sites in Ca^{2+} signal transduction in mitochondria from INS-1 clonal β-cells. J Biol Chem 284:515–524

McClenaghan NH (2007) Physiological regulation of the pancreatic β-cell: functional insights for understanding and therapy of diabetes. Exp Physiol 92:481–496

McClenaghan NH, Flatt PR (2000) Metabolic and K_{ATP} channel-independent actions of keto acid initiators of insulin secretion. Pancreas 20:38–46

McClenaghan NH, Barnett CR, Ah-Sing E, Abdel-Wahab YH, O'Harte FP, Yoon TW, Swanston-Flatt SK, Flatt PR (1996a) Characterization of a novel glucose-responsive insulin-secreting cell line, BRIN-BD11, produced by electrofusion. Diabetes 45:1132–1140

McClenaghan NH, Barnett CR, O'Harte FP, Flatt PR (1996b) Mechanisms of amino acid-induced insulin secretion from the glucose-responsive BRIN-BD11 pancreatic β-cell line. J Endocrinol 151:349–357

McClenaghan NH, Scullion SM, Mion B, Hewage C, Malthouse JP, Flatt PR, Newsholme P, Brennan L (2009) Prolonged L-alanine exposure induces changes in metabolism, Ca^{2+} handling and desensitization of insulin secretion in clonal pancreatic β-cells. Clin Sci (Lond) 116:341–351

McCluskey JT, Hamid M, Guo-Parke H, McClenaghan NH, Gomis R, Flatt PR (2011) Development and functional characterization of insulin-releasing human pancreatic β cell lines produced by electrofusion. J Biol Chem 286:21982–21992

McDaniel ML, Marshall CA, Pappan KL, Kwon G (2002) Metabolic and autocrine regulation of the mammalian target of rapamycin by pancreatic β-cells. Diabetes 51:2877–2885

Moffitt JH, Fielding BA, Evershed R, Berstan R, Currie JM, Clark A (2005) Adverse physiochemical properties of tripalmitin in β cells lead to morphological changes and lipotoxicity in vitro. Diabetologia 48:1819–1829

Morgan D, Oliveira-Emilio HR, Keane D, Hirata AE, Santos da Rocha M, Bordin S, Curi R, Newsholme P, Carpinelli AR (2007) Glucose, palmitate and pro-inflammatory cytokines modulate production and activity of a phagocyte-like NADPH oxidase in rat pancreatic islets and a clonal β cell line. Diabetologia 50:359–369

Morgan D, Rebelato E, Abdulkader F, Graciano MF, Oliveira-Emilio HR, Hirata AE, Rocha MS, Bordin S, Curi R, Carpinelli AR (2009) Association of NAD(P)H oxidase with glucose-induced insulin secretion by pancreatic β cells. Endocrinology 150(5):2197–2201

Nakazaki M, Kakei M, Koriyama N, Tanaka H (1995) Involvement of ATP-sensitive K^+ channels in free radical-mediated inhibition of insulin secretion in rat pancreatic β-cells. Diabetes 44:878–883

Newsholme P, Krause M (2012) Nutritional regulation of insulin secretion: implications for diabetes. Clin Biochem Rev 33:35–47

Newsholme P, Brennan L, Bender K (2006) Amino-acid metabolism, β cell function and diabetes. Diabetes 55(Suppl 2):S39–S47

Newsholme P, Keane D, Welters HJ, Morgan NG (2007a) Life and death decisions of the pancreatic β-cell: the role of fatty acids. Clin Sci (Lond) 112:27–42

Newsholme P, Bender K, Kiely A, Brennan L (2007b) Amino acid metabolism, insulin secretion and diabetes. Biochem Soc Trans 35:1180–1186

Newsholme P, Haber EP, Hirabara SM, Rebelato EL, Procopio J, Morgan D, Oliveira-Emilio HC, Carpinelli AR, Curi R (2007c) Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production and activity. J Physiol 583:9–24

Newsholme P, Rebelato E, Abdulkader F, Krause M, Carpinelli A, Curi R (2012) Reactive oxygen and nitrogen species generation, antioxidant defenses, and β-cell function: a critical role for amino acids. J Endocrinol 214:11–20

Nguyen CA, Akiba Y, Kaunitz JD (2012) Recent advances in gut nutrient chemosensing. Curr Med Chem 19:28–34

Nielsen K, Sorensen PG, Hynne F (1997) Chaos in glycolysis. J Theor Biol 186:303–306

Nielsen K, Sorensen PG, Hynne F, Busse HG (1998) Sustained oscillations in glycolysis: an experimental and theoretical study of chaotic and complex periodic behaviour and of quenching of simple oscillations. Biophys Chem 72:49–62

Nolan CJ, Prentki M (2008) The islet β-cell. Fuel responsive and vulnerable. Trends Endocrinol Metab 19:285–291

Oh DY, Lagakos WS (2011) The role of G-protein-coupled receptors in mediating the effect of fatty acids on inflammation and insulin sensitivity. Curr Opin Clin Nutr Metab Care 14:322–327

Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, Li P, Lu WJ, Watkins SM, Olefsky JM (2010) GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. Cell 142:687–698

Patterson S, Flatt PR, Brennan L, Newsholme P, McClenaghan NH (2006) Detrimental actions of metabolic syndrome risk factor, homocysteine, on pancreatic β-cell glucose metabolism and insulin secretion. J Endocrinol 189:301–310

Patterson S, Scullion SM, McCluskey JT, Flatt PR, McClenaghan NH (2007) Prolonged exposure to homocysteine results in diminished but reversible pancreatic β-cell responsiveness to insulinotropic agents. Diabetes Metab Res Rev 23:324–334

Persaud SJ, Muller D, Belin VD, Kitsou-Mylona I, Asare-Anane H, Papadimitriou A, Burns CJ, Huang GC, Amiel SA, Jones PM (2007) The role of arachidonic acid and its metabolites in insulin secretion from human islets of langerhans. Diabetes 56:197–203

Peterson G (2012) Current treatments and strategies for type 2 diabetes: can we do better with GLP-1 receptor agonists? Ann Med 44:338–349
Poitout V, Hagman D, Stein R, Artner I, Robertson RP, Harmon JS (2006) Regulation of the insulin gene by glucose and fatty acids. J Nutr 136:873–876
Prentki M, Joly E, El-Assaad W, Roduit R (2002) Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in β-cell adaptation and failure in the etiology of diabetes. Diabetes 51 (Suppl 3):S405–S413
Reid T (2012) Choosing GLP-1 receptor agonists or DPP-4 inhibitors: weighing the clinical trial evidence. Clin Diab 30:3–12
Renstrom E, Ding WG, Bokvist K, Rorsman P (1996) Neurotransmitter-induced inhibition of exocytosis in insulin-secreting β cells by activation of calcineurin. Neuron 17:513–522
Roche E, Farfari S, Witters LA, Assimacopoulos-Jeannet F, Thumelin S, Brun T, Corkey BE, Saha AK, Prentki M (1998) Long-term exposure of β-INS cells to high glucose concentrations increases anaplerosis, lipogenesis, and lipogenic gene expression. Diabetes 47:1086–1094
Rubi B, del Arco A, Bartley C, Satrustegui J, Maechler P (2004) The malate-aspartate NADH shuttle member Aralar1 determines glucose metabolic fate, mitochondrial activity, and insulin secretion in β cells. J Biol Chem 279:55659–55666
Salehi A, Flodgren E, Nilsson NE, Jimenez-Feltstrom J, Miyazaki J, Owman C, Olde B (2005) Free fatty acid receptor 1 (FFA(1)R/GPR40) and its involvement in fatty-acid-stimulated insulin secretion. Cell Tissue Res 322:207–215
Salvucci M, Neufeld Z, Newsholme P (2013) Mathematical model of metabolism and electrophysiology of amino acid and glucose stimulated insulin secretion: in vitro validation using a β-cell line. PLoS One 8:e52611
Sener A, Malaisse WJ (1980) L-Leucine and a non-metabolized analogue activate pancreatic islet glutamate dehydrogenase. Nature 288:187–189
Sener A, Best LC, Yates AP, Kadiata MM, Olivares E, Louchami K, Jijakli H, Ladriere L, Malaisse WJ (2000) Stimulus-secretion coupling of arginine-induced insulin release: comparison between the cationic amino acid and its methyl ester. Endocrine 13:329–340
Shapiro H, Shachar S, Sekler I, Hershfinkel M, Walker MD (2005) Role of GPR40 in fatty acid action on the β cell line INS-1E. Biochem Biophys Res Commun 335:97–104
Smith PA, Sakura H, Coles B, Gummerson N, Proks P, Ashcroft FM (1997) Electrogenic arginine transport mediates stimulus-secretion coupling in mouse pancreatic β-cells. J Physiol 499(Pt 3):625–635
Straub SG, Sharp GW (2002) Glucose-stimulated signaling pathways in biphasic insulin secretion. Diabetes Metab Res Rev 18:451–463
Tarasov A, Dusonchet J, Ashcroft F (2004) Metabolic regulation of the pancreatic β-cell ATP-sensitive K^+ channel: a pas de deux. Diabetes 53(Suppl 3):S113–S122
Tato I, Bartrons R, Ventura F, Rosa JL (2011) Amino acids activate mammalian target of rapamycin complex 2 (mTORC2) via PI3K/Akt signaling. J Biol Chem 286:6128–6142
Tengholm A, Gylfe E (2009) Oscillatory control of insulin secretion. Mol Cell Endocrinol 297:58–72
Tomita T, Masuzaki H, Iwakura H, Fujikura J, Noguchi M, Tanaka T, Ebihara K, Kawamura J, Komoto I, Kawaguchi Y, Fujimoto K, Doi R, Shimada Y, Hosoda K, Imamura M, Nakao K (2006) Expression of the gene for a membrane-bound fatty acid receptor in the pancreas and islet cell tumours in humans: evidence for GPR40 expression in pancreatic β cells and implications for insulin secretion. Diabetologia 49:962–968
Tremblay F, Lavigne C, Jacques H, Marette A (2007) Role of dietary proteins and amino acids in the pathogenesis of insulin resistance. Annu Rev Nutr 27:293–310
Westermark PO, Lansner A (2003) A model of phosphofructokinase and glycolytic oscillations in the pancreatic β-cell. Biophys J 85:126–139
Wiederkehr A, Wollheim CB (2006) Mini-review: implication of mitochondria in insulin secretion and action. Endocrinology 147:2643–2649
Wuttke A, Idevall-Hagren O, Tengholm A (2013) P2Y1 receptor-dependent diacylglycerol signaling microdomains in β cells promote insulin secretion. Faseb J 27:1610–1620

Xu J, Han J, Long YS, Epstein PN, Liu YQ (2008) The role of pyruvate carboxylase in insulin secretion and proliferation in rat pancreatic β cells. Diabetologia 51:2022–2030

Yaney GC, Corkey BE (2003) Fatty acid metabolism and insulin secretion in pancreatic β cells. Diabetologia 46:1297–1312

Yang J, Wong RK, Park M, Wu J, Cook JR, York DA, Deng S, Markmann J, Naji A, Wolf BA, Gao Z (2006) Leucine regulation of glucokinase and ATP synthase sensitizes glucose-induced insulin secretion in pancreatic β-cells. Diabetes 55:193–201

Yu JH, Kim KH, Kim H (2006) Role of NADPH oxidase and calcium in cerulein-induced apoptosis: involvement of apoptosis-inducing factor. Ann N Y Acad Sci 1090:292–297

8 Physiology and Pathology of the Anomeric Specificity for the Glucose-Induced Secretory Response of Insulin-, Glucagon-, and Somatostatin-Producing Pancreatic Islet Cells

Willy J. Malaisse

Contents

Abstract

This review deals with the anomeric specificity of the secretory response of insulin-, glucagon-, and somatostatin-producing cells to D-glucose and D-mannose. In a physiological perspective, emphasis is placed on the experimental findings documenting such an anomeric specificity and its possible determinants, including consideration on the essential role of the anomeric specificity of phosphoglucoisomerase, phosphomannoisomerase, and phosphoglucomutase. The possible role of mutarotase, glucokinase, and a sweet taste receptor is also discussed. The anomeric specificity of the metabolic and functional response to D-glucose in tumoral islet cells is briefly considered. In a pathological perspective, perturbations of such an anomeric specificity in both diabetic subjects and a number of animal models with altered pancreatic islet status are presented. This review thus represents an updated contribution on the anomeric specificity of the metabolic and functional responses of the three major types of islet endocrine cells to selected hexoses.

Keywords

Pancreatic islets α-, β-, and δ-cells • Insulin, glucagon, and somatostatin secretion • D-glucose anomeric specificity

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_52,

Introduction

The anomeric specificity of hexose metabolism represents a ubiquitous phenomenon, concerns distinct hexoses, affects several metabolic pathways, is operative under normal conditions – for instance, in intact cells exposed to equilibrated D-glucose and at normal body temperature – underwent a phylogenic evolution, participates in the fine regulation of metabolic events, coincides with the anomeric specificity of functional events, and may be perturbed in pathological situations (Malaisse et al. 1988).

The present article, which complements prior reviews on the same issue (Malaisse et al. 1983a, 1988; Malaisse 1990), aims mainly at an updated perspective on the physiology and pathology of the anomeric specificity of the glucose-induced secretory response of insulin-, glucagon-, and somatostatin-producing pancreatic islet cells.

Physiological Aspects

Anomeric Specificity of the Secretory Response to D-Glucose. In 1974–1975, several reports revealed that α-D-glucose is more potent than β-D-glucose in stimulating insulin release from the pancreatic islet β-cell (Niki et al. 1974; Grodsky et al. 1974, 1975; Rossini et al. 1974a; Matschinsky et al. 1975a), to stimulate cyclic 3′:5′-adenosine monophosphate accumulation in pancreatic islets (Grill and Cerasi 1975), to induce efflux to phosphate from islets prelabelled with [^{32}P]orthophosphate (Pierce and Freinkel 1975), to protect the β-cell against the diabetogenic action of alloxan (Rossini et al. 1974b, 1975; Niki et al. 1976; Tomita and Kobayashi 1976), to inhibit glucagon secretion from the isolated perfused pancreas (Rossini et al. 1974a; Grodsky et al. 1975; Matschinsky et al. 1975a), and to stimulate proinsulin biosynthesis (Niki et al. 1977).

The system responsible for this situation was first speculated to consist in the interaction of D-glucose anomers with a stereospecific receptor possibly located at the surface of islet cells (Grodsky et al. 1975). However, it was then proposed that glycolysis represents the key component of the sensor device through which the anomers of D-glucose are identified in the pancreatic β-cell as a stimulus for insulin release (Malaisse et al. 1976a, b). Thus, it was documented that the more marked insulinotropic action of α- as distinct from β-D-glucose is associated with a higher glycolytic flux, itself attributable to the stereospecificity of the islet phosphoglucose isomerase (Malaisse et al. 1976a, b). In this respect, it should be underlined that phosphoglucoisomerase indeed displays anomeric specificity towards the α-anomer of D-glucose 6-phosphate but, at variance with previous proposals, towards the β-anomer of D-fructose 6-phosphate as both a substrate and product (Willem et al. 1992). This would favor the direct channeling of β-D-fructose 6-phosphate from phosphoglucoisomerase to phosphofructokinase (Malaisse and Bodur 1991). In the same study, it was also documented that phosphoglucoisomerase displays dual

anomerase activity catalyzing the interconversion of the anomers of either D-glucose 6-phosphate or D-fructose 6-phosphate (Willem et al. 1992).

Three further pieces of information require attention.

First, the activity of mutarotase, as judged from the formation of β-D-glucose from α-D-glucose (10–20 mM), was barely detectable in sonicated rat pancreatic islets (1.0 ± 0.6 ml min^{-1} g^{-1}), while averaging 19.5 ± 1.7 and 13.2 ± 2.2 ml min^{-1} g^{-1} in rat kidney and liver homogenates, respectively (Rasschaert et al. 1987).

Second, in 1983, it was proposed that the anomeric specificity of hexose metabolism in islet cells is attributable to a limited preference of glucokinase for the α-anomer of either D-glucose or D-mannose (Meglasson and Matschinsky 1983; Meglasson et al. 1983). As reviewed elsewhere (Malaisse and Sener 1985), several findings argue against such a view. For instance, the three following sets of observations merit to be mentioned.

The view that the anomeric specificity of glycolysis in intact cells could not be predicted and did not necessarily depend on the anomeric preference of glucose-phosphorylating isoenzyme(s) was first supported by the finding that, in rat intact erythrocytes incubated for 60 min at 8 °C, the output of lactic acid was significantly higher in the cells exposed to α-D-glucose as compared to β-D-glucose, the increment in lactic acid output attributed to β-D-glucose, expressed relative to the mean corresponding value found with α-D-glucose, averaging at 4.0 and 7.0 mM D-glucose, respectively, 39.3 ± 4.2 and 73.7 ± 5.8 % as compared ($p < 0.05$ or less) to reference values of 100.0 ± 5.4 and 100.0 ± 10.4 %. Such a difference contrasted with the fact that in erythrocyte homogenates, which displayed no glucokinase activity, the phosphorylation of the hexose was about 60 % higher with β-D-glucose than α-D-glucose throughout a range of hexose concentrations between 10.0 μM and 10.0 mM (Malaisse et al. 1985a).

A second indication that the rate of D-glucose utilization by islet cells is not regulated solely by the activity of hexokinase and/or glucokinase emerged from a study conducted in either rat pancreatic islet homogenates or intact isolated pancreatic islets exposed to 40.0 mM D-glucose (Sener et al. 1985a). Thus, at 7 °C, the rate of phosphorylation by islet homogenates was lower with α- than β-D-glucose, whether in the absence or presence of exogenous D-glucose 6-phosphate. Yet, at the same high concentration of D-glucose (40.0 mM), the production of ^{3}HOH from α- or β-D-[5-^{3}H]glucose, the glucose-induced increment in lactate production, the oxidation of α- and β-D-[U-^{14}]glucose, and the glucose-induced increment in ^{45}Ca net uptake by intact islets all failed to display a significant anomeric preference, the trend being in favor of higher mean values with α- than β-D-glucose, in mirror image of the phosphorylation data collected in islet homogenates. It should be stressed that, in the same study, it was duly verified that the preference for α-D-glucose in terms of either D-[5-^{3}H]glucose utilization or ^{45}Ca net uptake could be evidenced at a lower concentration of D-glucose (6.0 mM). In fair agreement with these findings, no anomeric specificity of the insulin secretory response to 40.0 mM D-glucose could be documented in experiments conducted in rat isolated perfused pancreases (Sener et al. 1985a).

A further and strong argument against the possible role of glucokinase as a determinant of the anomeric specificity of the metabolic, cationic, and functional response of rat pancreatic islets to D-glucose was found in experiments conducted in either rat pancreatic islets or rat perfused pancreases (Sener et al. 1985b). Thus, in islet homogenates incubated for 60 min at 8 °C, the phosphorylation of the D-glucose anomers (3.3 mM), as judged by either a nonisotopic or radioactive procedure, was higher with β-D-glucose than with α-D-glucose, whether in the absence or presence of exogenous D-glucose 6-phosphate (0.02 mM in the nonisotopic procedure and 3.0 mM in the radioisotopic procedure). Yet, over 60-min incubation at 8 °C, the production of lactic acid by intact islets was much lower with β-D-glucose than α-D-glucose (3.3 mM each). Likewise, over 60-min incubation at 8 °C, the net uptake of ^{45}Ca averaged, respectively, 290 ± 12 fmol/islet in the absence of D-glucose (basal value), 318 ± 18 fmol/islets in the presence of 3.3 mM β-D-glucose, and 401 ± 24 fmol/islet in the presence of 3.3 mM α-D-glucose. Comparable results were obtained in the presence of L-leucine (10.0 mM), which itself augmented ^{45}Ca net uptake to 516 ± 13 fmol/islet. Last, whether in the presence of L-leucine (10.0 mM) or in the presence of 2.0 mM Ba^{2+} and 1.4 mM theophylline but absence of extracellular Ca^{2+}, the increment in insulin output from perfused rat pancreases evoked by α-D-glucose (3.3 mM) was twice higher than that evoked by β-D-glucose (also 3.3 mM) (Sener et al. 1985b).

These considerations are not meant to ignore that the perturbation of the anomeric specificity of glucose-stimulated insulin release in type 2 diabetes could conceivably be attributable, on occasion and at least in part, either to a mutation of the glucokinase gene resulting in alteration of its anomeric specificity (Sener et al. 1996) or to a perturbed modulation of glucokinase anomeric specificity by its regulatory protein (Courtois et al. 2000).

Last, the concept of a glucoreceptor, in its original definition (Matschinsky et al. 1975b), should not be ignored, as duly underlined already in 1987 (Malaisse 1987). And, indeed, investigations concerning the effect of sweet compounds on insulin release (Malaisse et al. 1998) as well as findings concerning the insulinotropic action of L-glucose pentaacetate (Malaisse 1998) drew attention to the possible role of sweet or bitter taste receptors in the insulin secretory response to these insulinotropic agents. Quite recently, it was documented that pancreatic β-cells express the sweet taste receptor and that activation of this receptor by artificial sweeteners stimulate insulin secretion (Nakagawa et al. 2009, 2013a). Moreover, the same investigators revealed that activation of this T1R3 receptor by sucralose or 3-O-methyl-D-glucose augments intracellular ATP content of MIN6 cells, whether in the presence of 5.5 mM D-glucose or higher D-glucose concentrations, as well as in the presence of the mitochondrial fuel methyl succinate (Nakagawa et al. 2013b). It was concluded that D-glucose by acting on T1R3 may promote its own metabolism.

Such a β-cell taste receptor is thought to display preference towards the α-anomer of D-glucose, as prevailing for the recognition of the sweet taste of D-glucose anomers and, hence, as a possible candidate for perturbation of the anomeric specificity of glucose-stimulated insulin release (Malaisse-Lagae and Malaisse 1986).

Anomeric Specificity of the Secretory Response to D-Mannose. The anomeric specificity of the insulinotropic action of hexoses was found to be also operative in the secretory response to D-mannose (Niki et al. 1979; Malaisse-Lagae et al. 1982b). For instance, over 5-min incubation at 37 °C in the presence of 11.1 mM D-mannose freshly dissolved in an iced medium, the output of insulin from isolated rat pancreatic islets averaged 4.15 ± 0.57 μU/islet in the case of α-D-mannose, as distinct ($p < 0.005$) from only 2.03 ± 0.33 μU/islet in the case of β-D-mannose (Malaisse-Lagae et al. 1982b). The α-anomer of D-mannose is also more potent than the β-anomer in augmenting the islet content of aldohexose-1,6-bisphosphates, known to activate phosphofructokinase in pancreatic islets (Sener et al. 1982a), in increasing the output of lactic acid from the islets, in raising the $NADH/NAD^+$ and $NADPH/NADP^+$ ratio in the islets, and in inhibiting ^{86}Rb efflux from prelabelled islets (Malaisse-Lagae et al. 1982b; Sener et al. 1982b). In fair agreement with these results, the α-anomers of either D-glucose or D-mannose were found more efficient than the corresponding β-anomers in protecting rat pancreatic islets against the inhibitory effect of alloxan upon glucose-stimulated insulin release (Sener et al. 1982b). It was eventually proposed that the anomeric specificity of the insulinotropic action of D-glucose and D-mannose is likely to be attributable to the well-established α-stereospecificity of phosphoglucomutase, this enzyme being capable of catalyzing in the islets, from either glucose 6-phosphate or mannose 6-phosphate and fructose-1, 6-bisphosphate, the synthesis of the corresponding aldose-1,6-bisphosphate. The resulting activation of phosphofructokinase by aldose-1, 6-bisphosphates would then lead to a more marked increase in glycolytic and oxidative fluxes in the islets exposed to the α-anomers, as distinct from β-anomers, of either D-glucose or D-mannose (Sener et al. 1982a; Malaisse-Lagae et al. 1982a). In the same perspective, it was documented that phosphomannoisomerase specifically catalyzes the reversible conversion of β-D-mannose 6-phosphate and β-D-fructose 6-phosphate (Malaisse-Lagae et al. 1992). It was thus concluded that the hexose-sensor device of the pancreatic β-cell should be conceived of as an integration of interrelated biochemical reactions in which the hexose serves as a precursor of both glycolytic intermediates and activators and key glycolytic enzymes (Malaisse et al. 1983b).

Anomeric Specificity of the Somatostatin Secretory Response toD-Glucose. The first study documenting the anomeric specificity of glucose-induced somatostatin secretion was published in 1987 (Leclercq-Meyer et al. 1987a). The experiments were performed in the perfused pancreas of six lean (192 ± 9 g body wt.) and six obese (284 ± 19 g) Zucker rats. The perfusate contained L-leucine (10.0 mM) throughout the experiment. In each type of rat, three animals received the D-glucose anomers (3.3 mM for 15 min) in an α1-β1-α2-β2 sequence (α1 referring to the first administration of α-D-glucose and α2 to its second administration) and the three other rats in the opposite order (β1-α1-β2-α2). No significant difference between lean and obese rats was found for the pancreas wet weight, its somatostatin content, the basal somatostatin output, and the integrated somatostatin release during the entire experiment. The interpretation of secretory data took into

account the priming action of D-glucose upon hormonal output as resulting from the repeated administration of the hexose. For such a purpose, the α1/β1 and α2/β2 ratios in hormonal secretion recorded in the α1-β1-α2-β2 series were compared, respectively, to the β1/α1 and β2/α2 ratios found in the β1-α1-β2-α2 series. The ratios recorded in the latter series were eventually expressed relative to the mean values found at the same time and in the same type of animals (lean or obese) in the former series. The biphasic stimulation of insulin and somatostatin release yielded for the β1/α1 and β2/α2 ratios in the β1-α1-β2-α2 series mean values of 38.6 ± 4.3 % (n = 12) for insulin and 56.9 ± 9.4 % (n = 10) for somatostatin, both significantly lower ($p < 0.02$ or less) than the corresponding reference values for the α1/β1 and α2/β2 ratios in the α1-β1-α2-β2 series, i.e., 100.0 ± 6.0 % (n = 12) and 100.0 ± 11.5 % (n = 12) for insulin and somatostatin, respectively. Likewise, in the case of the inhibitory action of D-glucose upon glucagon release, the β/α ratios averaged in the β1-α1-β2-α2 series 48.9 ± 5.6 % (n = 10) as distinct ($p < 0.001$) from 100.0 ± 10.7 % (n = 12) for the α/β ratios in the α1-β1-α2-β2 series. The identical anomeric specificity of this triple secretory response was considered compatible, in terms of the metabolic regulation of hormonal release, with a coordinated behavior of all islet cells (Leclercq-Meyer et al. 1987a).

Tumoral Insulin-Producing Cells. As a kind of transition from physiological to pathological considerations, the anomeric specificity of the metabolic and functional response of tumoral islet cells to D-glucose merits attention. When tumoral insulin-producing cells of the RINm5F line are exposed to low concentrations (0.14–0.83 mM) of the D-glucose anomers, the utilization of D-[5-^{3}H]glucose is significantly higher with α- than β-D-glucose (Malaisse et al. 1985b). At 2.8 mM D-glucose, however, no significant anomeric difference is anymore observed for the utilization of D-[5-^{3}H]glucose, the oxidation of D-[U-^{14}C]glucose or the increase in lactate output above basal value. At an even higher concentration of D-glucose (16.7 mM), the conversion of D-[5-^{3}H]glucose to ^{3}HOH is even significantly higher with β-D-glucose than with α-D-glucose (Malaisse et al. 1985b). Incidentally, in rat pancreatic islets, a rise in D-glucose concentration from 5.6 to 40.0 mM also causes a shift from an α-stereospecific pattern to a situation in which no significant anomeric preference is anymore detected. As a matter of fact, the increase of the β/α ratio for D-[5-^{3}H]glucose utilization by RINm5F cells in the 0.14–2.8 mM range of D-glucose concentrations (logarithmic scale) is grossly parallel to that found in rat pancreatic islets in the 5.6–40.0 mM range of hexose concentrations (also logarithmic scale).

Likewise, when the anomeric specificity of glucose-induced protein biosynthesis was examined in RINm5F cells incubated for 5 min at 37 °C in the presence of L-[4-^{3}H]phenylalanine, the incorporation of the tritiated amino acid into TCA-precipitable material was significantly higher with α- than β-D-glucose in the 0.15–0.40 mM range of anomer concentration, but no more so in the 1.4–16.7 mM range (Barreto et al. 1987). Incidentally, in RINm5F cells, the relative magnitude of the glucose-induced increase in protein biosynthesis relative to basal value is much higher than that recorded within the same experiments for the glucose-induced increment in insulin output also expressed relative to basal value (Valverde et al. 1988).

Last, in RINm5F cells exposed to 2.8 mM D-glucose, alike in rat pancreatic islets exposed to 5.6 mM D-glucose, the metabolism of the hexose in the pentose cycle also displays anomeric specificity, the ratio in $^{14}CO_2$ generation from D-[1-^{14}C] glucose/D-[6-^{14}C]glucose, the fraction of D-glucose metabolism occurring through the pentose cycle, and the flow rate through such a cycle being always higher with β- than α-D-glucose, as expected from the anomeric specificity of glucose-6-phosphate dehydrogenase (Malaisse et al. 1985c).

Pathological aspects

Diabetic Subjects. Attention was then paid to a possible alteration of the anomeric specificity of the secretory response to D-glucose in pathological situations. In terms of insulin release, a perturbation of the anomeric specificity was first documented by comparing in normal and non-insulin-dependent diabetic subjects the time course of changes in plasma insulin concentration after intravenous administration in randomized order and 60 min apart of either α- or β-D-glucose (Rovira et al. 1987). In seven normal subjects injected with 5.0 g of each anomer, the α/β paired ratio for plasma insulin concentration (expressed relative to the zero time reference value) averaged 160 ± 53, 129 ± 14, and 116 ± 9 % 2, 4, and 6 min after the injection of the D-glucose anomers, with an overall mean value of 134 ± 15 % (n = 21) indicating a higher insulin response to α- than β-D-glucose. This was confirmed in a further series of 8 normal subjects injected with only 3.5 g of D-glucose, with an α/β ratio 2–6 min after such an injection averaging 147 ± 11 % (n = 40). It should be stressed that, in this second series of normal subjects, as in the first one, the α/β ratio for plasma insulin concentration progressively decreased after injection of the D-glucose anomers. The first sample in which the concentration of insulin was significantly higher than basal value was collected 2 min after injection of the D-glucose anomers and yielded, in these two series of experiments conducted in normal subjects, the highest mean α/β ratio. In eight diabetic subjects, the secretory response to D-glucose was insufficient to allow characterization of its anomeric specificity. In the remaining five patients, a preferential response to α-D-glucose was observed in three cases, but not so in the other two cases. The severity of diabetes, as judged from both the basal plasma glucose and insulin concentrations, was more pronounced in the two diabetic subjects displaying a mean α/β ratio of only 88 ± 5 % 3–6 min after injection of 3.5 g D-glucose than in the three diabetic subjects in which the α/β ratio averaged over the same period 151 ± 11 % (n = 12). The eight patients in whom the anomeric specificity could not be assessed were, according to the same criteria, the most severely diabetic subjects. It was concluded that the apparent perturbation of the anomeric specificity of glucose-induced insulin release in certain diabetic subjects warrants more extensive investigation on its precise incidence and etiopathogenic significance (Rovira et al. 1987).

Further information on the possible perturbation of the anomeric specificity of the insulin and glucagon secretory response to D-glucose was collected in animal models.

Zucker Rats. First, in the experiments conducted in Zucker rats and already considered above, two anomalies of the hormonal secretory response to the anomers of D-glucose were observed. First, the data for somatostatin release collected for one lean rat in the β1-α1-β2-α2 series could not be used because the hormonal release remained undetectable (<0.1 pg/min) throughout the experiment, while in the other Zucker rats, the basal somatostatin output averaged 1.7 ± 0.6 pg/min (Leclercq-Meyer et al. 1987a). Second, the data for glucagon release collected for another lean rat in the β1-α1-β2-α2 series were also discarded because, in this animal, α-D-glucose failed on two successive occasions to affect glucagon output. More precisely, in this lean rat, β-D-glucose caused both a sizeable stimulation of insulin release and an inhibition of glucagon output, while α-D-glucose, which also stimulated insulin release, failed to inhibit glucagon output. Moreover, in the same lean rat, the mean rate of glucagon secretion recorded in the absence of D-glucose (201 pg/min) was much lower than in the other five lean rats (1,085 ± 158 pg/min). Incidentally, when ignoring the glucagon data recorded in the same lean rat, the mean values for the integrated insulin and glucagon output over a period of 155 min were 2–3 times higher ($p < 0.05$ in both cases) in obese animals than in lean rats (Leclercq-Meyer et al. 1987b).

BB Rats. An obvious alteration of the insulin secretory response to D-glucose anomers was then observed in diabetic BB rats (Leclercq-Meyer et al. 1987c). In this study, 13 normoglycemic and 11 diabetic BB rats of comparable age and body weight were examined in the fed state. The pancreases were perfused in the presence of L-leucine (13.0 mM). The anomers of D-glucose (3.3 mM) were injected for two periods of 15 min each (min 41–55 and 76–90) in either an α-β or a β-α sequence. Two diabetic rats in which D-glucose virtually failed to stimulate insulin release were not further considered. The glucose-induced increment in insulin output was judged from the integrated release during exposure to each anomer and the paired control output (min 38–40 and 73–75). Despite a much lower insulin content of the pancreas, the control insulin output (μU/min) was twice higher ($p < 0.001$) in diabetic than in normoglycemic BB rats. The glucose-induced increment in insulin output (μU/min) was twice lower, however, in the diabetic BB rats than in the control animals. In order to correct for the priming action of D-glucose at the occasion of the two administrations of the hexose anomers, the mean α/β ratio for the glucose-induced increment in insulin output recorded in the rats injected in the α-β sequence was expressed relative to the mean β/α ratio in the animals injected in the opposite β-α sequence. This calculation yielded, in the normoglycemic rats, a mean value of 2.35 ± 0.59 ($df = 11$; $p < 0.05$ as compared to unity). In the diabetic rats, the same variable was much lower ($p < 0.05$) not exceeding 0.64 ± 0.41 ($df = 7$), which is not significantly different from unity ($p > 0.3$). In summary, in the normoglycemic rats, α-D-glucose was about twice more potent than β-D-glucose in augmenting insulin output, while no preference of a-D-glucose could anymore be detected in the hyperglycemic BB rats (Leclercq-Meyer et al. 1987c).

The results obtained in normoglycemic and diabetic BB rats, taken from a local colony, raise the question whether the lack of anomeric specificity, observed in the

diabetic animals, is already present in diabetes-prone BB rats prior to the onset of hyperglycemia. In order to tackle the latter questions, two groups of 13 BW and BB rats each were obtained from colonies maintained at the Møllegaard Breeding Centre (Denmark). BW and BB rats differ from one another, the latter having a higher incidence of diabetes mellitus. Eight BW and seven BB rats aged 58–68 days were used as pancreatic islets donors. No significant difference was found between BW and BB rats, respectively, in terms of body weight, plasma D-glucose, and insulin concentrations or islet insulin content (Malaisse et al. 1988). None of the five remaining BW rats became glycosuric, even when last examined at the age of 124 ± 3 days. However, in the remaining six BB rats, four animals became diabetic between the 64th and 101st days of life, while only two rats remained aglycosuric when last examined at 126 ± 2 days of age. Groups of 100 islets each were perifused for 70 min in the presence of L-leucine (10.0 mM). During the first 45 min, the perifusate also contained 2.8 mM D-glucose in anomeric equilibrium. For the 46th min onwards, either α- or β-D-glucose (7.0 mM) was administered. In both BW and BB rats, the mean secretory response to α-D-glucose exceeded that evoked by β-D-glucose. After only 4–5 min exposure to the hexose anomers, the increment in insulin output, when expressed relative to the paired prestimulatory value (min 43–44), which did not differ significantly in BW and BB rats, was highly significant in islets exposed to α-D-glucose but failed to achieve statistical significance in islets exposed to β-D-glucose. Even after 25 min stimulation, the increment in insulin output, relative to the paired prestimulatory value, remained twice higher in islets exposed to α-D-glucose rather than β-D-glucose, and this in both BW and BB rats. These findings suggest that the perturbation of the anomeric response seen in diabetic BB rats (Leclercq-Meyer et al. 1987c) may be secondary to chronic hyperglycemia.

Neonatal Streptozotocin. Niki et al. then reported that in the rat model of non-insulin-dependent diabetes obtained by a neonatal streptozotocin injection, glucose tolerance at 8–10 weeks of age was obviously impaired, despite normal blood glucose concentration after an overnight fast (Niki et al. 1988). Either the α-anomer or β-anomer of D-glucose, used at a concentration of 10.0 mM, was administered for 20 min after 20-min preperfusion with a basal medium containing 1.67 mM equilibrated D-glucose in either control rats or the diabetic animals. In both cases, 5 rats received the α-anomer of D-glucose and 5 other rats its β-anomer. In the control rats, the α-anomer evoked in both the first peak-shaped and second sustained phases of insulin release, a significantly higher secretory response than the β-anomer. In contrast, the insulin response to α-D-glucose in the diabetic rats was virtually identical to the response to β-D-glucose. The integrated amount of insulin released during the 20-min perfusion with α-D-glucose in the diabetic rats only represented 30.6 ± 8.3 % of that in the control rats (120.7 ± 11.0 vs. 393.4 ± 30.7 ng; $p < 0.001$), while that with β-D-glucose in the diabetic rats was 79.0 ± 7.2 % of that in the control animals (94.4 ± 5.3 vs. 119.5 ± 6.7; $p < 0.01$). In relative terms, the secretory response to α-D-glucose was thus more severely affected than that to β-D-glucose in this animal model of non-insulin-dependent diabetes (Niki et al. 1988).

Duct-Ligated Rabbits. A comparable perturbation of the anomeric specificity of the insulin secretory response to D-glucose was then observed in a study conducted in the perfused pancreas prepared from either 14 control rabbits or nine rabbit which underwent ligation of the pancreatic duct 32–45 days before the perfusion experiments (Fichaux et al. 1991). Advantage was thus taken of the intolerance to glucose provoked in rabbits by ligation of the pancreatic duct (Catala et al. 1986). L-leucine (10.0 mM) was present in the perfusate throughout the experiments. The two anomers of D-glucose were administered for 20 min in a randomized order to achieve final hexose concentrations of either 5.6 or 8.3 mM.

In response to the administration of 5.6 mM D-glucose, the glucose-induced increment in insulin output, expressed relative to paired basal value, was significantly higher, in the control rabbits, with α- than β-D-glucose; as a matter of fact, it did not achieve statistical significance with the β-anomer. In response to the administration of 8.3 mM D-glucose, the increment in insulin output, expressed in the same manner, was again significantly higher, in the control rabbits, with α- than β-D-glucose. A vastly different situation prevailed in the duct-ligated rabbits. First, the basal insulin output recorded prior to the administration of the D-glucose anomers was about thrice higher in the duct-ligated rabbits than in the control animals. Second, the time course of the secretory response to the D-glucose anomers was clearly altered in the duct-ligated rabbits. Thus, a significant increase in insulin output above basal value occurred at a later time in duct-ligated than control animals. Moreover, in the duct-ligated rabbits, no distinction could anymore be made between an early peak-shaped response and a later steady-state output, in sharp contrast to the biphasic pattern found in control rabbits. Last, when the administration of D-glucose was halted, the output of insulin declined more slowly in duct-ligated than control rabbits. Relative to basal value, the insulin secretory response to 8.3 mM (pooled data obtained with α- and β-D-glucose) was not significantly different, however, in five duct-ligated rabbits which displayed, whether in the fed state or after an 18-h period of food deprivation, a mean plasma D-glucose concentration virtually identical to that found in control rabbits, and in eight control animals averaging, respectively, 67.7 ± 15.1 % ($n = 10$) and 66.4 ± 11.1 % ($n = 16$). In four of these euglycemic duct-ligated rabbits, the insulin secretory response to α-D-glucose exceeded ($p < 0.001$) that evoked by β-D-glucose. Such was not the case, however, in the fifth animal, so that the preference for α-D-glucose failed to achieve statistical significance when this subgroup of five rabbits was considered as a whole. In the four other duct-ligated rabbits, frank hyperglycemia was recorded in the fasted and/or fed state. In these four hyperglycemic duct-ligated rats, the insulin secretory response to the anomers of D-glucose (pooled data obtained with α- and β-D-glucose) amounted to no more than 40.4 ± 7.4 % of paired basal value. Moreover, in the same four animals, the secretory response to β-D-glucose always exceeded that evoked by the α-anomer ($p < 0.001$). Interestingly, when the anomer-induced increment in insulin output was expressed relative to the paired basal output, the response to β-D-glucose was not significantly different in normal and duct-ligated rabbits, whether the latter animals were euglycemic or diabetic, and pooling all available data averaged

55.3 ± 8.8 % (n = 17). The response to α-D-glucose, however, decreased from 84.4 ± 18.6 % (n = 8) in control rabbits and 65.1 ± 20.7 % (n = 5) in euglycemic duct-ligated amounts to 30.8 ± 2.1 % (n = 4) in diabetic duct-ligated rabbits ($p < 0.05$ as judged by rank correlation) (Fichaux et al. 1991).

In obvious contrast to the altered anomeric specificity of the insulin secretory response to D-glucose found in the duct-ligated rabbits, the inhibition of glucagon secretion by the anomers of D-glucose was quite similar in control and duct-ligated rabbits, whether in its time course, relative magnitude or anomeric specificity. The sole difference between control and duct-ligated rabbits consisted in a higher basal glucagon output in duct-ligated rabbits (0.95 ± 0.10 ng/min; n = 18) than in control animals (0.52 ± 0.07 ng/min; n = 16). Both α- and β-D-glucose (8.3 mM) significantly decreased glucagon secretion in all cases. As judged by paired comparison, the mean extent of such an inhibition, whether expressed as ng/min or as a percentage of basal glucagon output, was greater with α- than β-D-glucose in control animals (n = 8), in duct-ligated rabbits considered as a whole (n = 9), and even in the hyperglycemic duct-ligated rabbits (n = 4).

These findings were considered as compatible with the view that chronic hyperglycemia leads to a severe alteration of the anomeric preference for α-D-glucose in insulin-producing cells, but not so in glucagon-producing cells (Fichaux et al. 1991).

Diazoxide-Treated Rats. In the light of the findings so far reviewed, it could be expected that, in the transition from the normal to the diabetic behavior, an in-between situation could be encountered, in which the anomeric preference of insulin release for α-D-glucose, while not yet lost, would already be attenuated. Such was indeed found to be the case in rats made moderately hyperglycemic and, for only 48 h, by repeated administration of diazoxide and D-glucose (Leclercq-Meyer et al. 1991). Five rats received, during the 2 days (day 1 and day 2) which preceded the pancreas perfusion experiments, at 8.00 a.m., 4.00 p.m., and 12.00 p.m., an oral administration of diazoxide (50 mg per rat) and D-glucose (500 mg per rat). A seventh last oral dose of the diazoxide-glucose mixture was given to the animals at 8.00 a.m. on the day of the experiments (day 3). The glycemia progressively increased in the diazoxide-treated rats, being eventually on day 3 twice higher than that found in control rats, this coinciding with a thrice lower plasma insulin concentration in the diazoxide-treated rats than in the control animals. The pancreases were perfused for 100 min in the presence of L-leucine (10.0 mM). The α- and β-anomer of D-glucose were administered for 15 min each (min 41–56 and 76–91) with a 20 min interval, either in the α-β or β-α sequence. In addition to six control rats previously examined under the same experimental conditions (Sener et al. 1985b), further experiments were conducted in control rats either in the α-β or β-α sequence. The basal insulin release prior to the administration of the D-glucose anomers and the insulin output found during exposure to such anomers were nearly 50 times higher in the diazoxide-treated rats than in the two control animals, this being tentatively ascribed to a rebound closure of ATP-sensitive K^+ channels after removal of the pancreas from the diazoxide-treated rats. In the diazoxide-treated rats, α-D-glucose was more potent than β-D-glucose in stimulating insulin release.

The anomeric specificity of insulin release was evaluated by calculating the α/β ratios, as judged from the integrated insulin output (min 41–60 and min 76–95) and after correction for basal output (min 40–41 or 75–76). In order to make allowance for the priming action of D-glucose under the present experimental conditions, the individual α/β ratios obtained in the diazoxide rats were eventually expressed relative to the mean value recorded in control animals examined in the same anomeric sequence, i.e., either α-β or β-α. The evaluation of the α/β ratios in insulin output yielded a mean value which was much lower in the diazoxide-treated rats (1.56 ± 0.15; $n = 5$) than in the control rats (3.26 ± 0.67; $n = 8$). When these values were normalized in relation to the sequence of administration of the D-glucose anomers, the a/β ratio in diazoxide-treated rats only represented 49.8 ± 7.6 % ($n = 5$; $p < 0.05$) of the mean control value (100.0 ± 14.4 %; $n = 8$). In this study, the basal glucagon output was comparable in control and diazoxide-treated rats. Both α- and β-D-glucose inhibited glucagon output in either the two control rats or the five diazoxide-treated rats. In the latter rats, as distinct from the two control animals, no anomeric difference in the inhibition of glucagon release was observed. Moreover, the D-glucose anomers appeared to inhibit the release of glucagon to a lesser relative extent in the diazoxide-treated rats than in the control animals. These findings were considered as compatible with the proposal that the anomeric malaise documented in several animal models of diabetes represents the outcome of a progressive process in which the anomeric specificity is first attenuated, then abolished, and eventually even reversed. This sequence of change might thus depend on the severity and duration of the prior hyperglycemic state and, as such, reflect the extent of glycogen accumulation in the islet cells (Marynissen et al. 1990; Malaisse et al. 1991, 1993; Malaisse 1991).

Partially Pancreatectomized Rats. In order to explore the possible significance of insulin-producing cell secretory hyperactivity as a determinant of the process of glucotoxicity, the anomeric specificity of glucose-induced insulin release was then examined in normoglycemic partially pancreatectomized rats (Leclercq-Meyer et al. 1992). Female rats approximately 2 months old were anesthetized, and the splenic portion and the major duodenal part of the pancreas were removed by electrical cauterization. About 80–85 % of the pancreas was considered to be removed in the operated animals. Pancreatic perfusion experiments were performed 48–211 days after partial pancreatectomy (PPX-rats). The perfusate contained L-leucine (10.0 mM) throughout the experiment. The α- and β-anomers of D-glucose (3.3 mM) were administered at a 20-min interval for 15 min (min 41–56 and 76–91) in either an α1-β2 or a β1-α2 sequence. Perfusions using PPX-rats were also performed in which equilibrated D-glucose was administered twice at comparable times and concentrations. Because of the smaller amount of pancreatic tissue present, the flow rate was adjusted to lower value in the PPX-rats than in the control animals. The insulin secretory response to D-glucose was judged from the integrated hormonal output and corrected for the corresponding basal output. The plasma D-glucose concentration on the experimental day was slightly, albeit not significantly, higher in the PPX-rats than control animals, while the plasma insulin concentration was significantly higher in the PPX-rats than control rats. Prior to the first

administration of D-glucose, the output of insulin recorded in the sole presence of L-leucine was higher in PPX-rats (0.40 ± 0.09 ng/min; n = 21) than in control animals (0.06 ± 0.01 ng/min; n = 6). The administration of D-glucose (3.3 mM) always provoked a biphasic stimulation of insulin release. Pooling all available data, the glucose-induced increment in insulin output was not significantly different in control rats (2.47 ± 0.55 ng/min; n = 12) and PPX-rats (1.49 ± 0.24 ng/min; n = 42). Whether in control animals of PPX-rats, an equal number of experiments were conducted in the α1-β2 and β1-α2 sequences. The anomeric specificity of the secretory response was judged from the paired α1/β2 and β1/α2 ratios in glucose-stimulated insulin release. In both control rats and PPX-rats the α1/β2 ratio largely exceeded ($p < 0.01$ or less) the β1/α2 ratio. Such an anomeric preference was not significantly different in control rats and PPX-rats. In the experiments including two successive administrations of equilibrated D-glucose to the pancreas of PPX-rats, the ratio between the first and second secretory response averaged 66.3 ± 8.6 % (n = 7; $p < 0.02$ versus unity), documenting a priming phenomenon. The latter ratio exceeded ($p < 0.005$) the β1/α2 ratio and was lower ($p < 0.09$) than the α1/β2 ratio recorded in the PPX-rats. These findings support the view that sustained hyperglycemia and its undesirable metabolic consequences in insulin-producing pancreatic islet cells, e.g., glycogen accumulation, rather than the secretory status of these cells, in terms of normal or prolonged hyperactivity, play a key role in the perturbation of the anomeric specificity of glucose-stimulated insulin release often found in type 2 diabetes (Leclercq-Meyer et al. 1992).

Starved Rats. Somehow in mirror image of the preceding study, a last set of investigations aimed at exploring whether starvation affects the anomeric specificity of glucose-induced insulin secretion in the perfused rat pancreas (Leclercq-Meyer et al. 1993). Ten female Wistar rats fasted for 48 h were used in these investigations. The experimental design was the same as that used in the preceding study, and the control fed rats of the same sex and strain were also the same as those which served as control animals in the preceding study (Leclercq-Meyer et al. 1992). The data relative to glucagon secretion in these six fed control rats had not yet been reported and were provided in this last report. The plasma D-glucose and insulin concentrations were lower in fasted rats than in fed animals. The basal glucagon output (ng/min) and total glucagon output (ng) were both significantly higher ($p < 0.001$) in fasted rats than in fed animals. The administration of the D-glucose anomers (3.3 mM), whether in the α1-β2 or β1-α2 sequence, induced a biphasic stimulation of insulin release in the 48-h fasted rats. In these rats, the α1/β2 ratio was twice higher than the β1/α2 ratio ($p < 0.01$), indicating that α-D-glucose was more potent than β-D-glucose in stimulating insulin output. No significant difference was found between either the α1/β2 or β1/α2 ratio when comparing the results recorded in fed and starved rats. At the most, a trend towards a slight attenuation of the anomeric specificity of glucose-induced insulin release was observed in starved rats, the response to α-D-glucose relative to β-D-glucose (taken as the square root of the quotient obtained after division of the mean α1/β2 ratio by the mean β1/α2 ratio) averaging 1.48 ± 0.17 in starved rats, as compared to 2.18 ± 0.33 in fed animals. Both α- and β-D-glucose inhibited glucagon secretion,

whether in fed or starved rats. There was only a trend towards a lesser relative inhibitory effect of β-D-glucose, as compared to α-D-glucose, upon glucagon output in both fed and starved rats. The relative magnitude of the inhibitory action of the D-glucose anomers upon glucagon release was less pronounced, however, in starved than fed rats in the case of both the α-anomer ($p < 0.01$) and β-anomer ($p < 0.01$). It was concluded that the influence of starvation upon the secretory response of both insulin-producing and glucagon-producing cells to D-glucose anomers indicates that the inhibitory action of D-glucose upon glucagon release is not secondary to stimulation of insulin secretion by the hexose and further suggests that the anomeric malaise of insulin release in models of β-cell glucotoxicity cannot be solely attributed to an impaired utilization of exogenous D-glucose by islet cells, as indeed observed in starvation (Levy et al. 1976; Malaisse and Malaisse-Lagae 1992).

Conclusion

In conclusion, the present review provides an updated overview of the anomeric specificity of the secretory response of insulin-, glucagon-, and somatostatin-producing cells to such hexoses as D-glucose and D-mannose, with emphasis on both physiological aspects, especially the biochemical determinants of such an anomeric specificity, and pathological aspects in either diabetic subjects or various animal models of disturbed pancreatic islet function.

References

Barreto M, Valverde I, Malaisse WJ (1987) Anomeric specificity of glucose-stimulated protein biosynthesis in tumoral pancreatic islet cells. Med Sci Res 15:1103–1104

Catala J, Bonnafous R, Hollande E (1986) Disturbances in the regulation of glycaemia in rabbits following pancreatic duct-ligation. Biochemical and immunocytochemical studies. Diabet Metab 12:203–211

Courtois P, Bource F, Sener A, Malaisse WJ (2000) Anomeric specificity of human liver and β-cell glucokinase: modulation by the glucokinase regulatory protein. Arch Biochem Biophys 373:126–134

Fichaux F, Marchand J, Yaylali B, Leclercq-Meyer V, Catala J, Malaisse WJ (1991) Altered anomeric specificity of glucose-induced insulin release in rabbits with duct-ligated pancreas. Int J Pancreatol 8:151–167

Grill V, Cerasi E (1975) Glucose-induced cyclic AMP accumulation in rat islets of Langerhans: preferential effect of the α anomer. FEBS Lett 54:80–83

Grodsky GM, Fanska R, West L, Manning M (1974) Anomeric specificity of glucose-stimulated insulin release: evidence for a glucoreceptor? Science 186:536–538

Grodsky GM, Fanska R, Lundquist J (1975) Interrelationships between α and β anomers of glucose affecting both insulin and glucagon secretion by the perfused rat pancreas. Endocrinology 97:573–580

Leclercq-Meyer V, Woussen-Colle M-C, Lalieu C, Marchand J, Malaisse WJ (1987a) Anomeric specificity of glucose-induced somatostatin secretion. Experientia 43:1216–1218

Leclercq-Meyer V, Marchand J, Malaisse WJ (1987b) Anomeric specificity of the insulin and glucagon secretory response to D-glucose in lean and obese Zucker rats. Pancreas 2:645–652

Leclercq-Meyer V, Marchand J, Malaisse WJ (1987c) Alteration of the insulin secretory response to D-glucose anomers in diabetic BB rats. Med Sci Res 15:1535–1536
Leclercq-Meyer V, Marchand J, Malaisse WJ (1991) Attenuated anomeric difference of glucose-induced insulin release in the perfused pancreas of diazoxide-treated rats. Horm Metab Res 23:257–261
Leclercq-Meyer V, Malaisse-Lagae F, Coulic V, Akkan AG, Malaisse WJ (1992) Preservation of the anomeric specificity of glucose-induced insulin release in partially pancreatectomized rats. Diabetologia 35:505–509
Leclercq-Meyer V, Marchand J, Malaisse WJ (1993) Effect of starvation upon the anomeric specificity of glucose-induced insulin release. Diabet Nutr Metab 6:129–134
Levy J, Herchuelz A, Sener A, Malaisse WJ (1976) The stimulus-secretion coupling of glucose-induced insulin release. XX. Fasting: a model for altered glucose recognition by the β-cell. Metabolism 25:583–591
Malaisse WJ (1987) Insulin release: the glucoreceptor myth. Med Sci Res 15:65–67
Malaisse WJ (1990) Physiology and pathology of the pancreatic β-cell glucose-sensor device. The Morgagni prize lecture. In: Crepaldi G, Tiengo A, Enzi G (eds) Diabetes, obesity and hyperlipidemias. Elsevier Science, Amsterdam, pp 3–22
Malaisse WJ (1991) The anomeric malaise: a manifestation of β-cell glucotoxicity. Horm Metab Res 23:307–311
Malaisse WJ (1998) The riddle of L-glucose pentaacetate insulinotropic action. Int J Mol Med 2:383–388
Malaisse WJ, Bodur H (1991) Hexose metabolism in pancreatic islets. Enzyme-to-enzyme tunnelling of hexose 6-phosphates. Int J Biochem 23:1471–1481
Malaisse WJ, Malaisse-Lagae F (1992) Hexose metabolism in pancreatic islets. Oxidative response to D-glucose in fed and starved rats. Acta Diabetol 29:94–98
Malaisse WJ, Sener A (1985) Glucokinase is not the pancreatic β-cell glucoreceptor. Diabetologia 28:520–527
Malaisse WJ, Sener A, Koser M, Herchuelz A (1976a) The stimulus-secretion coupling of glucose-induced insulin release. XXIV. The metabolism of α- and β-D-glucose in isolated islets. J Biol Chem 251:5936–5943
Malaisse WJ, Sener A, Koser M, Herchuelz A (1976b) Identification of the α-stereospecific glucosensor in the pancreatic β-cell. FEBS Lett 65:131–134
Malaisse WJ, Malaisse-Lagae F, Sener A (1983a) Anomeric specificity of hexose metabolism in pancreatic islets. Physiol Rev 63:321–327
Malaisse WJ, Deleers M, Malaisse-Lagae F, Sener A (1983b) Anomeric specificity of hexose metabolism in pancreatic islets. In: Mngola EN (ed) Diabetes 1982, vol 600. Excerpta medica ICS. pp 345–351
Malaisse WJ, Giroix M-H, Dufrane SP, Malaisse-Lagae F, Sener A (1985a) Anomeric specificity of glucose metabolism in a non glucokinase-containing cell. Biochem Int 10:233–240
Malaisse WJ, Giroix M-H, Dufrane SP, Malaisse-Lagae F, Sener A (1985b) Environmental modulation of the anomeric specificity of glucose metabolism in normal and tumoral cells. Biochim Biophys Acta 847:48–52
Malaisse WJ, Giroix M-H, Sener A (1985c) Anomeric specificity of glucose metabolism in the pentose cycle. J Biol Chem 260:14630–14632
Malaisse WJ, Malaisse-Lagae F, Leclercq-Meyer V (1988) Anomeric specificity of hexose metabolism in health and disease. Neth J Med 33:247–256
Malaisse WJ, Giroix M-H, Zähner D, Marynissen G, Sener A, Portha B (1991) Neonatal streptozotocin injection : a model of glucotoxicity? Metabolism 40:1101–1105
Malaisse WJ, Maggetto C, Leclercq-Meyer V, Sener A (1993) Interference of glycogenolysis with glycolysis in pancreatic islets from glucose-infused rats. J Clin Invest 91:432–436
Malaisse WJ, Vanonderbergen A, Louchami K, Jijakli H, Malaisse-Lagae F (1998) Effects of artificial sweeteners on insulin release and cationic fluxes in rat pancreatic islets. Cell Signal 10:727–733

Malaisse-Lagae F, Malaisse WJ (1986) Abnormal identification of the sweet taste of D-glucose anomers. Diabetologia 29:344–345 (Letter)
Malaisse-Lagae F, Sener A, Malaisse WJ (1982a) Phospho-glucomutase: its role in the response of pancreatic islets to glucose epimers and anomers. Biochimie 64:1059–1063
Malaisse-Lagae F, Sener A, Lebrun P, Herchuelz A, Leclercq-Meyer V, Malaisse WJ (1982b) Réponse sécrétoire, ionique et métabolique des îlots de Langerhans aux anomères du D-mannose. CR Acad Sci (Paris) 294(III):605–607
Malaisse-Lagae F, Willem R, Penders M, Malaisse WJ (1992) Dual anomeric specificity of phosphomannoisomerase assessed by 2D phase sensitive ^{13}C EXSY NMR. Mol Cell Biochem 115:137–142
Marynissen G, Leclercq-Meyer V, Sener A, Malaisse WJ (1990) Perturbation of pancreatic islet function in glucose-infused rats. Metabolism 39:87–95
Matschinsky FM, Pagliara AS, Hover BA, Haymond MW, Stillings SN (1975a) Differential effects of α- and β-D-glucose on insulin and glucagon secretion from the isolated perfused rat pancreas. Diabetes 24:369–372
Matschinsky FM, Ellerman J, Stillings S, Rayband F, Pace C, Zawalich W (1975b) Hexoses and insulin secretion. In: Hasseblatt A, von Bruchhausen F (eds) Insulin, part 2. Springer, Heidelberg, pp 79–114
Meglasson MD, Matschinsky FM (1983) Discrimination of glucose anomers by glucokinase from liver and transplantable insulinoma. J Biol Chem 258:6705–6708
Meglasson DM, Schinco M, Matschinsky FM (1983) Mannose phosphorylation by glucokinase from liver and transplantable insulinoma. Cooperativity and discrimination of anomers. Diabetes 32:1146–1151
Nakagawa Y, Nagasawa M, Yamada S, Hara A, Mogami M, Nikolaev VO et al (2009) Sweet taste receptor expressed in pancreatic β-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion. PLoS ONE 4:e5106
Nakagawa Y, Nagasawa M, Mogami H, Lohse M, Ninomiya Y, Kojima J (2013a) Multimodal function of sweet taste receptor expressed in pancreatic β-cells: generation of diverse patterns of intracellular signals by sweet agonists. Endocr J 60:1191–1206
Nakagawa Y, Ohtsu Y, Nagasawa M, Shibata H, Kojima I (2013b) Glucose promotes its own metabolism by acting on the cell surface glucose-sensing receptor T1R3. Endocr J. doi:10.1507
Niki A, Niki H, Miwa I, Okuda J (1974) Insulin secretion by anomers of D-glucose. Science 186:150–151
Niki A, Niki H, Miwa I, Lin BJ (1976) Interaction of alloxan and anomers of D-glucose on glucose-induced insulin secretion and biosynthesis in vitro. Diabetes 25:574–579
Niki A, Niki H, Ozawa T (1977) Induction of insulin biosynthesis by anomers of D-glucose. FEBS Lett 80:5–8
Niki A, Niki H, Miwa I (1979) Effect of anomers of D-mannose on insulin release from perfused rat pancreas. Endocrinology 105:1051–1054
Niki A, Niki H, Niki I, Kunoh Y (1988) Insulin release by glucose anomers in a rat model of non-insulin-dependent diabetes. Diabetologia 31:65–67
Pierce M, Freinkel N (1975) Anomeric specificity for the rapid transient efflux of phosphate ions from pancreatic islets during secretory stimulation with glucose. Biochem Biophys Res Commun 63:870–874
Rasschaert J, Zähner D, Malaisse WJ (1987) Low mutarotase activity in normal and tumoral pancreatic islet cells. Med Sci Res 15:621–622
Rossini AA, Soeldner JS, Hiebert JM, Weir GC, Gleason RE (1974a) The effect of glucose anomers upon insulin and glucagon secretion. Diabeologia 10:795–799
Rossini AA, Berger M, Shadden J, Cahill GF Jr (1974b) β cell protection to alloxan necrosis by anomers of D-glucose. Science 183:424
Rossini AA, Arcangeli MA, Cahill GF Jr (1975) Studies of alloxan toxicity on the β cell. Diabets 24:516–522

Rovira A, Garrotte FJ, Valverde I, Malaisse WJ (1987) Anomeric specificity of glucose-induced insulin release in normal and diabetic subjects. Diabet Res 5:119–124

Sener A, Malaisse-Lagae F, Malaisse WJ (1982a) Glucose-induced accumulation of glucose-1, 6-bisphosphate in pancreatic islets: its possible role in the regulation of glycolysis. Biochem Biophys Res Commun 104:1033–1040

Sener A, Malaisse-Lagae F, Lebrun P, Herchuelz A, Leclercq-Meyer V, Malaisse WJ (1982b) Anomeric specificity of D-mannose metabolism in pancreatic islets. Biochem Biophys Res Commun 108:1567–1573

Sener A, Giroix M-H, Leclercq-Meyer V, Marchand J, Malaisse WJ (1985a) Anomeric dissociation between glucokinase activity and glycolysis in pancreatic islets. Biochem Int 11:77–84

Sener A, Leclercq-Meyer V, Marchand J, Giroix M-H, Dufrane SP, Malaisse WJ (1985b) Is glucokinase responsible for the anomeric specificity of glycolysis in pancreatic islets? J Biol Chem 260:12978–12981

Sener A, Malaisse-Lagae F, Xu LZ, Pilkis SJ, Malaisse WJ (1996) Anomeric specificity of the native and mutant forms of human β-cell glucokinase. Arch Biochem Biophys 328:26–34

Tomita T, Kobayashi M (1976) Differential effects of α- and β-D-glucose on protection against alloxan toxicity in isolated islets. Biochem Biophys Res Commun 73:791–798, 22

Valverde I, Barreto M, Malaisse WJ (1988) Stimulation by D-glucose of protein biosynthesis in tumoral insulin-producing cells (RINm5F line). Endocrinology 122:1443–1448

Willem R, Biesemans M, Hallenga K, Lippens G, Malaisse-Lagae F, Malaisse WJ (1992) Dual anomeric specificity and dual anomerase activity of phosphoglucoisomerase quantified by two-dimensional phase sensitive ^{13}C EXSY NMR. J Biol Chem 267:210–217

Physiological and Pathophysiological Control of Glucagon Secretion by Pancreatic α-Cells

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_37,

Abstract

Glucagon is a major hyperglycemic hormone secreted by pancreatic α-cells. It plays a key role in glucose homeostasis by counteracting the action of the hypoglycemic hormone insulin and strongly contributing to the correction of hypoglycemia. Its main effect is to stimulate glucose output from the liver. The mechanisms by which glucose controls glucagon secretion are still largely unknown and hotly debated. Glucagon secretion is impaired in diabetes since there is a relative hyperglucagonemia in all forms of diabetes which strongly aggravates hyperglycemia and there is a reduced or absent glucagon response to hypoglycemia particularly in type 1 diabetes. The reasons of these defects are poorly known. This article presents a short overview of the role of glucagon and the proposed mechanisms of control of glucagon secretion in normal conditions and diabetes and briefly comments the anti-glucagon therapies in diabetes.

Keywords

α-Cells • Diabetes • Electrophysiology • Glucagon • Glucose • Hyperglycemia • Islets of Langerhans • K_{ATP} channels • Metabolism • Secretion • Sulfonylurea

Introduction

The glucagon story began in the 1920s when Banting and Best tested the effect of a pancreatic extract in fully pancreatectomized dogs (Lefebvre 2011; Gromada et al. 2007). They observed a mild and transient hyperglycemia preceding the insulin-induced hypoglycemia. In 1923, Kimball and Murlin attributed this hyperglycemia to a specific substance that they named glucagon, or the mobilizer of glucose. For many years, the insulin preparations extracted from pancreas were contaminated by glucagon, but the hyperglycemic effect of glucagon was neglected. Even though the amino acid sequence of glucagon was established by Bomer and his collaborators in 1956, a more complete understanding of the role of glucagon in physiology and disease was not established until the 1970s, when a

specific radioimmunoassay was developed by Unger and his collaborators. Glucagon secretion by pancreatic α-cells was found to be stimulated by hypoglycemia and inhibited by hyperglycemia. Its first and main target is the liver from which it stimulates glucose output to correct hypoglycemia. Therefore, its metabolic actions are opposite to those of insulin. It ensures that the minimal energetic supply is available to the body, particularly to working muscles during exercise, and the brain which is the main consumer of glucose and relies almost entirely on a continuous supply of glucose (Bolli and Fanelli 1999).

Soon after the development of the radioimmunoassay for glucagon, it was found that glucagon secretion was impaired in diabetes. In particular, the glucagon response to hypoglycemia was blunted in type 1 diabetic patients, and a chronic hyperglucagonemia was observed in type 2 diabetic patients which was found to largely contribute to chronic hyperglycemia. These observations reinforced the need to understand the mechanisms of control of glucagon secretion and of impaired secretion in diabetes. However, the scarcity of α-cells, the difficulty to isolate and recognize them in the living state, and the complexity of their interactions with the other cells of the islet have hampered fast progress in the understanding of their physiology. As a result, their study has been strongly neglected for almost 40 years. It is only recently that several observations have revived interest in glucagon. In particular, provocative studies have suggested that glucagon is the *sine qua non* condition of diabetes (Lee et al. 2011, 2012; Unger and Cherrington 2012) and that α-cells can transdifferentiate to β-cells under certain conditions (Thorel et al. 2010; Al-Hasani et al. 2013).

In this chapter, we will review the physiology of glucagon, going from the mechanisms controlling glucagon secretion to the mechanisms of glucagon action. We will mainly focus on the control of glucagon secretion by glucose. We will also briefly review the defects of glucagon secretion in diabetes and the possible therapeutic interventions. The readers who want to have complementary information on the physiology of α-cells can consult several excellent reviews on the topic (Gromada et al. 2007; Quesada et al. 2008; Kawamori et al. 2010).

Biosynthesis of Proglucagon-Derived Peptides and Localization

Glucagon is a 29-amino acid peptide (3,485 Da) belonging to the secretin family. Its amino acid sequence is similar in various mammalian species, including human, pig, cow, hamster, mouse, rat, etc. It is synthetized from a large precursor peptide, pre-proglucagon encoded by chromosome 2 in human. Pre-proglucagon is expressed in pancreatic α-cells, intestinal L-cells (endocrine cells scattered throughout the small and large intestine, with the highest density in the distal ileum and colon), and several regions of the brain (Parker et al. 2010; Gromada et al. 2009). The mRNA of the pre-proglucagon gene is identical in all these tissues (Novak et al. 1987; Drucker and Asa 1988). Many transcription factors control the

expression of the pre-proglucagon gene (Gromada et al. 2007). Interestingly, the promoter region of the pre-proglucagon gene varies between tissues. Thus, in the rat, a sequence of ~1,250 nucleotides of the promoter region directs the expression to pancreatic α-cells, whereas a longer sequence of ~2,250 nucleotides is required to direct the expression of the gene to L-cells (Lee et al. 1992). This tissue-specific regulation of the gene might be different in the human (Nian et al. 1999, 2002). In pancreatic α-cells, insulin represses glucagon gene expression (Philippe 1991), whereas cAMP-producing agents increase it (Philippe 1996).

Human pre-proglucagon is a polypeptide of 180 amino acids residues that is cleaved by a signal peptidase to release a signal peptide made of 20 amino acids and proglucagon made of 160 amino acids (Mayo et al. 2003; Bataille 2007). As shown in Fig. 1, proglucagon contains several pairs of dibasic amino acid residues (Lys-Arg (KR) and Arg-Arg (RR)) which are recognition sites of prohormone convertases (PC) which cleave proglucagon into several peptides with various biological activities. The nature of these peptides is different in L- and α-cells because both cell types express distinct PCs.

In α-cells, the predominant expression of PC2 (PCSK2) leads to the production of glicentin-related pancreatic polypeptide (GRPP or proglucagon 1–30), glucagon (proglucagon 33–61), intervening peptide 1 (IP-1, proglucagon 64–69), and the major proglucagon fragment MPGF (proglucagon 72–160) (Dey et al. 2005; Fig. 1). All these products are secreted in parallel upon stimulation (Holst et al. 1994). It seems that PC2 is necessary and sufficient for cleaving proglucagon in all these fragments (Rouille et al. 1994). In agreement with this, PC2 knockout mice cannot cleave proglucagon in α-cells. Glucagon can be processed by a specific endopeptidase (MGE: miniglucagon-generating endopeptidase) to miniglucagon which corresponds to the C-terminal part of the peptide (Philippe 1996; Mayo et al. 2003; Bataille 2007; Dey et al. 2005; Holst et al. 1994; Rouille et al. 1994; Dalle et al. 2002; Conlon 1988; Orskov et al. 1987, 1994; Holst et al. 2009). Its half-life is very short. It has been suggested that about 3.5 % of the α-cell glucagon is cleaved to miniglucagon which exerts a potent inhibitory effect on β-cells, thus opposing the action of glucagon itself. Miniglucagon is also formed in target tissues. A small percentage of glucagon (<10 %) would be transformed into miniglucagon in one pass through the liver where it would inhibit the plasma membrane Ca^{2+} pump independently of cAMP. The activity of the peptides other than glucagon and miniglucagon is unclear or poorly characterized.

In L-cells, the predominant expression of PC1/3 (PCSK1) generates glicentin (proglucagon 1–69), glucagon-like peptide 1 (GLP-1, proglucagon 72–108 = GLP-1 [1–37]), IP-2 (proglucagon 111–123), and GLP-2 (proglucagon 126–158) (Conlon 1988; Orskov et al. 1987). GLP-1 [1–37] is inactive, requiring *N*-terminal truncation of amino acids 1–6 for activation. It is thus processed to GLP-1 [7–37] (proglucagon 78–108) and GLP-1 [7–36] amide (Holst et al. 2009). Actually, in the current literature, the unqualified designation of GLP-1 often refers to the truncated peptide. In humans, almost all of the GLP-1 secreted from the gut is amidated (Orskov et al. 1994), whereas in many animal species (rodents, pigs), part of the secreted peptide is GLP-1 (7–37). In L-cells, glicentin remains uncleaved or is partially

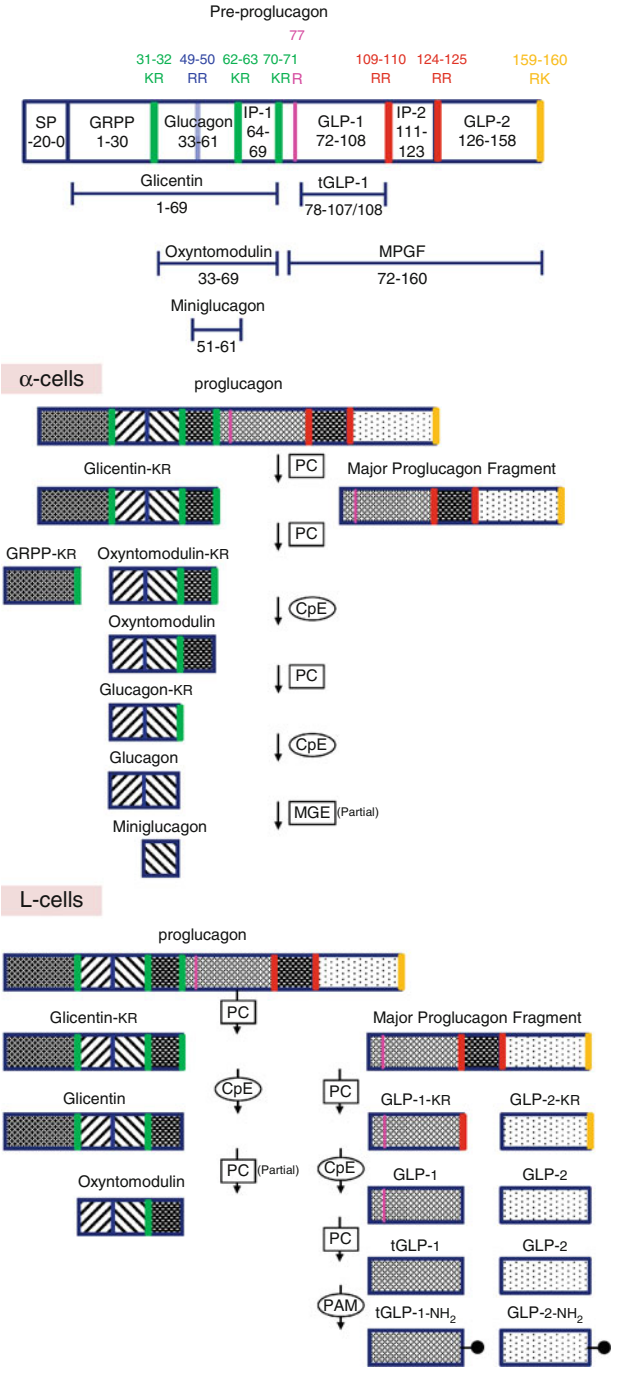

Fig. 1 Structure of the mammalian pre-proglucagon gene and the proglucagon-derived peptides liberated in pancreatic α-cells and intestinal L-cells. See text for details. *CpE* carboxypeptidase-E,

processed to GRPP and oxyntomodulin (proglucagon 33–69). The close homologies between glucagon, GLP-1 and GLP-2 suggest that an ancestral glucagon- or GLP-encoding exon duplicated and then triplicated during evolution (Philippe 1996). The proglucagon-derived peptides with a well-established biological activity in L-cells are thus GLP-1 [7–37], GLP-1 [7–36] amide, GLP-2, and oxyntomodulin. It seems that PC1/3 is both necessary and sufficient to produce these peptides (Zhu et al. 2002; Ugleholdt et al. 2004). Interestingly, adenovirus-forced expression of PC1/3 in pancreatic α-cells leads to GLP-1 production, improving glucose-induced insulin secretion and enhancing β-cells survival (Wideman et al. 2006).

In the central nervous system, both PC1/3 and PC2 are expressed, but regional variations exist, leading to region-specific differences of posttranslational processes (Schafer et al. 1993). As a consequence, both glucagon and GLP-1 are produced in the central nervous system, though predominantly GLP-1 (Kieffer and Habener 1999; Lui et al. 1990; Larsen et al. 1997).

The differential processing of proglucagon in α- and L-cells described above is not a strict rule. Indeed, small amounts of gut-specific peptides can also be found in the pancreas, such as GLP-1 [1–37], GLP-1 [1–36] amide (proglucagon 72–108 or 72–107 amide), and GLP-2 (Holst et al. 1994). Moreover, in the rat pancreas and in the fetal and neonatal pancreas, some pancreatic α-cells express PC1/3, leading to the production of GLP-1 [7–36/37] (Masur et al. 2005; Nie et al. 2000). It is noteworthy that some pathological conditions, such as increased levels of interleukin-6 (Ellingsgaard et al. 2011) or hyperglycemia (Hansen et al. 2011; Whalley et al. 2011), stimulate the expression of PC1/3 and the production of GLP-1 in α-cells.

It is unclear whether extrapancreatic glucagon is produced. Glucagon immunoreactivity was reported in the stomach and gut of various animal species (Sundler et al. 1976; Alumets et al. 1983; Larsson et al. 1975; Grimelius et al. 1976). However, it was later claimed that it could reflect immunoreactivity to glicentin, oxyntomodulin, or other proglucagon-derived peptides but not true glucagon. Therefore, more specific antibodies, directed against the C-terminal part of glucagon, were used. They showed the existence of glucagon-producing cells in the stomach of the dog (Hatton et al. 1985; Lefebvre and Luyckx 1980). Electron microscopy demonstrated that these cells have secretory granules with a structure similar to that of pancreatic α-cells, i.e., with a dense core surrounded by a small pale halo (Sundler et al. 1976). However, in humans, endocrine cells containing glucagon were only found in the stomach of fetuses or neonates, but not in adults (Tsutsumi 1984; Ravazzola et al. 1981; Holst et al. 1983a). It was argued that, in humans, glucagon-producing cells could be outside of the pancreas and the stomach since normal levels of immunoreactive glucagon were reported in totally

Fig. 1 (continued) GRPP glicentin-related pancreatic polypeptide, *GLP* glucagon-like peptide, *IP* intervening peptide, *MGE* miniglucagon-generating endopeptidase, *MPGF* major proglucagon-derived fragment, *PAM* peptidylglycine α-amidating monooxygenase, *PC* prohormone convertase, *SP* signal peptide (Adapted with permission from Bataille (2007))

pancreatectomized and gastrectomized patients (Bringer et al. 1981). Because antibody directed against the C-terminal part of glucagon could also detect glucagon-related products, chromatographic profiles of peptides trapped with a specific glucagon antibody were performed to detect the peak corresponding to true glucagon (Baldissera and Holst 1984). The results are again conflicting. True glucagon was detected in small amounts in the ileum (rich in L-cells) but not in the stomach of normal individuals (Baldissera and Holst 1984). The presence of plasmatic true glucagon was also studied in fully pancreatectomized human patients. Some studies support its presence (Holst et al. 1983b), and others its absence (Bajorunas et al. 1986; Ohtsuka et al. 1986; Barnes and Bloom 1976). The reasons for these discrepancies are unknown and thus leave open the question about the presence of extrapancreatic true glucagon in humans. An indirect argument supporting the possibility that there is indeed bioactive extrapancreatic glucagon is that infusion of somatostatin in pancreatectomized human patients improved glucose tolerance to an oral glucose load (Bringer et al. 1981).

Moreover, if there is really true extrapancreatic glucagon, it is unclear to which extent it contributes to glucagonemia and how its secretion is controlled by glucose. Using antisera specific and nonspecific to glucagon, it was found that oral glucose load elicited a decrease in plasma glucagon and a remarkable rise in plasma total immunoreactive glucagon in pancreatectomized dogs, as in control dogs (Ohneda et al. 1984), whereas arginine increased the plasma concentration of both glucagon and glucagon-like peptides. It should be kept in mind that pancreatectomy might have promoted by an unknown mechanism the production of extrapancreatic glucagon. The same is true for diabetes since PC2 expression was found to be increased in jejunal biopsies of type 2 diabetic patients (Knop et al. 2011). It is however unclear whether this increased expression is present in L-cells. Removal of the duodenum and Roux-en-Y gastric bypass are two other situations associated with an increase in glucagonemia, reflecting again the high plasticity of glucagon production in various (patho)physiological situations (Jorgensen et al. 2013; Muscogiuri et al. 2013).

GLP-1 is rapidly eliminated from the plasma because it is essentially degraded by the enzyme dipeptidyl peptidase IV (DPP-4) which cleaves the two *N*-terminal amino acids, generating a metabolite that is inactive, at least on insulin secretion. Glucagon half-life in the plasma is short (~5 min). Approximately 20 % of glucagon is removed from the circulation by the liver. A larger proportion (~30 %) is removed by the kidney which degrades it. This explains the high glucagonemia of patients suffering from kidney failure. The remaining 50 % of glucagon is destroyed in the circulation by several proteases, including serine and cysteine proteases, cathepsin B, and DPP-4.

Main Physiological Factors Controlling Glucagon Release

Glucagon secretion is physiologically controlled by nutrients, neurotransmitters, and hormones (many of which are also neurotransmitters).

Nutrients

Glucose inhibits glucagon secretion and attenuates glucagon release elicited by various secretagogues such as arginine. By contrast, hypoglycemia (due to fasting, physical exercise, or insulin) strongly stimulates glucagon secretion. These feedback loops minimize fluctuations in plasma glucose levels to protect against severe and possibly life-threatening consequences. The effects of free fatty acids are unclear since inhibitory and stimulatory effects have been reported (see below). Ketone bodies (β-hydroxybutyrate and acetoacetate) inhibit glucagon secretion (Edwards et al. 1969, 1970; Ikeda et al. 1987). By contrast, most amino acids stimulate glucagon release, but their glucagonotropic effects vary between the different types of amino acids (Rocha et al. 1972). Arginine is considered as one of the most effective ones and is often employed in provocative tests of both α- and β-cell functions (Rorsman et al. 1991; Pipeleers et al. 1985a, b; Gerich et al. 1974a). The glucagonotropic effect of amino acids may be physiologically relevant to prevent hypoglycemia after protein intake since amino acids also stimulate insulin secretion (Unger et al. 1969).

Hormones and Neurotransmitters

Many hormones affect glucagon secretion. Adrenaline released by the adrenals is one of the most potent glucagonotropic agents. GIP (gastric-inhibitory polypeptide or glucose-dependent insulinotropic polypeptide), CCK (cholecystokinin), GRP (gastrin-releasing peptide), PACAP (pituitary adenylate cyclase-activating polypeptide), VIP (vasoactive intestinal polypeptide), oxytocin, and vasopressin also stimulate glucagon release (Gromada et al. 2007; Quesada et al. 2008; Gao et al. 1991, 1992; Dunning et al. 2005). By contrast, somatostatin is one of the most potent glucagonostatic agents. Insulin and GABA have frequently been reported to inhibit glucagon release (Dunning et al. 2005) but these effects are debated (see below). GLP-1 [7–36] amide, leptin, ghrelin, and amylin might also exert glucagonostatic effects (Gromada et al. 2007; Unger and Cherrington 2012; Quesada et al. 2008; Tuduri et al. 2009; Marroqui et al. 2011; Chen et al. 2011). For several of these molecules, it is unclear whether the observed effects result from a direct action on α-cells and/or an indirect action via a paracrine factor or another modulator.

Activation of both branches of the autonomic nervous system (parasympathetic and sympathetic) stimulates glucagon secretion, particularly when hypoglycemia is profound. This involves acetylcholine (ACh), noradrenaline, and also, very likely, neuropeptides (Dunning et al. 2005; Havel and Taborsky 1994; Taborsky and Mundinger 2012; Ahren 2000). As it will be seen in the next paragraphs, glucagon is not only controlling glycemia but also the availability of several substrates. It is therefore not surprising that stresses other than hypoglycemia, such as hypoxia (Baum et al. 1979), hyperthermia (Moller et al. 1989), physical stress (Jones et al. 2012; Ramnanan et al. 2011), sepsis, inflammation, trauma, or burns (McGuinness 2005), stimulate glucagon release, i.e., in conditions where it is advantageous to mobilize fuels.

Action of Glucagon

The Glucagon Receptor

Glucagon is the hormone of energetic need. Its main targets are the hepatocytes, the adipocytes, the pancreatic β-cells, the hypothalamus, the gastrointestinal tract, the heart, and the kidney (Svoboda et al. 1994). It exerts its activity by binding to the glucagon receptor which is a G protein-coupled receptor (GPCR) composed of seven transmembrane domains and belonging to the class B GPCR superfamily (Mayo et al. 2003; Siu et al. 2012). The rat and mouse glucagon receptors have 485-amino acids, whereas the human receptor is shorter, with 477 amino acids. Mouse and rat receptors are very similar (93 % identity in amino acid sequence), whereas the human receptor is only 80 % identical to the mouse receptor (Sivarajah et al. 2001). Activation of the glucagon receptor exerts complex effects. By activating Gs, glucagon stimulates adenylate cyclase, leading to production of cAMP and activation of PKA, Epac, cAMP-responsive element-binding protein (CREB), and CREB-regulated transcription coactivator 2 (CRTC2; TORC2) (Jelinek et al. 1993; Koo et al. 2005; Erion et al. 2013). By another pathway, glucagon has been suggested to increase $[Ca^{2+}]_c$. The underlying mechanisms are debated. It might involve the stimulation of phospholipase C (via Gq and/or Gi/o (Xu and Xie (2009)) leading to IP_3 production. However, some investigators have attributed this effect to a cAMP-dependent potentiation of the action of IP_3-producing agents (Mayo et al. 2003; Jelinek et al. 1993; Wakelam et al. 1986; Rodgers 2012; Wang et al. 2012; Authier and Desbuquois 2008; Aromataris et al. 2006; Hansen et al. 1998; Pecker and Pavoine 1996). Experiments on cell lines expressing the glucagon receptor have shown that stimulation of IP_3 production requires high glucagon concentrations (Xu and Xie 2009). Through mechanisms that remain incompletely understood, glucagon also activates AMP-activated protein kinase (AMPK) via Ca^{2+}/calmodulin-dependent protein kinase kinase-β (CaMKKβ or CaMKK2) (Kimball et al. 2004; Peng et al. 2012) and p38 MAPK (Cao et al. 2005), and it induces acetylation of Foxa2, an activator of lipid metabolism and ketogenesis (von Meyenn et al. 2013).

Main Effects of Glucagon and Mechanisms of Action

The major effects of glucagon on glucose homeostasis are prevention and correction of hypoglycemia. It should be stressed that glucagon is not the only hormone involved in these effects. Indeed, prevention and correction of hypoglycemia result from both decrease of insulin, the major hypoglycemic hormone of the body, and increase of several counterregulatory factors which raise glucose: glucagon, catecholamines (i.e., adrenaline, noradrenaline), cortisol, and growth hormone. These factors do not play the same role. Glucagon and catecholamines allow a rapid recovery from acute hypoglycemia and are critical for counterregulation because hypoglycemia develops or progresses when both glucagon and catecholamines are lacking and insulin is present, despite the actions of other glucose-

counterregulatory factors. They exert redundant effects because the lack of glucagon or catecholamime allows recovery from hypoglycemia. However, the role of glucagon is more critical for acute recovery because blockade of catecholamine action (without glucagon deficiency) does not affect the speed of recovery from acute hypoglycemia, whereas glucagon deficiency (without blockade of catecholamine action) slows down the recovery from acute hypoglycemia. Hence, catecholamines are not normally critical, but become critical to glucose counterregulation when glucagon is deficient. It seems however that the role of glucagon is less important if hypoglycemia develops slowly. Growth hormone and cortisol have more long-term effects and are involved in defense against prolonged hypoglycemia (Cryer 1981, 1993, 1996; Gerich 1988).

The effect of glucagon on its main target, the liver, is to stimulate gluconeogenesis and glycogenolysis and to inhibit glycolysis and glycogen synthesis, leading to hepatic efflux of glucose. These effects involve PKA-dependent phosphorylations which are either activating or inactivating (Fig. 2). Three mechanisms explain the stimulation of gluconeogenesis. PKA phosphorylates the bifunctional enzyme fructose-2,6-bisphosphatase (FBP2)/phosphofructokinase-2 (PFK2) leading to activation of FBP2 and inactivation of PFK2. Because FBP2 hydrolyses fructose-2,6-bisphosphate ($F(2,6)P_2$) and PFK2 synthetizes $F(2,6)P_2$, their phosphorylation induces a drop in the concentration of $F(2,6)P_2$. Since $F(2,6)P_2$ is an allosteric inhibitor of fructose-1,6-bisphophatase (FBP1), its drop increases the activity of FBP1 and thus the conversion of fructose 1,6-bisphosphate ($F(1,6)P_2$) into F-6-P. Moreover, by CREB activation, glucagon stimulates the transcription of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6P) (Herzig et al. 2001; Yoon et al. 2001; Barthel and Schmoll 2003). PEPCK catalyzes the conversion of oxaloacetate into phosphoenolpyruvate (PEP), whereas G6P catalyzes the transformation of glucose-6-P into glucose. It should be noted that the stimulatory effect of glucagon on gluconeogenesis depends on the provision of substrates (such as lactate released by muscles, red blood cells or other tissues, glycerol released by adipocytes, and amino acids released by muscles or other tissues) which are under the control of other hormones such as adrenaline. In other words, in the absence of provision of gluconeogenic substrates, the glucagon-induced increase in hepatic glucose production is entirely attributable to an enhancement of glycogenolysis (Ramnanan et al. 2011).

Three mechanisms explain the inhibition of glycolysis. By phosphorylating pyruvate kinase (PKLR), glucagon inhibits the conversion of PEP into pyruvate. It also inhibits the transcription of the PKLR gene. Additionally, since $F(2,6)P_2$ stimulates phosphofructokinase-1 (PFK1), the decrease in $F(2,6)P_2$ described above reduces the activity of PFK1, downregulating glycolysis. Glucagon stimulates glycogenolysis by phosphorylating (via PKA) phosphorylase kinase which phosphorylates and activates glycogen phosphorylase (GP), the enzyme responsible for hydrolysis of glycogen into glucose 1-P, the precursor of glucose. Glucagon inhibits glycogen synthesis by phosphorylating and inactivating glycogen synthase (GS). All these effects are antagonized by insulin.

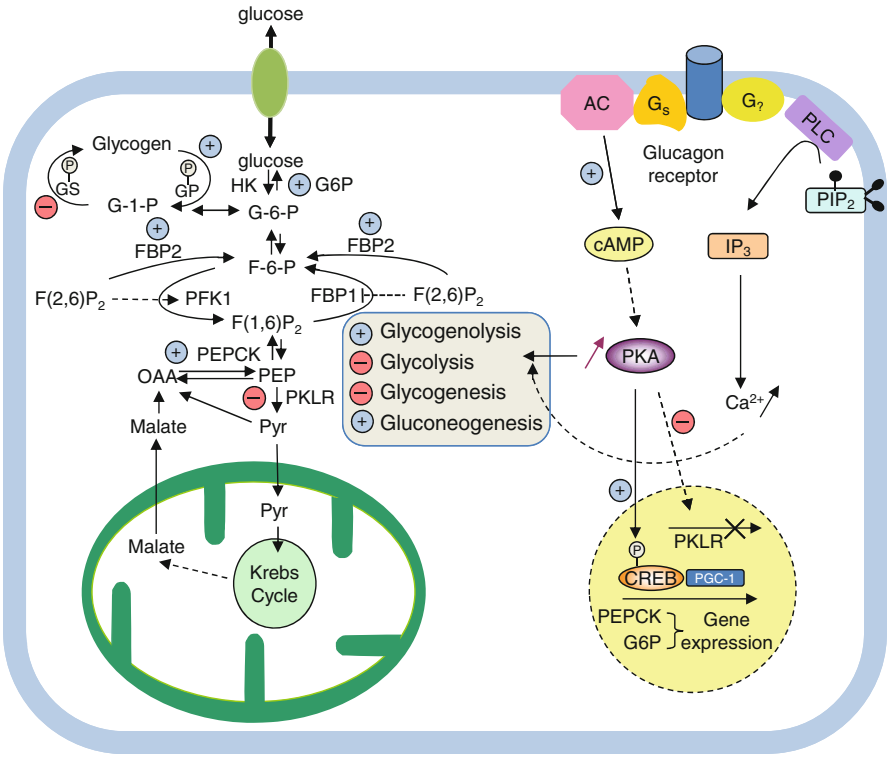

Fig. 2 Mechanisms by which glucagon stimulates gluconeogenesis and glycogenolysis and inhibits glycolysis and glycogen synthesis in the liver. See text for details. *AC* adenylate cyclase, *CREB* cAMP-responsive element-binding protein, *F(1,6)P*$_2$ fructose-1,6-bisphosphate, *F(2,6)P*$_2$ fructose-2,6-bisphosphate, *F-6-P* fructose-6-phosphate, *FBP1* fructose-1,6-bisphosphatase, *FBP2* fructose-2,6-bisphosphatase, *G-1-P* glucose-1-phosphate, *G-6-P* glucose-6-phosphate, *G6P* glucose-6-phosphatase, *GP* glycogen phosphorylase, *GS* glycogen synthase, *HK* hexokinase, *IP*$_3$ inositol 1,4,5-trisphosphate, *OAA* oxaloacetate, *PEP* phosphoenolpyruvate, *PEPCK* phosphoenolpyruvate carboxykinase, *PFK1* phosphofructokinase-1, *PGC-1* peroxisome proliferator-activated receptor-γ-coactivator-1, *PIP*$_2$ phosphatidylinositol 4,5-bisphosphate, *PKLR* pyruvate kinase, *PLC* phospholipase C, *Pyr*, pyruvate (Adapted with permission from Quesada et al. (2008))

It should be stressed that the effect of glucagon on hepatic glucose output is transient. Recent experiments on rodents have suggested that glucagon can cross the blood–brain barrier and act on the mediobasal hypothalamus to suppress, via the vagus nerve, hepatic glucose production, thereby limiting the direct stimulatory effect of glucagon on the liver to a transient phase (Mighiu et al. 2013).

Glucagon inhibits triacylglycerol synthesis and stimulates fatty acid β-oxidation in the liver (von Meyenn et al. 2013; Chen et al. 2005). Inhibition of fatty acid synthesis results from an inactivating phosphorylation and a decreased expression of acetyl-CoA carboxylase (ACC) which transforms acetyl-CoA into malonyl-CoA, the precursor of fatty acids. This lowers plasmatic triglyceride levels. Stimulation of fatty acid β-oxidation results from the drop in malonyl-CoA

concentration which is a potent allosteric inhibitor of carnitine palmitoyltransferase I (CPT1), the pace-setting step in β-oxidation of long-chain fatty acids. In conditions of caloric restriction or pathological insulinopenia (i.e., when lipolysis is stimulated leading to supply of fatty acids), glucagon stimulates the production by the liver of ketone bodies, such as acetoacetate and β-hydroxybutyrate, which provide energy to various tissues including skeletal and cardiac tissues and the brain (fatty acids do not cross the blood–brain barrier). Stimulation of ketogenesis results from the increased concentration of acetyl-CoA which accumulates because of the stimulation of fatty acid oxidation and the inhibition of ACC by glucagon. The increased levels of acetyl-CoA inhibit pyruvate dehydrogenase and activate pyruvate carboxylase. Oxaloacetate thus produced is used for gluconeogenesis rather than for the Krebs cycle, and accumulated acetyl-CoA is condensed into acetoacetyl-CoA, the first intermediate product of ketogenesis. Accumulation of ketone bodies (including acetone) leads to ketoacidosis, a major complication of type 1 diabetes (Unger and Cherrington 2012; Gerich et al. 1975a; Muller et al. 1973).

Glucagon stimulates the uptake of amino acids for gluconeogenesis in the liver. Of the amino acids transported from muscle to the liver during starvation, alanine predominates. The effect of glucagon on amino acid levels is well illustrated by rare cases of glucagonomas in which patients can develop plasma hypoaminoacidemia, especially of amino acids involved in gluconeogenesis, such as alanine, glycine, and proline (Kawamori et al. 2010; Cynober 2002). As a result, glucagon inhibits protein synthesis and stimulates ureogenesis.

Via an increase in cAMP levels and the activation of the hormone-sensitive lipase, glucagon stimulates lipolysis, thereby hydrolyzing triacylglycerol into free fatty acids and glycerol, which are released from adipocytes. Glycerol can then be used by the liver for gluconeogenesis. Again, glucagon-stimulated lipolysis is antagonized by insulin. It is therefore more easily observed in conditions of insulinopenia, and it is not clear whether or not it stimulates lipolysis under normal physiological conditions in human (Ranganath et al. 2001; Arafat et al. 2013; Gravholt et al. 2001). Glucagon also inhibits fatty acid synthesis via an AMPK-dependent inactivating phosphosphorylation of ACC in adipocytes (Peng et al. 2012).

Because most of the effects of glucagon are antagonized by insulin, the insulin/glucagon ratio is physiologically more relevant than the absolute level of glucagon. Other effects of glucagon have been reported, such as positive inotropic and chronotropic effects on the heart, increased thermogenesis, increased glomerular filtration in the kidney, decreased gut motility, etc. (Jones et al. 2012; Bansal and Wang 2008; Ali and Drucker 2009). They often require supraphysiological glucagon concentrations. Glucagon also stimulates insulin release, but the physiological relevance of this effect is questionable because stimulation of insulin secretion is associated with inhibition of glucagon release. Moreover, near-total α-cell ablation did not impair β-cell function (Thorel et al. 2011).

Glucagon and Diabetes

In normal physiological conditions, hypoglycemia is a potent stimulus for glucagon release, and hyperglycemia rapidly decreases plasma glucagon level. Type 1 (T1DM) and type 2 (T2DM) diabetes are characterized by two major defects: an impaired glucagon response to hypoglycemia (Cryer 2002, 2012) and a chronic hyperglucagonemia which has been shown to aggravate hyperglycemia (Unger and Cherrington 2012; Dunning and Gerich 2007; Cho et al. 2012).

Impaired Glucagon Response to Hypoglycemia

In patients with T1DM and advanced T2DM who have β-cell failure or absolute endogenous insulin deficiency, glucagon secretion is not stimulated by hypoglycemia (usually iatrogenic following an excess of insulin caused by treatment with insulin, sulfonylureas, or glinides) (Fig. 3a–b; Cryer 2012; Gerich et al. 1973; Ohneda et al. 1978). This impaired glucagon response to hypoglycemia can lead to life-threatening hypoglycemic episodes. The mechanisms of this defective response are hotly debated. It has been suggested that it results from an impaired activation of the autonomic nervous system (reviewed in Taborsky and Mundinger (2012)), a lack of decrement of insulin secretion (i.e., corresponding to a lack of alleviation of the inhibitory effect of insulin on α-cells = "switch-off" hypothesis, see below) (review by Cryer (2012)), an excessive somatostatin secretion (Yue et al. 2012; Karimian et al. 2013), or an impaired direct action of glucose on α-cells. All these possible mechanisms are not necessarily exclusive.

The defective glucagon response can lead the diabetic patients to be non-adherent to intensive insulin therapy. Moreover, it can generate, after repetitive hypoglycemic episodes, a viscous circle in which lower plasma glucose concentrations are required to trigger counterregulation and hypoglycemia attenuates defenses (including increased epinephrine secretion and symptomatic defenses) against subsequent hypoglycemia. This led to the concept of hypoglycemia-associated autonomic failure (HAAF) according to which hypoglycemia, in combination with an attenuated increment in sympathoadrenal activity and the syndrome of reduced hypoglycemia awareness, induces recurrent hypoglycemia. These problems represent a major barrier preventing blood glucose normalization, and major hypoglycemia can be the direct cause of 2–4 % of deaths in type 1 diabetic patients (Cryer 2002, 2012, 2013). HAAF is generally largely reversible by scrupulous avoidance of hypoglycemia.

Hyperglucagonemia

Several studies showed that plasma glucagon levels are greatly elevated in diabetic ketoacidosis (Muller et al. 1973) and that hyperglucagonemia occurs in type 1

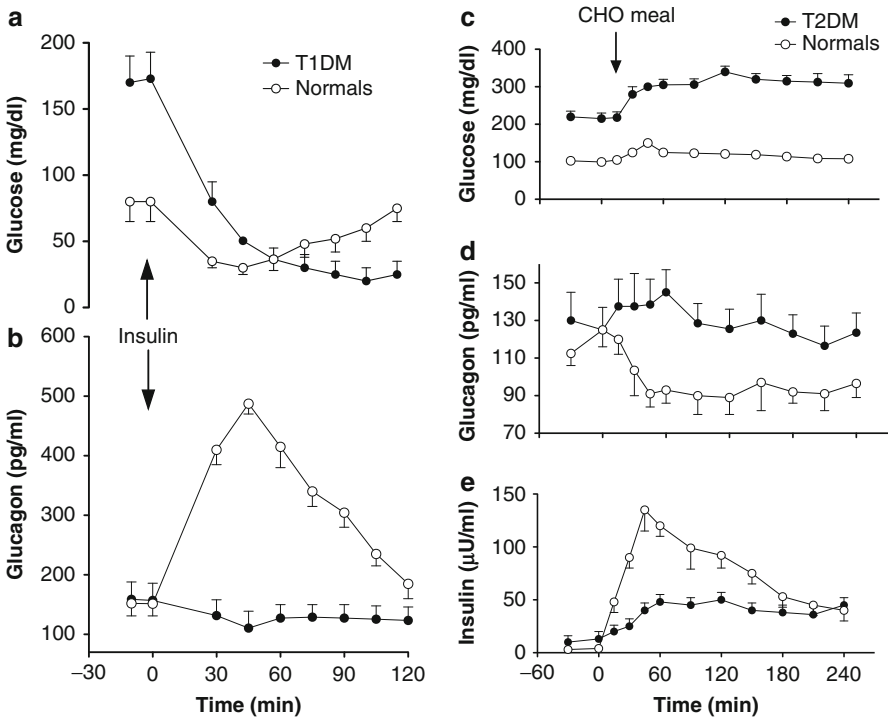

Fig. 3 (**a**–**b**). Plasma glucose and glucagon responses to insulin-induced hypoglycemia in normoglycemic subjects (*open circles*) and in patients with T1DM (*closed circles*). Insulin administration induced a large rise in glucagonemia in response to hypoglycemia in normoglycemic subjects. However, in type 1 diabetic patients, it failed to affect glucagonemia although it induced a prominent hypoglycemia (Adapted with permission from Gerich et al. (1973)). (**c**–**e**). Plasma glucose, glucagon, and insulin responses to a large carbohydrate meal in normoglycemic subjects (*open circles*) and in patients with T2DM (*closed circles*). Ingestion of a large carbohydrate (CHO) meal induced a drop in glucagonemia associated with a robust insulin response in normoglycemic subjects. However, it elicited a transient rise in glucagonemia associated with a larger rise in glycemia and a blunted insulin response in type 2 diabetic patients (Adapted with permission from Dunning and Gerich (2007))

(Raskin and Unger 1978a) and type 2 diabetes (Dunning and Gerich 2007; Raskin and Unger 1978a, b; Solomon et al. 2012; Knop et al. 2007a; Reaven et al. 1987) since plasma glucagon is often inappropriately high in the context of hyperglycemia. Moreover, the decrease of glucagonemia observed in normal individuals during a carbohydrate meal does not occur in patients with T1DM (Hare et al. 2010a) or T2DM (Fig. 3c–e; Dunning and Gerich 2007; Ohneda et al. 1978; Knop et al. 2007a, b). This defect is specific to glucose since these patients display a larger glucagon response to arginine than control individuals (Unger et al. 1970).

The absolute or relative hyperglucagonemia in diabetes plays a key role in the establishment of fasting and postprandial hyperglycemia (see Dunning and Gerich (2007) for review, but see also Raju and Cryer (2005)). The fact that glucagon

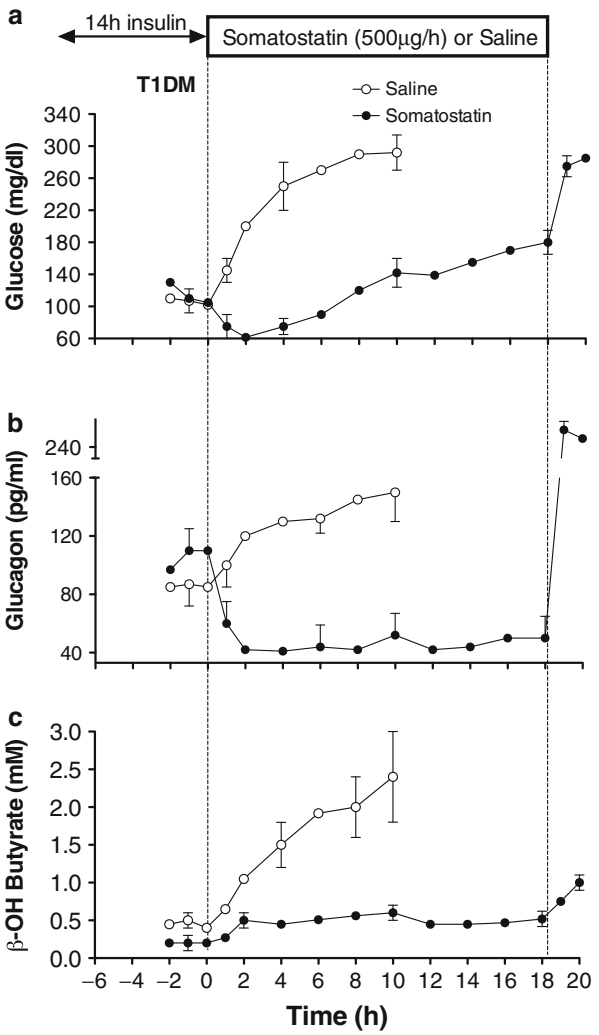

Fig. 4 Effect of suppression of glucagon secretion on plasma levels of glucose, glucagon, and β-hydroxybutyrate during withdrawal of insulin and infusion of somatostatin or saline in patients with T1DM. See text for description (Adapted with permission from Dunning and Gerich (2007))

strongly contributes to hyperglycemia in the fasted state has elegantly been demonstrated in a study performed on patients with T1DM (Fig. 4). In these patients, euglycemia could be maintained during insulin infusion. Insulin withdrawal induced a marked increase in plasma glucose, ketone bodies, and glucagon levels. However, suppression of glucagon secretion by somatostatin infusion prevented the development of diabetic ketoacidosis and the marked fasting hyperglycemia. This also suggests that hypoinsulinemia *per se* does not lead to fasting hyperglycemia and ketoacidosis, but glucagon, by means of its glycogenolytic, gluconeogenic, ketogenic, and lipolytic actions, is necessary for the full development of this condition. The role of hyperglucagonemia in the maintenance of increased rates

of hepatic glucose production in the fasting state has also been amply documented in T2DM (Consoli et al. 1989; Baron et al. 1987).

Likewise, the lack of suppression of glucagon largely contributes to postprandial hyperglycemia in both T1DM (Dinneen et al. 1995) and T2DM (Shah et al. 2000, 1999) and in the impaired glucose tolerance state (Dunning and Gerich 2007; Larsson and Ahrén 2000).

Many recent studies on transgenic mice which have been engineered to block the production or the action of glucagon corroborate these findings. Studies from the group of Unger have even led to the provocative proposal that glucagon is the *sine qua none* condition of diabetes. Indeed, glucagon receptor knockout mice do not develop diabetes upon destruction of their β-cells by streptozotocin (Lee et al. 2011, 2012; Unger and Cherrington 2012).

The mechanism responsible for the excessive glucagon secretion in diabetes is not yet clear. One possibility could be an increased number of α-cells. There is no doubt that the proportion of α-cells per islet is importantly increased in T1DM because of β-cell destruction. However, the absolute number of α-cells per pancreas was reported to be similar in control and T1DM (Orci et al. 1976a; Rahier et al. 1983). Quantifications of α-cell mass in a large number of type 2 diabetic subjects showed that the proportion of α-cells per islet was also increased because of the ~35 % decrease in the β-cell mass (Deng et al. 2004; Henquin and Rahier 2011). However, the absolute α-cell mass was identical in type 2 diabetic and nondiabetic subjects (Henquin and Rahier 2011). Another possibility that has often been mentioned in the literature to explain the chronic hyperglucagonemia in diabetes is based on the suggestion that insulin inhibits glucagon secretion. Hence, hyperglucagonemia would result from an absolute lack of insulin or a resistance of the α-cell to insulin. This concept is also taken into account by the "switch-off" hypothesis (see below). Given that this would involve a loss of paracrine action of insulin on α-cells, diabetes has been qualified as a disease of paracrinopathy (Unger and Orci 2010). Other mechanisms could also contribute to hyperglucagonemia, such as an alteration of paracrine factors other than insulin, an impaired direct action of glucose on α-cells, and gluco- and/or lipotoxic effects on α-cells. A recent study showed that chronic glucose infusion in rats induced a hyperglucagonemia that preceded a decline in insulin secretion, suggesting that glucose toxicity may first manifest as α-cell dysfunction prior to any measurable deficit in insulin secretion (Jamison et al. 2011). However, another study in humans reported that experimental hyperglycemia impaired pancreatic β-cell function but did not acutely impair α-cell glucagon secretion in normal glucose-tolerant subjects (Solomon et al. 2012). Another report showed that glucose stimulated, rather than inhibited, glucagon release of isolated islets of type 2 diabetic patients suggesting that the impaired secretion still occurs ex vivo (Walker et al. 2011). It was also suggested that α-cells of streptozotocin-induced diabetic mice hypersecrete because of increased electrical activity associated with altered electrophysiological characteristics (Huang et al. 2013).

Other observations question the fact that α-cells are not inhibited by glucose in diabetes. Indeed, in T1DM and T2DM, glucagonemia transiently increased and

then decreased during an oral glucose tolerance test, whereas it immediately decreased, like in normoglycemic individuals, during isoglycemic intravenous glucose infusion (Knop et al. 2007a, b; Hare et al. 2010a). This suggests that a gastrointestinal factor, released during the oral glucose load, might be responsible for the increase in glucagonemia (Knop et al. 2007a, 2011). This possibility is supported by the observations that PC2 expression is increased in the jejunum (possibly in L-cells) of patients with T2DM (Knop et al. 2011) and that oral glucose stimulates secretion from L-cells (Diakogiannaki et al. 2012). Hence, the transient rise in glucagonemia observed in diabetes could result from an initial abnormal release of glucagon from L-cells followed by a normal suppression of glucagon release from α-cells. This attractive hypothesis however requires confirmation. Likewise, the chronic hyperglucagonemia found in diabetes could also result from extrapancreatic glucagon production.

As mentioned above, the effect of glucagon on hepatic glucose output is transient in healthy individuals, possibly because glucagon in the hypothalamus counteracts the direct stimulatory effect of glucagon on the liver. It has recently been shown that this feedback loop is disrupted in rodents fed a high-fat diet (hypothalamic glucagon resistance). This suggests that, in pathological states like diabetes, chronic hyperglucagonemia leads to continuous hepatic glucose production and hence to chronic hyperglycemia (Mighiu et al. 2013).

Microanatomy of the Islets of Langerhans

The distribution and abundance of α-cells within islets has been extensively reviewed in the chapter entitled The Comparative Anatomy of Islets of this book. Briefly, it is species dependent. In normal laboratory mice and rats, islets are composed of a central core of β-cells representing ~75 % of the cells (60–90 % depending on the islets) and an outer layer of other endocrine cells including ~20 % of α-cells (10–30 % depending on the islets), <10 % of somatostatin-secreting δ-cells, and 1–5 % of pancreatic polypeptide-secreting PP-cells. The existence of a fifth islet cell type, the ghrelin-producing ε-cells (or X-cells), has sometimes been suggested. It would represent <5 % of the islet cells. However, ghrelin-immunoreactive cells are primarily found during gestational development and would not be terminally differentiated endocrine cells since they give rise to significant numbers of α- and PP-cells (Arnes et al. 2012). The distribution of α-cells can be very different in some transgenic mice. For instance, α-cells are frequently located in central regions of islets in K_{ATP}-deficient mouse models, such as SUR1 (Marhfour et al. 2009) or Kir6.2 knockout mice (Seino et al. 2000; Winarto et al. 2001) or in mice completely or partially deficient in specific adhesion molecule (Esni et al. 1999). The reasons for this altered distribution are unknown. In humans, the proportion of β- and α-cells is more variable between islets than in mice. Moreover, in average, the proportion of β-cells is lower (~55 %, 30–80 % depending on the islets), while that of α-cells is higher (~35 %, 10–60 % depending on the islets) than in mice, predisposing human islets to intense paracrine signaling (Cabrera et al. 2006; Brissova et al. 2005; Bosco et al. 2010;

Steiner et al. 2010; Kilimnik et al. 2009). β-, α-, and δ-cells are found both in the periphery and in the center of the islet (Cabrera et al. 2006). This aspect of random distribution might only concern large islets since small islets (40–60 μm in diameter) would have a similar structure to that of rodent islets with β-cells in the core and α-cells in the periphery, whereas larger islets would be organized in trilaminar epithelial plates with most β-cells occupying a central layer and α-cells being located in the periphery of the plates (Bosco et al. 2010). These plates would be folded with different degrees of complexity to form the islets and bordered by vessels on both sides. The structure of each plate would thus somehow resemble that of rodent islets (Kawamori et al. 2010; Bosco et al. 2010).

In humans, dogs, and rodents, the distribution of α- and PP-cells varies as a function of the region of the pancreas. The head of the pancreas contains islets rich in PP-cells and poor in α-cells, whereas the body and the tail of the pancreas contain islets rich in α-cells and poor in PP-cells (Rahier et al. 1983; Gersell et al. 1979; Louw et al. 1997; Wang et al. 2013; Orci et al. 1976b).

The coupling between islet cells is also different between rodent and human islets. In mouse islets, β-cells are remarkably electrically coupled because of gap junctions made of conexin36 (Ravier and Rutter 2005). In humans, coupling is restricted to small clusters of β-cells within the islet (Cabrera et al. 2006; Nadal et al. 1999; Quesada et al. 2006a). In both rodents and human islets, α- and δ-cells seem to be uncoupled (Nadal et al. 1999; Quesada et al. 2006a).

The blood supply of the pancreas is through the superior mesenteric artery for the head and the splenic artery for the body and the tail of the pancreas. Each islet is richly vascularized. Although the organization of the vascularization has been extensively studied in the past, it is still unclear whether or not there is a directional blood flow from β- to α- and δ-cells supporting an endocrine influence between the different cell types (Cabrera et al. 2006; Samols et al. 1988; Stagner et al. 1988; Brunicardi et al. 1996; Bonner-Weir and Orci 1982). Paracrine interactions do nevertheless exist and allow mutual crosstalk between adjacent cells.

Rodent islets are densely innervated by the sympathetic and parasympathetic nervous system (Ahren 2000; Gilon and Henquin 2001). Recent studies revealed however major differences between mouse and human islets (Rodriguez-Diaz et al. 2011a). In mouse islets, parasympathetic and sympathetic axons densely innervate β-, α-, and δ-cells. In human islets, endocrine cells are barely innervated. Very few parasympathetic axons innervate islet cells, and most axons innervating islet cells are sympathetic and they preferentially contact smooth muscle cells of the vasculature. Surprisingly, contrary to mouse α-cells, human α-cells secrete acetylcholine (Rodriguez-Diaz et al. 2011b).

Glucose Transport and Metabolism in α-Cells

As in β-cells, the transport of glucose into α-cells occurs by facilitated diffusion. However, unlike β-cells, rodent α-cells express mostly GLUT1 (SLC2A1) but not GLUT2 (SLC2A2). Although glucose uptake is 10 times lower in α-cells than in

β-cells, it is not rate limiting for glucose metabolism since it is 5–10 times higher than that of glucose utilization (Heimberg et al. 1995; Gorus et al. 1984). The rate of glucose metabolism in α-cells is only 20–40 % of that observed in β-cells (Gorus et al. 1984; Detimary et al. 1998; Schuit et al. 1997; Mercan et al. 1993). α-Cells possess the low K_m hexokinase I and the β-cell variant of the high Km hexokinase IV, glucokinase (liver- and β-cell-specific glucokinases differ in their 15 amino-terminal residues) (Heimberg et al. 1996; Tu et al. 1999). Hexokinase I is sensitive to its product of reaction since it is inhibited by glucose 6-phosphate and is saturated already at 1 mM glucose, i.e., in the subphysiological range. In contrast to hexokinase I, catalysis via glucokinase is resistance to feedback inhibition by glucose 6-phosphate, allowing glucokinase to sustain high metabolic flux despite elevated intracellular concentration of glucose 6-phosphate. The presence of hexokinase I in α-cells might explain why these cells already respond to very low glucose concentrations (Heimberg et al. 1996; Sekine et al. 1994). Estimations of metabolic fluxes in FACS sorted β- and non-β-cells (mostly α-cells) suggested that glucose metabolism in α- and β-cells is similar at the level of glycolysis but diverges markedly beyond pyruvate formation (Heimberg et al. 1995, 1996; Schuit et al. 1997). Indeed, glucose is almost fully oxidized in β-cells, whereas non-β-cells exhibit rates of glucose oxidation that are 1/3–1/6 those of the total glucose utilization (Gorus et al. 1984; Detimary et al. 1998; Schuit et al. 1997). This difference between the two cell populations is associated with a two to eightfold lower lactate dehydrogenase activity and a twofold higher mitochondrial glycerol-3-phosphate dehydrogenase activity in β-cells *versus* non-β-cells (Schuit et al. 1997; Sekine et al. 1994). The fraction of total glucose utilization that is oxidized to CO_2 strongly increases in β-cells when glycolysis accelerates but only weakly (Detimary et al. 1998) or not at all (Schuit et al. 1997) in non-β-cells. Glucose strongly stimulates anaplerosis (which corresponds to the filling of the Krebs cycle with intermediates that are channeled into anabolic pathways) in β-cells, but not in non-β-cells, possibly because the latter ones have a much lower expression of the anaplerotic enzyme pyruvate carboxylase than β-cells. All these observations suggest that, in contrast to β-cell, the metabolism of glucose in α-cell is mainly anaerobic.

Accordingly, glucose-induced increases in cytosolic ATP (Ravier and Rutter 2005; Ishihara et al. 2003) and NAD(P)H fluorescence (Quesada et al. 2006b; Le Marchand and Piston 2010; Quoix et al. 2009; Mercan et al. 1999) are much smaller in α- than in β-cells. Other studies have even reported that glucose failed to affect the FAD fluorescence in α-cells within islets (Quesada et al. 2006b) and the ATP/ADP ratio in purified α-cells (Detimary et al. 1998). It is possible that the ATP concentration is already very high at low glucose in α-cells. Thus the ATP concentration in rat α-cells was found to be twofold higher (6.5 mM) than that of their β-cell counterparts and the ATP/ADP ratio to be much higher than that of β-cells at low glucose. Interestingly, monomethyl succinate which enters directly into the Krebs cycle causes a similar increase in ATP levels in both α- and β-cells, whereas glucose induces a smaller increase in α-cells than in β-cells (Ishihara et al. 2003). This demonstrates that oxidative metabolism is possible in α-cells, but the passage from glycolysis to the Krebs cycle is inefficient.

The view that glucose metabolism in α-cells is mainly anaerobic is however at variance with a few observations reporting that lactate dehydrogenase and mitochondrial glycerol phosphate dehydrogenase activity are similar in β- and non-β-cells (Jijakli et al. 1996) and that the monocarboxylate transporter 1 (transporting lactate, SLC16A1) are equally low in α- and β-cells (Zhao et al. 2001). The reasons for these discrepancies are unknown.

Uncoupling protein 2 (UCP2) is an inner mitochondrial protein expressed both in α- and β-cells. It was recently shown to mildly dissipate the proton motive force generated during mitochondrial electron transport and to limit mitochondrial reactive oxygen species (ROS) production in α-cells (Allister et al. 2013).

Electrophysiology of α-Cells

Like β-cells, α-cells are electrically excitable, and glucagon secretion is triggered by action potential firing (Gromada et al. 2007; Rorsman et al. 2008; Braun and Rorsman 2010). Their electrical activity depends on the activity of a number of ion channels present in the plasma membrane (Fig. 5). Different types of ion channels have been identified in α-cells, either by their pharmacological sensitivities or by their electrical properties in patch-clamp experiments. Unfortunately, several discrepancies have been reported in the literature. The reasons for these discrepancies are not clear and might be due to the different strains or model of cells used (rat, mouse, human, or guinea pig α-cells or cell lines), cell preparation (isolated α-cells or α-cells in intact isolated islets or in *in situ* pancreas slices; cultured cells or freshly isolated cells), experimental conditions, method of identification/selection of the α-cells (electrical properties, size/whole-cell capacitance, single-cell RT-PCR, fluorescent protein specifically expressed in α-cells (GYY mouse, Quoix et al. (2007)) or in β-cells (MIP mouse, Leung et al. (2005) – in this model, α-cells are selected by exclusion), immunodetection post-experiment, FACS purified cells, expected electrical activity at low glucose), etc.

Capacitance measurements of mouse islet cells have reported a smaller value for α-cells (~4 pF) than for β-cells (~6.2 pF), consistent with their smaller size (Leung et al. 2005; Huang et al. 2011a). The capacitance of α-cells is close to that of δ-cells (~5 pF). Contrary to β-cells, the action potentials of α-cells present very often overshooting phases (>0 mV).

K_{ATP} Channels

Being sensitive to ATP and ADP, ATP-sensitive K^+ channels (K_{ATP} channels) couple cell metabolism to electrical activity because a rise of the ATP/ADP ratio inhibits the K_{ATP} current. In pancreatic β-cells, K_{ATP} channels transduce variations in the blood glucose concentration (and hence in the intracellular ATP/ADP ratio) to changes in insulin secretion. K_{ATP} channels are octamers composed of two unrelated subunits, Kir (K^+ inward rectifier) and SUR (sulfonylurea receptor),

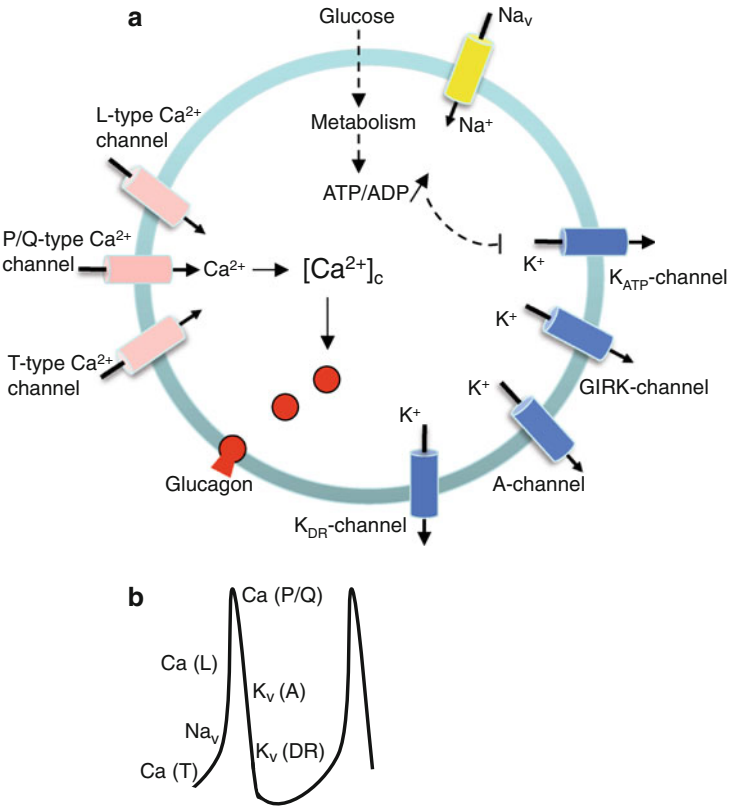

Fig. 5 (**a**) Main types of channels involved in the control of the membrane potential of α-cells. Opening of Na_v and voltage-dependent Ca^{2+} channels (*on the left*) depolarizes the cells, whereas opening of K^+ channels (*on the right*) hyperpolarizes the cells. See text for details. (**b**). Schematic action potentials in human α-cells with the contribution of the main channels to the different phases of a spike. Low-threshold voltage-dependent channels (T-type Ca^{2+} channels and Na^+ channels) allow the membrane potential to reach the threshold for activation of high-threshold voltage-dependent Ca^{2+} channels (L and P/Q type). See text for details (Adapted with permission from Rorsman et al. (2012))

associated in a 4:4 stoichiometry. The channel pore is composed of four Kir6.x subunits and is surrounded by four regulatory SUR subunits (Shyng and Nichols 1997). To have a functional channel, it is necessary that each SUR subunit binds to one Kir6.x subunit. There are two types of Kir6.x subunits, Kir6.1 and Kir6.2, and two types of SUR, SUR1 and SUR2. Their expression depends on the cell type. In β-cells, K_{ATP} channels are composed of Kir6.2 and SUR1 subunits. By binding to SUR1, some drugs directly affect the K_{ATP} current. This is the case of sulfonylureas (tolbutamide, glibenclamide) and non-sulfonylurea compounds (repaglinide, nateglinide) which close K_{ATP} channels and diazoxide which opens them.

Mouse, rat, human α-cells, and clonal glucagon-secreting α-TC6 cells express K_{ATP} channels (Walker et al. 2011; Quoix et al. 2009; Leung et al. 2005; Huang

et al. 2011a, b; Barg et al. 2000; Gromada et al. 2004; Rorsman et al. 2012; Bokvist et al. 1990; Olsen et al. 2005; Macdonald et al. 2007; Rajan et al. 1993; Ronner et al. 1993). Guinea pig α-cells seem however to lack these channels (Rorsman and Hellman 1988). K_{ATP} channels of α-cells have the same subunit composition as in β-cells, i.e., the pore-forming subunit Kir6.2 and the sulfonylurea receptor SUR1 (Bokvist et al. 1999; Franklin et al. 2005; Shiota et al. 2005; Leung et al. 2006a; Suzuki et al. 1997).

Experiments on single α-cells have reported very different densities of K_{ATP} channels (Leung et al. 2005; Barg et al. 2000; Bokvist et al. 1990; Gopel et al. 2000a), which have sometimes been attributed to species differences (rat *vs.* mouse) (Gromada et al. 2007). This kind of comparison should however be taken with care because the results were obtained by different groups with different experimental conditions and a limited number of cells. Indeed, a more recent study performed on a larger number of α-cells in mouse pancreatic slices has reported that the density of the K_{ATP} channels was extremely variable between α-cells and in average slightly lower than that of β-cells (Huang et al. 2011b). In the mouse, the sensitivity of the K_{ATP} channels to ATP is higher in α- than β-cells (Huang et al. 2011b; Leung et al. 2006a), whereas in the rat, it is similar in both cell types (Gromada et al. 2007). Hence, the sensitivity of α-cell K_{ATP} channels to ATP has been suggested to be higher in the mouse than in the rat. However, this conclusion has never been confirmed by direct comparison. If true, the reasons of this differential sensitivity are obscure. In particular, it is unclear whether PIP_2 which decreases the ATP sensitivity of the β-cell K_{ATP} channel is involved since it has been shown to increase (Bokvist et al. 1990) or to not affect (Leung et al. 2005) the α-cell K_{ATP} channel activity.

K_v Channels

Voltage-dependent K^+ channels (K_v) play a major role in the control of the excitability of the cells and are responsible for the repolarizing phase of action potentials. They are encoded by 40 genes in humans and are divided into 12 subfamilies (K_v1–12) (Wulff et al. 2009). They are composed of four α subunits that form the pore of the channel and can be assembled as homo- or heterotetramers and four regulatory β subunits. Identification of the K_v channels in a specific cell type is a real challenge and often requires several approaches. Gene and protein expression analyses are very powerful tool. However, they can hardly be applied to the identification of K_v channels in primary α-cells because they require many cells and it is extremely difficult to obtain pure primary α-cells in large amounts. These analyses have therefore been applied only to the glucagon-secreting α-TC6 cell line. Immunodetections of the α subunits of K_v channels have been performed on primary islet cells but for only a limited number of K_v subtypes. The biophysical properties of all K_v channels have been characterized in details, but it remains a challenge to precisely determine what type of channels underlies a specific K^+

current in a specific cell because several K_v channels are often coexpressed, the biophysical characteristics of some types are very similar, and within subfamilies, such as the K_v1- or K_v7-family, the α-subunits can heteromultimerize resulting in a wide variety of possible channel tetramers. Pharmacological tools can help but they often display partial or poor specificity because of the high homology of many K_v channels.

Gene and protein expression analyses and immunodetections revealed the presence of several K_v channel isoforms in α-TC6 cells (Xia et al. 2007; Hardy et al. 2009): $K_v2.1$, $K_v3.2$, $K_v3.3$, $K_v3.4$, $K_v4.1$, $K_v4.3$, $K_v6.3$, $K_v11.1$ (Erg1), and $K_v11.2$ (Erg2). Immunohistochemistry demonstrated the presence of some K_v channels in primary α-cells, such as $K_v3.1$ and $K_v6.1$ in human α-cells (Yan et al. 2004) and $K_v4.3$ and $K_v3.4$ in mouse α-cells (Gopel et al. 2000b).

On the basis of the biophysical and pharmacological properties, two types of K_v currents in α-cells have been characterized but with species differences:

- K_{DR}: a tetraethylammonium (TEA)-sensitive delayed rectifier slowly inactivating K_v current in mouse (Leung et al. 2005; Barg et al. 2000; Gopel et al. 2000a; Xia et al. 2007; Spigelman et al. 2010) and human α-cells (Spigelman et al. 2010; Ramracheya et al. 2010). On the basis of the use of the $K_v2.1/2.2$ blocker stromatoxin, it has been suggested that K_v2 channels contribute to most of the K_v current in mouse α-cells and to half (Spigelman et al. 2010) or even more (Ramracheya et al. 2010) of the current in human α-cells. $K_v3.2/3.3$ could also contribute to this current in the mouse (Xia et al. 2007).
- A current: several studies, except one (Spigelman et al. 2010), reported the presence of an A-type TEA-resistant and 4-aminopyridine (4-AP)-sensitive transient K_v current in mouse (Leung et al. 2005; Gopel et al. 2000a; Xia et al. 2007), rat, and human α-cells (Ramracheya et al. 2010). Because the A current is absent in δ-cells, it has sometimes been used to recognize α-cells (Gopel et al. 2000b). This A current undergoes steady-state inactivation already at fairly negative voltages, being half-maximal at −68 mV in the mouse (Gopel et al. 2000a; Kanno et al. 2002) and −49 mV in human α-cells (Ramracheya et al. 2010), i.e., at potentials much lower than those inactivating K_{DR}. However, it recovers from inactivation much more rapidly than K_{DR} (Ramracheya et al. 2010). The $K_v4.1/4.3$ subtypes have been suggested to contribute to the A current in mouse α-cells (Leung et al. 2005; Gopel et al. 2000a; Xia et al. 2007; Kanno et al. 2002). This would also be the case in human α-cells since the A current is inhibited by the selective $K_v4.x$-antagonist heteropodatoxin-2 (Ramracheya et al. 2010).

The contribution of both currents to the repolarizing phase of the action potential is shown in Fig. 5b. Because of the peculiar electrophysiological characteristics of the α-cells, it was suggested that, contrary to what occurs in other cells, K_v channels might be positive regulators of glucagon secretion by relieving depolarization-induced inactivation of Na_v or T-type currents involved in the generation of action potentials (see below in the paragraph discussing the direct inhibitory effects of glucose on α-cells).

Other K Channels

- BK channels (Big K), also called Maxi-K or slo1, are encoded by genes other than those of K_v channels. They are activated by depolarization and by a rise in $[Ca^{2+}]_c$ and are involved in the control of the excitability of the cells. The existence of BK in α-cells has been suggested on the observation that iberiotoxin inhibits a voltage-dependent current. Because BK are activated by depolarization, they are activated together with K_v channels. It has been suggested that BK significantly contribute to the K^+ current activated by the depolarization in human α-cells but not in mouse α-cells (Spigelman et al. 2010; Ramracheya et al. 2010).
- G protein-gated inwardly rectifying K^+ channels (GIRK) are activated by G protein-coupled receptors, leading to hyperpolarization of the cell. Four classes have been described (GIRK1–4 corresponding to Kir3.1–3.4). A GIRK current composed of Kir3.2c and Kir3.4 and activated by somatostatin has been described in α-cells (Yoshimoto et al. 1999; Gromada et al. 2001a; Kailey et al. 2012).

HCN Channels

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels play a pacemaker role in cardiac sinoatrial node cells and certain neurons. They are activated by hyperpolarization and cAMP, are permeable to both Na^+ and K^+ ions, and produce a slowly activating inward current (I_f (f for funny because of its peculiar electrophysiological characteristic) or I_h). Hence, they depolarize the cells and initiate action potential firing. They are encoded by four different genes (HCN1–4). The four isoforms have been detected in α-TC6 cells and immunodetection has detected HCN2 in rat α-cells (Zhang et al. 2008). As expected, their activation by hyperpolarization has been shown to elicit an inward current.

Na_v Channels

Voltage-dependent Na^+ channels (Na_v) play a major role in the upstroke phase of the action potential of many electrically excitable cells. Because of their very fast kinetics of activation and often large amplitude, they allow a rapid and coordinated depolarization in response to a change in membrane potential and the propagation of the electrical signal over long distances such as in neurons. They are composed of one α subunit that forms the pore of the channel and is sufficient to generate a Na^+ current. The α subunit is often associated with at least one auxiliary β subunit that modulates the channel activity (Johnson and Bennett 2006). The α subunits are encoded by ten genes. They are all voltage gated except the tenth one (Catterall 2012). All nine voltage-gated channels are blocked by tetrodotoxin but with different potencies (Nieto et al. 2012). Four types of β subunits have been

described. Na_v currents have been recorded in guinea pig (Rorsman and Hellman 1988), mouse (Leung et al. 2005; Gopel et al. 2000a; Vignali et al. 2006), rat (Gromada et al. 1997), and human α-cells (Ramracheya et al. 2010). The Na_v current activates at potentials more positive than -30 mV in mouse (Huang et al. 2013; Leung et al. 2005; Barg et al. 2000) and human α-cells (Ramracheya et al. 2010) and -50 mV in guinea pig α-cells (Rorsman and Hellman 1988). Like their neuronal counterparts, the Na^+ currents in α-cells exhibit steady-state inactivation which occurs at around -40 mV in the mouse (Huang et al. 2011a, b, 2013; Leung et al. 2005; Barg et al. 2000; Gopel et al. 2000a, b; Kanno et al. 2002) and human, and at around -60 mV in guinea pigs (Rorsman and Hellman 1988). These values are much more negative in β-cells (around -100 mV in the mouse (Kanno et al. 2002; Plant 1988)) and have sometimes been used to distinguish both cell types in the mouse (Gopel et al. 2000b; Kanno et al. 2002). Thus, when holding at -80 mV, I_{Na} can be fully activated in α-cells but not in β-cells. Single-cell RT-PCR experiments have suggested that $Na_v1.7$ is expressed in mouse α-cells (Vignali et al. 2006). It has been suggested that the large Na_v current contributes to the generation of the overshoot of the action potentials (Fig. 5b). One study (Leung et al. 2005), but not the others (Barg et al. 2000; Vignali et al. 2006), has reported a lack of Na_v channels in cultured dispersed mouse α-cells suggesting that, under certain circumstances, the Na_v current could be lost.

Ca_v Channels

Voltage-dependent Ca^{2+} channels (VDCC) are key transducers of depolarization into rise in $[Ca^{2+}]_c$ that initiate many physiological events. They are composed of five subunits named α_1, α_2–δ, β, and γ (Catterall 2011). The α_1 subunit, also named Ca_v, is the most important one as it constitutes the channel pore and its expression is sufficient to generate a Ca^{2+} current (Perez-Reyes et al. 1989). Ten different isoforms of the α_1 subunit have been identified and are subdivided in three families ($Ca_v1.1$–1.4, $Ca_v2.1$–2.3, $Ca_v3.1$–3.3). The other subunits are regulatory subunits which modify the expression level and the biophysical and pharmacological properties of VDCC (Lacerda et al. 1991; Singer et al. 1991).

The molecular identity of the Ca_v subunit determines the main biophysical properties of the VDCC (Catterall 2011; Walker and De Waard 1998). According to these properties, it is possible to distinguish two main classes of VDCC: LVA (low-voltage activated) and HVA (high-voltage activated) channels. As indicated by their name, LVA channels are activated at fairly negative voltages usually just above the resting potential and carry a current named T type (transient) because it inactivates quite rapidly. They are composed of the α_1 subunits of the Ca_v3 ($Ca_v3.1$–3.3) family and are specifically inhibited by NNC 55–0396 (Alvina et al. 2009). HVA channels are activated by higher membrane potentials than those activating LVA channels (approximately −40 mV). Because of their slow inactivation, they are the main conduits for Ca^{2+} entry from the external medium. Several types of HVA channels have been described which can be distinguished by

their pharmacological sensitivity: L, P/Q, N, and R types. L-type channels (long lasting) are composed of the α_1 subunits of the Ca_v1 ($Ca_v1.1$–1.4) family. They are specifically blocked by dihydropyridines (Carosati et al. 2006). P/Q-type channels are composed of the $Ca_v2.1$ subunit. The difference between the two types of channels results from different alternative splicing of the α_1 subunit and the type of β subunit associated to the channel. They are inhibited by ω-agatoxin (from the venom of the funnel web spider, *Agelenopsis aperta*) (Catterall et al. 2005). N-type channels are composed of the $Ca_v2.2$ subunit and are specifically and irreversibly blocked by ω-conotoxin GVIA (from the venom of the marine snail *Conus geographus*) (Catterall et al. 2005). R-type channels are composed of the $Ca_v2.3$ subunit. Although they are classified as HVA type channels, their activation potential is between that of HVA and LVA channels (Tottene et al. 1996). They are specifically blocked by SNX-482, a toxin extracted from the venom of the spider tarantula *Hysterocrates gigas* (Catterall et al. 2005).

Gene expression analysis suggested the presence of $Ca_v1.2$ (L type) and $Ca_v2.2$ (N type) in α-TC6 cells (Xia et al. 2007). In primary α-cells, VDCC were first identified by patch clamp in guinea pig α-cells but their precise nature was not determined (Rorsman and Hellman 1988). Later, it was found that rat α-cells have HVA channels which are sensitive to ω-conotoxin GVIA (N type) and nifedipine (L type) (Gromada et al. 1997). The nature of the VDCC channels was studied in detail in mouse α-cells. Except in one report (Vignali et al. 2006), these cells express LVA (T-type channels) (Leung et al. 2005, 2006b; Gopel et al. 2000a). The T-type current is activated at voltages greater than −50 mV and undergoes half-maximal steady-state voltage-dependent inactivation at −45 mV (Gopel et al. 2000a). One study reports that there are two populations of α-cells, a major one possessing T-type channels with an activation threshold of −40 mV and a small one with T-type channels having a lower activation threshold, −60 mV, consistent with a pacemaker role typical of T-type channels (Leung et al. 2006b). Mouse α-cells also express HVA Ca^{2+} channels (Barg et al. 2000; Macdonald et al. 2007; Gopel et al. 2000a; Leung et al. 2006b). As in the rat, 50–60 % of the HVA current would be flowing through L-type channels (nifedipine or isradipine sensitive) (Barg et al. 2000; Macdonald et al. 2007; Vignali et al. 2006) which would be of the $Ca_v1.2$ and $Ca_v1.3$ subtypes (Vignali et al. 2006). It is unclear which channels carry the remaining HVA current. Based on the sensitivity to ω-conotoxin, it has been suggested that it would be carried by N-type channels (Barg et al. 2000; Macdonald et al. 2007; Gopel et al. 2000a). However, this was not confirmed in another study (Vignali et al. 2006), and it was recently acknowledged that the data using ω-conotoxin could have been misinterpreted because of some unspecific effects of the drug (Rorsman et al. 2012). The partial blockade of HVA currents by ω-agatoxin IVA (Rorsman et al. 2012; Vignali et al. 2006) suggests that P/Q Ca^{2+} channels (rather than N type) carry part of the HVA current. The expression of SNX-482-sensitive R-type channels in mouse α-cells is controversial since one study supports its presence (Vignali et al. 2006) and another one its absence (Jing et al. 2005). A fairly similar equipment of Ca^{2+} channels would exist in human α-cells (T-, L-, and P/Q-type Ca^{2+} channels) except that, contrary to the situation in the mouse, the P/Q type would contribute

more than the L type to the HVA current (Rorsman et al. 2012). The steady-state inactivation of the T-type current would be half-maximal at −71 mV in human α-cells (Ramracheya et al. 2010). Fig. 5b illustrates the contribution of the different VDCC to the upstroke phase of the action potential.

Only HVA current allows sufficient Ca^{2+} influx to stimulate exocytosis. However, the role of the various types of Ca^{2+} channels in the control of exocytosis and $[Ca^{2+}]_c$ is complex and controversial. Blockade of L-type channels strongly decreases $[Ca^{2+}]_c$ but seems to have little effect on glucagon release (Quoix et al. 2009; Macdonald et al. 2007; Vieira et al. 2007). Inversely, blockade of non-L-type channels (presumably P/Q type) barely decreases $[Ca^{2+}]_c$ but strongly inhibits exocytosis (Macdonald et al. 2007; Vieira et al. 2007). The proposed explanation for this apparent discrepancy is that the P/Q channels are close to the exocytotic sites, and a tiny increase in their activity is sufficient to dramatically affect exocytosis although it escapes detection by global $[Ca^{2+}]_c$ measurements. By contrast, the L-type channels would be localized further away from the exocytotic sites, and a large increase in their activity would be required to raise $[Ca^{2+}]_c$ enough at the exocytotic sites (Rorsman et al. 2012; Macdonald et al. 2007). However, in the presence of adrenaline which depolarizes the cells and mobilizes Ca^{2+} from the endoplasmic reticulum (Vieira et al. 2004; Liu et al. 2004), Ca^{2+} influx through L-type channels would become the most important to control exocytosis (Rorsman et al. 2012; Gromada et al. 1997; De Marinis et al. 2010). All these hypotheses require confirmation.

Store-Operated Channels

Ca^{2+} channels activated by the emptying of intracellular Ca^{2+} pools are usually called SOCs (store-operated channels). Among the currents elicited by SOCs, some of them named I_{CRAC} (Ca^{2+} release-activated Ca^{2+} current) have a high selectivity for Ca^{2+}, while others called I_{CRAN} (Ca^{2+} release-activated nonselective cationic current) have a poor ion selectivity (Parekh and Putney 2005). Store-operated Ca^{2+} entry (SOCE) is an important Ca^{2+} influx pathway in many non-excitable and some excitable cells. It is regulated by the filling state of intracellular Ca^{2+} stores, notably the endoplasmic reticulum (ER). Reduction in $[Ca^{2+}]_{ER}$ results in activation of plasma membrane Ca^{2+} channels that mediate sustained Ca^{2+} influx which is required for many cell functions as well as refilling of Ca^{2+} stores. Two molecular partners are essential for SOCE: Orai as the pore-forming subunit of the store-operated channel located in the plasma membrane and stromal interaction molecule (STIM) located in the membrane of the endoplasmic reticulum (ER) (Feske 2010). Three Orai isoforms (Orai1–3) and two STIM isoforms (STIM1–2) have been identified. STIM are single-pass transmembrane molecules and contain Ca^{2+}-sensing EF-hand motifs in their N-terminal end facing the ER lumen. STIM1-Orai1 interaction is the best characterized. Emptying of Ca^{2+} from the ER results in dissociation of the ion from STIM1, which rapidly moves and aggregates in the ER in a region close to the plasma membrane to interact with Orai1.

This interaction activates Ca^{2+} entry through Orai1. It has been suggested that another protein, TRPC1 (transient receptor potential channel 1), can also activate SOCE in some cell types (Cheng et al. 2011).

The presence of SOCs in α-cells was suggested by experiments showing that the emptying of the ER by thapsigargin or cyclopiazonic acid (CPA), two inhibitors of SERCAs, induces an influx of Ca^{2+} into the cell (Vieira et al. 2007; Liu et al. 2004). A recent study using mouse and human islets showed that ER Ca^{2+} depletion trigger accumulation of STIM1 puncta in the subplasmalemmal ER where they cocluster with Orai1 in the plasma membrane and activate SOCE (Tian et al. 2012).

Effects of Nutrients Other than Glucose on Glucagon Secretion

Amino Acids

Unlike glucose, which inhibits glucagon secretion and stimulates insulin secretion, amino acids induce the release of both hormones, but their stimulatory effect on glucagon release is more effective at low glucose concentration (Rocha et al. 1972; Pipeleers et al. 1985c; Leclercq-Meyer et al. 1985; Ostenson and Grebing 1985). Arginine, asparagine, glutamate, and alanine strongly stimulate glucagon release, with the first two being the most potent (Rocha et al. 1972; Rorsman et al. 1991; Gerich et al. 1974a; Pipeleers et al. 1985c; Dumonteil et al. 1999).

It is unclear whether the glucagonotropic effect of amino acids results from an activation of α-cell metabolism, a depolarization of the plasma membrane because of charge effects, or another mechanism. It has been suggested that the amino acids with the most potent glucagonotropic effects are the ones which enter the gluconeogenic pathway as pyruvate and are believed to provide most of the amino acid-derived glucose (Rocha et al. 1972). However, charge-dependent effects are also very important for amino acids which are positively charged at physiological pH or are cotransported with Na^+. For instance, glycine which is cotransported with Na^+ increases $[Ca^{2+}]_c$. A similar but less pronounced effect was obtained when Na^+ was cotransported with 10 mM of the non-metabolizable amino acid α-amino-isobutyric acid (Berts et al. 1997).

In the case of arginine, three mechanisms have been suggested. (a) The electrogenic entry of positively charged arginine induces membrane depolarization and activation of high-threshold VDCC, which triggers glucagon release (Gromada et al. 2007). The reason whereby arginine exerts a strong glucagonotropic effect at low glucose is that its depolarizing effect largely depends on the resistance of the plasma membrane and that the resistance of the α-cell plasma membrane is high because most K_{ATP} channels are already closed at low glucose. (b) The activation of NO synthase by nitric oxide (NO) derived from the metabolism of arginine could contribute to the glucagonotropic effect of arginine (Henningsson and Lundquist 1998). (c) A stimulation of PKC by arginine has also been suggested. However, this conclusion was mainly based on the use of an unspecific inhibitor of PKC, H-7 (Yamato et al. 1990). Because the effects of arginine on glucagon secretion and

α-cell membrane potential are of very fast onset, the first mechanism is probably the most important one. A metabolic effect is expected to be of slower onset.

It should be noted that some amino acids could also exert a glucagonotropic effect by acting on specific receptors, similarly to excitatory amino acids in the brain. This possibility has been suggested for glutamate and glycine (Li et al. 2013; Cabrera et al. 2008).

Fatty Acids

The effects of free fatty acids are unclear. Most of the old studies performed *in vivo* or *in vitro*, and in animal species or in man, have reported clear inhibitory effects of free fatty acids (FFAs) on glucagon secretion (Edwards et al. 1969, 1970; Gerich et al. 1974b, 1976a; Seyffert and Madison 1967; Gross and Mialhe 1974; Luyckx et al. 1975), but see also (Andrews et al. 1975). However, more recent studies performed *in vitro* demonstrated glucagonotropic effects of FFA. In particular, short-term exposure to palmitate stimulates glucagon secretion from murine islets (Bollheimer et al. 2004; Hong et al. 2005; Olofsson et al. 2004). It is unclear whether these discrepancies are due to differences of species, experimental conditions, or assays used. It has been suggested that the stimulatory effect of palmitate results from a direct effect on α-cells through an increase of the L-type Ca^{2+} current and an indirect effect through the relief of a paracrine inhibition by somatostatin secreted by δ-cells (Olofsson et al. 2004). Other studies have shown that the chain length of FFAs influences their stimulatory effect on glucagon secretion in mouse islets. It was also shown that the glucagonotropic effect of FFAs increases with the chain length of saturated FFAs and is stronger with saturated than with unsaturated FFAs (Hong et al. 2005).

Contrary to the situation found in β-cells, long-term exposure to palmitate or oleate increases glucagon secretion of mouse and rat islets and αTC1-cells (Hong et al. 2006, 2007) and abolishes the glucagonostatic effect of glucose (Collins et al. 2008; Dumonteil et al. 2000). These effects raise the question whether elevated plasma FFA levels may aggravate the hyperglucagonemia associated with type 2 diabetes.

The Control of Glucagon Secretion by Glucose

The mechanisms by which glucose regulates glucagon secretion are still unclear. In particular, it is unknown whether glucose exerts a direct and/or an indirect effect on α-cells. Three mechanisms have been suggested. (a) Activation of the autonomic nervous system stimulates glucagon secretion in response to hypoglycemia. (b) Secretory products from neighboring cells within the islet (paracrine factors) influence glucagon secretion in response to glucose. (c) Glucose exerts a direct control on α-cell. In this section, we discuss these three possibilities.

Autonomic Regulation of Glucagon Secretion

There are three major autonomic inputs to the islet α-cell: the sympathetic system which secretes noradrenaline, galanin, and neuropeptide Y (NPY); the parasympathetic system which secretes acetylcholine, GRP (gastrin-releasing peptide), PACAP (pituitary adenylate cyclase-activating peptide), and VIP (vasoactive intestinal peptide); and the adrenal medulla which releases adrenaline in response to activation of the sympathetic nervous system (Dunning et al. 2005; Taborsky and Mundinger 2012; Gilon and Henquin 2001; Taborsky et al. 1998; Miki et al. 2001; Burcelin and Thorens 2001; Evans et al. 2004; Minami et al. 2004). Acetylcholine stimulates glucagon secretion via M_3 muscarinic receptors (Duttaroy et al. 2004) and increases $[Ca^{2+}]_c$ (Berts et al. 1997). Adrenaline and noradrenaline stimulate glucagon secretion (Ahren 2000; Ahren et al. 1987) by mobilizing intracellular Ca^{2+}, by increasing cAMP levels, by enhancing Ca^{2+} influx through L-type Ca^{2+} channels via the activation of the low-affinity cAMP sensor Epac2, and by accelerating granule mobilization (Gromada et al. 1997; Vieira et al. 2004; De Marinis et al. 2010). These effects are attributed to β- and α_1-adrenergic receptors (Gromada et al. 1997; Vieira et al. 2004). All three inputs are activated by hypoglycemia and stimulate glucagon secretion (Ahren 2000; Havel et al. 1991, 1993; Havel and Valverde 1996). The magnitude of the activation of these pathways increases as glucose falls from euglycemia to near fatal levels, and their relative contributions to the glucagon response depend on the severity of hypoglycemia (Taborsky and Mundinger 2012; Taborsky et al. 1998; Miki et al. 2001; Burcelin and Thorens 2001; Evans et al. 2004; Minami et al. 2004).

Activation of the autonomic nervous system depends on glucose-sensing neurons in various brain regions and in particular in the ventromedial hypothalamus (VMH) (Borg et al. 1997, 1995; de Vries et al. 2003) and in brainstem neurons of the dorsal motor nucleus of the vagus (DMNX) (Trapp and Ballanyi 1995; Karschin et al. 1997) and the nucleus of the tractus solitarius (NTS) (Dallaporta et al. 2000; Thorens 2011, 2012). Many of the crucial components of the β-cell glucose-sensing mechanism, such as glucose transporters, glucokinase, and K_{ATP} channels, have been identified in neurons within the VMH (Thorens 2011; Kang et al. 2004; Ashcroft and Rorsman 2004). It has been shown that K_{ATP} channels play a key role in sensing hypoglycemia and triggering a counterregulatory glucagon response (Miki et al. 2001; Evans et al. 2004; McCrimmon et al. 2005). AMPK (Han et al. 2005; Alquier et al. 2007; McCrimmon et al. 2004) and GLUT2 in cerebral astrocytes (Marty et al. 2005) are also involved in the counterregulatory response, but the precise mechanisms are still poorly known.

Paracrine Regulation of Glucagon Secretion

It is unclear and still debated whether blood flows from one cell type to another within the islet (Cabrera et al. 2006; Samols et al. 1988; Brunicardi et al. 1996;

Stagner and Samols 1992; Stagner et al. 1989). However, it is quite evident that intense paracrine influences occur inside the islets.

The idea of the existence of a paracrine control of glucagon secretion comes from the observation that type 1 diabetic patients have lost all types of glucagon response to a change in blood glucose (Aguilar-Parada et al. 1969; Unger 1985), suggesting that a factor released from β-cells is responsible for the effect of glucose on glucagon secretion. Many other arguments supporting this view have been reported but only a few will be mentioned here. Two elegant studies performed on isolated islets showed that the specific activation of the metabolism of β-cells using transcriptional targeting strategy induced a suppression of glucagon secretion (Ishihara et al. 2003; Takahashi et al. 2006). Moreover, the specific destruction of β-cells by diphtheria toxin induced a larger glucagon secretion, reflecting the relief of an inhibition by β-cells (Ishihara et al. 2003). The existence of an inhibitory paracrine factor released from islet cells was further suggested by some observations (but not all) that glucose stimulates glucagon secretion from purified α-cells, i.e., in the absence of any possible paracrine influence from non-α-cells (Le Marchand and Piston 2010; Olsen et al. 2005; Franklin et al. 2005). The nature of the β-cell-derived factor is still largely controversial and several of them have been suggested. Since somatostatin is a potent glucagonostatic agent, an alternative hypothesis has also suggested that δ-cells, rather than β-cells, are responsible for the inhibitory effect of glucose.

The paragraphs below briefly review the effects of various paracrine factors on glucagon secretion and discuss their possible involvement in the glucagonostatic effect of glucose.

Pulsatility of Insulin, Glucagon, and Somatostatin Secretion

Two main periods of *in vivo* oscillations of islet hormone secretion have been described. Ultradian oscillations in insulinemia and glucagonemia with a period length of 70–140 min have been attributed to feedback mechanisms of glucose and seem to be entrained to the non-rapid eye movement (non-REM)/REM sleep cycle (Kern et al. 1996). More rapid oscillations of insulinemia, glucagonemia, and glycemia with a period of 5–15 min have been documented (Goodner et al. 1977, 1982; Lang et al. 1979, 1982; Weigle 1987; Jaspan et al. 1986; Meier et al. 2006; Menge et al. 2011; Rohrer et al. 2012). Different opinions have been expressed whether the pulsatile release of insulin and glucagon is a coupled or an independent process. Because plasma insulin cycled nearly in and glucagon nearly out of phase with glucose (Goodner et al. 1977, 1982; Menge et al. 2011; Rohrer et al. 2012), it was suggested that pulses of glucagon/insulin induce pulses of glucose production by the liver and that the resulting pulses of glycemia might in turn induce pulses of hormone release by the pancreas. However, other studies failed to find such relationships between the oscillations of insulin, glucagon, and glucose, suggesting that these oscillations are not controlled by feedback loops (Jaspan et al. 1986; Hansen et al. 1982). One study has also reported synchronous insulin and glucagon oscillations (Lang et al. 1982).

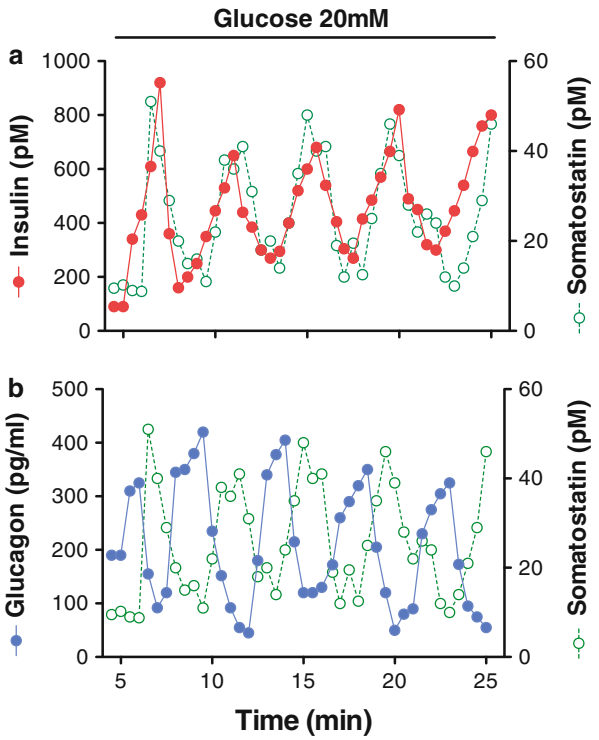

Fig. 6 Comparison of glucose-induced oscillations of somatostatin release with oscillations of insulin (**a**) and glucagon (**b**) secretion from the perfused rat pancreas. Glucagon oscillations are antiparallel to those of insulin and somatostatin (Adapted with permission from Salehi et al. (2007))

The observation that insulin, glucagon, and somatostatin are secreted in a pulsatile manner (period of 8–10 min/cycle) by the perfused rat, dog, monkey, and human pancreas (Stagner et al. 1980; Goodner et al. 1991; Salehi et al. 2007; Grapengiesser et al. 2006) during exposure to a constant glucose concentration ruled out the hypothesis that hormone secretions are driven by glucose oscillations (Fig. 6). In some studies, the three islet hormones were found to oscillate independently of each other, suggesting that each cell type possesses independent episodic secretory mechanism (Stagner et al. 1980; Goodner et al. 1991). However, other studies performed on the perfused rat pancreas showed a perfect dependence between the oscillations of the three hormones (period of 4–5 min/cycle) (Salehi et al. 2007; Grapengiesser et al. 2006), with pulses of somatostatin overlapping those of insulin with a delay of 30 s and being antisynchronous to those of glucagon (Salehi et al. 2007). Interestingly, a similar correlation between the oscillations of the three islet hormones (period of 5–8 min/cycle) was observed on batches of 5–15 isolated perifused human or mouse islets, i.e., in conditions where the only possible interactions between islets are paracrine in nature (Hellman et al. 2012, 2009).

Actually, at 3 mM glucose, hormone secretion was found stable with no detectable pulses of glucagon, insulin, or somatostatin, whereas the three hormones

started to oscillate in the presence of 20 mM glucose. Despite the fact that glucagon oscillated at 20 mM glucose, the average glucagon release was inhibited by 20 mM glucose because the nadirs between glucagon pulses were lower than the basal secretion at 3 mM glucose (Hellman et al. 2012). Given that only the β-cells are electrically coupled (Nadal et al. 1999), it is likely that pulsatile release of glucagon and somatostatin is determined by paracrine factors (Hellman et al. 2012). Since somatostatin is a potent glucagonostatic factor, it is tempting to speculate that each pulse of somatostatin secretion inhibits glucagon secretion. However, this might not be the sole factor since the antisynchrony between glucagon and somatostatin pulses was lost in the perfused pancreas of adenosine A_1 receptor knockout mice (Hellman et al. 2012; Salehi et al. 2009). Several other potential candidates have been proposed. They are briefly reviewed below, as well as their proposed mechanism of action.

Pulsatility might have several functional advantages. For the α-cell itself, it is possible that a cyclic elevation of $[Ca^{2+}]_c$ might be less energetically costly than a sustained $[Ca^{2+}]_c$ elevation and reduce the risk of Ca^{2+}-induced cytotoxicity. An oscillatory secretion is also suitable for α-cells since, contrary to β-cells, they cannot secrete continuously at a high sustained rate but display a rather phasic secretion upon exposure to a continuous stimulus. Pulsatility might also be beneficial to optimize the efficacy of glucagon on target cells, for instance, by preventing glucagon receptor downregulation or desensitization. *In vitro* experiments have shown that glucagon has a greater enhancing effect on hepatic glucose output and optimizes glucose production when administrated in pulses rather than continuously (Weigle and Goodner 1986; Komjati et al. 1986; Weigle et al. 1984). However *in vivo* experiments are conflicting (for review, see Lefebvre et al. (1996)). Thus glucagon infused in a pulsatile fashion exerted greater hyperglycemic, lipolytic (estimated by plasmatic levels of free fatty acid and glycerol), and ketogenic (β-hydroxybutyrate levels) effects than continuous hormone delivery (Paolisso et al. 1990) in man, but not in dog (Dobbins et al. 1994). Beneficial effects of pulsatile hormone release have been documented for insulin (Lefèbvre et al. 1987; Gilon et al. 2002).

Several studies reported a loss of antiparallelism between insulin and glucagon oscillations in T2DM (Menge et al. 2011) and prediabetic individuals (Rohrer et al. 2012). This latter observation suggests that the loss of pulsatile insulin-glucagon crosstalk precedes the actual manifestation of hyperglycemia.

Insulin

Several arguments support the concept that insulin inhibits glucagon secretion. (a) α-cells have a high density of insulin receptors (Franklin et al. 2005; Patel et al. 1982). (b) Exogenous insulin infusion inhibits glucagon secretion of type 1 diabetic patients whose β-cell function is extinct (Gerich et al. 1975b; Asplin et al. 1981) and in insulinopenic animal models (Weir et al. 1976). (c) Insulin suppresses glucagon secretion, decreases $[Ca^{2+}]_c$, and increases the K_{ATP} current in

α-cells, and blockade of insulin signaling by the phosphatidylinositol 3-kinase inhibitor, wortmannin, reverses the effects of insulin (Ravier and Rutter 2005; Olsen et al. 2005; Franklin et al. 2005; Leung et al. 2006a; Ramracheya et al. 2010; Xu et al. 2006). (d) There is a relative or an absolute hyperglucagonemia in insulin-deficient states (Gerich et al. 1973, 1976a, b; Kawamori et al. 2011) and increased glucagon secretion in perfused pancreas of streptozotocin-treated rats (Weir et al. 1976). (e) Neutralization of intra-islet insulin by an anti-insulin antibody strongly stimulates glucagon release (Maruyama et al. 1984). (f) α-cell-specific insulin receptor knockout (αIRKO) mice exhibit hyperglucagonemia and hyperglycemia in the fed state and a larger response to arginine (Kawamori et al. 2009, 2011; Kawamori and Kulkarni 2009).

Several mechanisms have been suggested to explain acute inhibition of the α-cell by insulin (reviewed in Bansal and Wang 2008). Insulin has been reported to decrease the α-cell K_{ATP} channel sensitivity to ATP inhibition in a phosphatidylinositol 3-kinase-dependent manner causing a hyperpolarization of the plasma membrane (Franklin et al. 2005; Leung et al. 2006a). Insulin could also promote the translocation of $GABA_A$ receptors from intracellular pools to the cell surface and enhance GABA-mediated Cl^- influx, leading again to a hyperpolarization of the cell (see below) (Xu et al. 2006).

Whether insulin is responsible for the glucagonostatic effect of glucose is another question. Several arguments support this proposal. (a) Due to the inverse regulation of the secretion of glucagon and insulin by glucose, insulin has long been considered the most likely candidate molecule to induce an indirect inhibition of glucagon secretion. (b) The glucagon response to a drop of the glucose concentration is completely lost in type 1 diabetic patients (Gerich et al. 1973) or alloxan-treated dogs and pigs (Meier et al. 2006; Braaten et al. 1974) and dramatically impaired in perifused islets or the perfused pancreas of streptozotocin-treated rats (Weir et al. 1976; Hope et al. 2004). (c) A meal rich in carbohydrates decreases glucagon secretion from healthy individuals with normal insulin secretion but tends to increase it in diabetic patients with absolute (as T1DM) or relative (insulin resistance of T2DM) insulinopenia. (d) As mentioned above, several experiments reported an antiparallel pulsatility of insulin and glucagon secretion during perifusion with a stable glucose concentration. (e) The glucagon response to hypoglycemia is attenuated by greater hyperinsulinemia (Galassetti and Davis 2000). (f) Knockdown of insulin receptors by small interfering RNA or neutralization of intra-islet insulin by an anti-insulin antibody strongly impairs the glucagonotropic effect of low glucose on isolated rodent islets (Franklin et al. 2005; Diao et al. 2005). Many other experiments have been performed *in vivo* to test the role of insulin in the control of glucagon secretion, but the interpretations are difficult because of the indirect metabolic effects and because some strategies used to change insulin secretion (sulfonylureas, diazoxide, somatostatin, etc.) can also exert a direct effect on α-cells and δ-cells (see below). Agents like streptozotocin which are used to kill β-cells are also toxic for the liver and the kidney and might affect glucose metabolism by such side effects. Moreover, many commercially available types

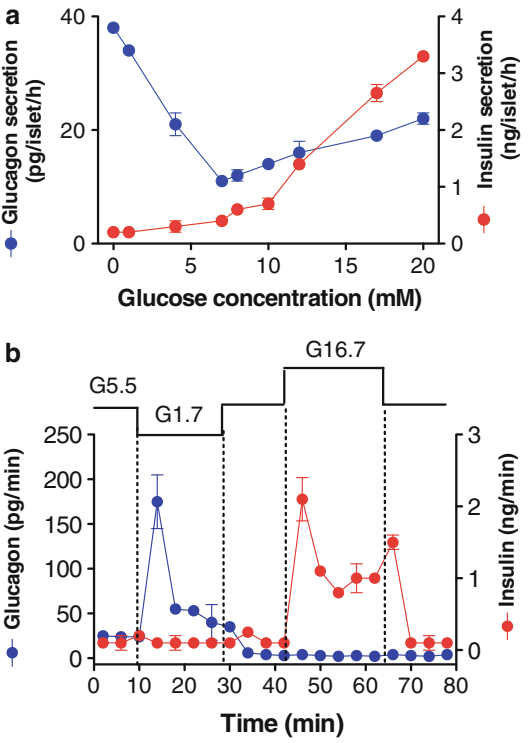

Fig. 7 (**a**–**b**). Glucose dependence of glucagon and insulin secretion from mouse pancreatic islets incubated for 1 h in the presence of 0–20 mM glucose (Adapted with permission from Vieira et al. (2007)). (**c**–**d**). Example of dissociations between glucose-induced glucagonostatic and insulinotropic effects on perifused mouse islets. Decreasing the glucose (G) concentration from the medium from 5.5 to 1.7 mM stimulated glucagon release although insulin secretion remained basal. By contrast, increasing the glucose concentration from the medium from 5.5 to 16.7 mM stimulated insulin release without affecting glucagon release (Adapted from Shiota et al. (2005))

of insulin are contaminated with zinc which can also have an effect on its own (see below).

The idea that paracrine insulin is involved in the glucagonostatic effect of glucose has been extended to the "switch-off" hypothesis according to which insulin exerts a tonic inhibition on α-cells, and removal of this brake under conditions of hypoglycemia would be necessary to trigger glucagon secretion (review in Cryer (2012) and Hope et al. (2004)). An elegant study performed in a model of insulin deficiency localized to the ventral lobe of the pancreas has also suggested that insulin has a permissive effect, permitting glucose to suppress glucagon secretion during hyperglycemia (Greenbaum et al. 1991).

Although very appealing, several observations suggest that insulin is not responsible for the glucagonostatic effect of glucose. (a) Probably one of the strongest arguments is that glucagon secretion starts to be inhibited by glucose concentrations that do not yet stimulate insulin release (Gerich et al. 1974a; Gao et al. 1992; Walker et al. 2011; Ravier and Rutter 2005; Vieira et al. 2007) (Fig. 7a). Moreover, there are plenty of other examples where glucagon secretion is inhibited by glucose concentrations that do not stimulate insulin release (Shiota et al. 2005; Cheng-Xue et al. 2013) (Fig. 7b). (b) Insulin does neither affect glucagon secretion from alloxan-treated lobe of the dog pancreas (Greenbaum et al. 1991) nor glucagon secretion of mouse islets (Quoix et al. 2009). (c) Exposure of islets to a high

concentration of insulin does not prevent the glucagonostatic effect of glucose (Quoix et al. 2009). (d) The inhibitory effect of glucose on glucagon secretion and $[Ca^{2+}]_c$ in mouse islets and single α-cells is not prevented by wortmannin (Ravier and Rutter 2005). (e) Glucagonemia and glycemia are increased to a similar level after streptozotocin treatment in control and αIRKO mice, and these levels are normalized by treatment with phlorizin, a competitive inhibitor of the Na^+-dependent glucose transport which prevents glucose reabsorption from the kidney (Kawamori et al. 2011). Moreover, in hyperinsulinemic-hypoglycemic clamp experiments, hypoglycemia induces a large increase in glucagon secretion under supraphysiological hyperinsulinemia in both control and αIRKO mice (Kawamori and Kulkarni 2009). (f) Finally, in humans, glucose recovery from hypoglycemia can occur in the absence of decrements in portal insulin below baseline and despite mild peripheral hyperinsulinemia (Heller and Cryer 1991). Clearly all these data nicely demonstrate that a change in glycemia can control glucagon secretion independently of an insulin action on α-cells. They therefore demonstrate that insulin is not the sole paracrine factor responsible for the glucagonostatic effect of glucose, but they do not exclude a contribution of insulin in this effect.

Zn^{2+}

Zn^{2+} is accumulated in secretory granules of islet cells, thanks to the ZnT8 transporter (Chimienti et al. 2006; Lemaire et al. 2009). In β-cells, it cocrystallizes with insulin in the dense-core secretory granules with two atoms of Zn^{2+} for six molecules of insulin, and it is necessary for insulin crystallization (Dodson and Steiner 1998; Wijesekara et al. 2010). Upon exocytosis, these hexameric crystals are exposed to a change in pH from 5.5 to 7.4, become dissociated, and release both atoms of zinc. High extracellular local concentrations of zinc (μM) are therefore anticipated within the islet (Huang et al. 1995). It has been suggested that Zn^{2+} released from stimulated β-cells inhibits glucagon release (Ishihara et al. 2003) and might therefore be responsible for the inhibitory effect of glucose (Egefjord et al. 2010; Hardy et al. 2011). A "switch-off" hypothesis involving Zn^{2+} instead of insulin has also been suggested (Zhou et al. 2007; Robertson et al. 2011). However, these hypotheses are not unanimously admitted.

The direct inhibitory effect of Zn^{2+} on α-cells is controversial since some studies reported an inhibitory effect of Zn^{2+} on $[Ca^{2+}]_c$ oscillations or glucagon secretion (Olsen et al. 2005; Franklin et al. 2005; Hardy et al. 2011; Gyulkhandanyan et al. 2008), whereas others reported no effect (Quoix et al. 2009) or even a stimulatory effect (Ravier and Rutter 2005; Ramracheya et al. 2010; Vieira et al. 2007). One mechanism provided to explain the inhibitory effect is an activation of K_{ATP} channels leading to α-cell repolarization (Franklin et al. 2005), but another study failed to see such effect (Gyulkhandanyan et al. 2008).

The study of the involvement of Zn^{2+} in the glucagonostatic effect of glucose started with the use of Zn^{2+} chelators. Chelation of Zn^{2+} was shown to prevent the

inhibition of glucagon secretion by insulinotropic agents (Ishihara et al. 2003; Franklin et al. 2005). However, another study reported that glucose retains its glucagonostatic effect in the presence of Zn^{2+} chelators (Macdonald et al. 2007). The study of the role of Zn^{2+} in the control of glucagon secretion was greatly helped by the use of the ZnT8 knockout mouse model that was initially developed to study the role of ZnT8 in diabetes since genome-wide association studies have linked a polymorphism in the ZnT8 gene to higher risk of developing type 2 diabetes (Rutter 2010). Since, in this mouse model, Zn^{2+} is no longer accumulated in secretory granules of islet cells, it is no longer released (Lemaire et al. 2009), and it is a very useful model to evaluate the role of Zn^{2+} released from β-cells in the glucagonostatic effect of glucose. Both global ZnT8 and β-cell-specific-ZnT8 knockout mice display no alteration of their fasting glucagonemia and of the control of glucagon secretion by glucose (Cheng-Xue et al. 2013; Lemaire et al. 2009; Hardy et al. 2011; Nicolson et al. 2009). This strongly indicates that Zn^{2+} is not responsible for the glucagonostatic effect of glucose. Since Zn^{2+} is coreleased with insulin, this conclusion is in full agreement with the argument mentioned above that glucose inhibits glucagon secretion at glucose concentrations that do not yet stimulate insulin release. ZnT8 appears largely dispensable for α-cell function because α-cell-specific ZnT8 knockout mice show no evident abnormalities in plasma glucagon and glucose homeostasis (Wijesekara et al. 2010). Nevertheless, since tiny amounts of Zn^{2+} can still be present in the cells independently of ZnT8 (Egefjord et al. 2010; Gyulkhandanyan et al. 2008), these experiments do not exclude the remote possibility that Zn^{2+} can affect somehow glucagon secretion.

GABA and γ-Hydroxybutyrate

In the central nervous system, γ-aminobutyric acid (GABA) is a major inhibitory neurotransmitter. It is mainly synthesized from glutamate by glutamic acid decarboxylase (GAD). Two types of GABA receptors have been identified. $GABA_A$ receptors (including $GABA_{A\text{-}\rho}$, previously named $GABA_C$) are ligand-gated Cl^- channels which exert both phasic and tonic currents, while $GABA_B$ receptors are G protein-coupled receptors. In islets, GABA is present in high concentrations in β-cells, but not in α- or δ-cells (Gilon et al. 1988; Thomas-Reetz et al. 1993; Taniguchi et al. 1979). GAD is present exclusively in β-cells, and not in α-, δ-, and PP-cells (Gilon et al. 1991a, b; Reetz et al. 1991). More than 20 years ago, it was suggested that glucose-induced inhibition of glucagon secretion involves activation of $GABA_A$ receptor Cl^- channels (Rorsman et al. 1989). Many experiments have been performed thereafter, but yielded conflicting results. In order to interpret them, it should be kept in mind that two routes of GABA release from β-cells are possible: a Ca^{2+}-dependent release from secretory vesicles or a Ca^{2+}-independent release via plasma membrane transporters. The first route is compatible with the observations that GABA is mainly accumulated in synaptic-like microvesicles (SLMVs, ~diameter of ~90 nm) (Gilon et al. 1988; Thomas-Reetz et al. 1993; Reetz et al. 1991) and possibly too in a few insulin-containing

large dense-core vesicles (LDCVs, ~diameter of ~300 nm) (Braun et al. 2007, 2010). Exocytosis of both SLMVs and LDCVs is Ca^{2+}-dependent and stimulated by glucose (Braun et al. 2004a, 2007; MacDonald et al. 2005). However, this makes unlikely that GABA is involved in the glucagonostatic effect of glucose concentrations that do not yet increase $[Ca^{2+}]_c$ in β-cells. The second, Ca^{2+}-independent route of release of GABA, is in theory compatible with its possible involvement in the glucagonostatic effect of low glucose concentrations. Indeed, it simply suffices that glucose promotes its release by transporters.

Several studies support the proposal that GABA inhibits glucagon secretion (Olsen et al. 2005; Gilon et al. 1991b; Rorsman et al. 1989; Franklin and Wollheim 2004; Wendt et al. 2004). This likely results from $GABA_A$ receptor activation since these receptors were detected in α-cells (Rorsman et al. 1989; Wendt et al. 2004; Bailey et al. 2007; Jin et al. 2013) and the glucagonostatic effect of GABA was reproduced by $GABA_A$ receptor agonists (Gilon et al. 1991b). However, other studies do not support an inhibitory action of GABA on α-cells. Thus, it was shown that GABA did not affect $[Ca^{2+}]_c$ (Quoix et al. 2009; Vieira et al. 2007; Berts et al. 1996) or the membrane potential (Hjortoe et al. 2004) of mouse α-cells. The role of $GABA_B$ receptors is enigmatic. Although their presence has been documented in α-cells (Braun et al. 2004b), $GABA_B$ receptor agonists and antagonists were ineffective on glucagon release (Gilon et al. 1991b; Braun et al. 2004b).

The contribution of GABA to the glucagonostatic effect of glucose has been evaluated in several experiments, but, again, the results are controversial. Thus, it was found that $GABA_A$ antagonists did (Wendt et al. 2004) or did not prevent (Macdonald et al. 2007; Gilon et al. 1991b; Taneera et al. 2012) the inhibitory effect of glucose on glucagon release. It is unclear whether these discrepancies reflect differences of species and/or experimental conditions. Moreover, if GABA alone is the paracrine factor responsible for the glucagonostatic effect of glucose, it is necessary that its release is stimulated by glucose. However, the glucose dependence of GABA release by β-cells is debated since it was found that glucose reduces the islet GABA content (Li et al. 2013; Pizarro-Delgado et al. 2010), whereas it inhibits (Pizarro-Delgado et al. 2010; Smismans et al. 1997; Winnock et al. 2002; Wang et al. 2006), stimulates (MacDonald et al. 2005; Gaskins et al. 1995), or does not affect its release (Nagamatsu et al. 1999). A release of GABA independent of SLMVs and LDCVs (through transporters) has been claimed to explain the high rate of secretion at low glucose (Braun et al. 2010; Jin et al. 2013; Taneera et al. 2012; Smismans et al. 1997). All these experiments suggest that GABA is unlikely essential for the control of glucagon secretion by glucose.

In the brain, GABA can be sequentially transformed by GABA transaminase (GABA-T) to succinate semialdehyde (SSA) and then by NADPH-dependent SSA reductase to γ-hydroxybutyrate (GHB). GHB is considered as a potent inhibitory neurotransmitter. It has recently been suggested that GHB released from β-cells is responsible for the glucagonostatic effect of glucose (Li et al. 2013). Indeed, (a) SSA reductase is present in β-cells, (b) glucose stimulates both the content and the release of GHB from islets, (c) blockade of GABA-T by vigabatrin prevents the glucagonostatic effect of glucose, (d) the putative GHB receptor (TSPAN-17) is

expressed in islet cells other than β-cells (presumably α-cells), (e) activation of the GHB receptor by 3-chloropropanoic acid inhibits glucagon release, and (f) the GHB receptor antagonist NCS-382 prevents the glucagonostatic effect of glucose. Since the release of GHB is stimulated by glucose concentrations that do not yet stimulate insulin release, this appealing model might explain the glucagonostatic effect of fairly low glucose concentrations. It nevertheless requires confirmation.

Somatostatin

Somatostatin is secreted by endocrine cells of the endocrine pancreas, the gastrointestinal tract, the pituitary gland, and the brain. Because of this high diversity of source of somatostatin, it is important to keep in mind that plasma somatostatin is not reflecting pancreatic somatostatin alone. It was even reported that pancreatic somatostatin contributes very little to circulating somatostatin (Gutniak et al. 1987; Ensinck et al. 1989; D'Alessio and Ensinck 1990). Hence, assessment of the pancreatic δ-cell physiology can only be accurately done in *in vitro* experiments on the perfused pancreas or isolated islets or δ-cells. Somatostatin inhibits both insulin and glucagon secretion (Gerich et al. 1974c, d; Starke et al. 1987; Barden et al. 1977) through local islet microcirculation or paracrine communication (Gromada et al. 2007; Stagner and Samols 1986). It has two active forms produced by alternative cleavage of a single preproprotein: one of 14 amino acids (somatostatin-14) and the other of 28 amino acids (somatostatin-28) (Weckbecker et al. 2003). Somatostatin-14 is the predominant form in pancreatic islets (Patel et al. 1981; Reichlin 1983a, b). Five types of somatostatin receptor (SSTR1–SSTR5) have been described. Somatostatin receptors are present in both α- and β-cells. There seems to be a consensus, based on immunodetections of the receptors, the use of specific agonists and the SSTR2 knockout mouse model, that rodent and human α-cells express mainly SSTR2 (Kailey et al. 2012; Cejvan et al. 2003; Strowski et al. 2000, 2006; Singh et al. 2007; Hunyady et al. 1997; Rossowski and Coy 1994). However, even if it is the main receptor, there are some evidences that other SSTR might also be expressed in α-cells, such as SSTR1 and/or SSTR5 (Kailey et al. 2012; Singh et al. 2007; Ludvigsen et al. 2004, 2007; Ludvigsen 2007; Strowski and Blake 2008). The type of SSTR present in β-cells seems more controversial, some studies reporting a predominance of SSTR5 and others of SSTR2 (Kailey et al. 2012; Kumar et al. 1999). It might represent species differences but discrepancies have been reported within the same species (Kailey et al. 2012; Kumar et al. 1999).

Somatostatin inhibits glucagon secretion by activating a pertussis toxin-sensitive protein (Gi/o) (Walker et al. 2011; Yoshimoto et al. 1999; Cheng-Xue et al. 2013; Gromada et al. 2001b; Kendall et al. 1995; Gopel et al. 2004). Three different, but not exclusive, mechanisms of inhibition have been documented. (a) Electrophysiological studies have shown that somatostatin activates a G protein-gated inwardly rectifying K^+ (GIRK) current in α-cells (coupled to G_{i2}), leading to membrane hyperpolarization, suppression of electrical activity, and inhibition of Ca^{2+}-dependent exocytosis (Yoshimoto et al. 1999; Gromada et al. 2001a; Kailey et al. 2012). (b) Somatostatin directly inhibits exocytosis by

activating, via G_{i2}, the serine/threonine protein phosphatase calcineurin (Gromada et al. 2001a). (c) It also inhibits adenylate cyclase activity, leading to a reduction of cAMP levels and PKA-stimulated glucagon secretion (Schuit et al. 1989; Fehmann et al. 1995; Hahn et al. 1978).

Somatostatin exerts a tonic inhibition on glucagon secretion (Gromada et al. 2007; Cheng-Xue et al. 2013; Hauge-Evans et al. 2009) which is revealed by the higher rate of glucagon release found in SSTR2 or somatostatin knockout mice (Cheng-Xue et al. 2013; Strowski et al. 2000), after blockade of SSTR2 receptors (Vieira et al. 2007), immunoneutralization of somatostatin by antibodies (Barden et al. 1977; Brunicardi et al. 2001; de Heer et al. 2008), or after treatment with pertussis toxin (Cheng-Xue et al. 2013; Gopel et al. 2004). This tonic effect of somatostatin probably explains the difficulty to see an acute inhibitory effect of exogenously applied somatostatin on glucagon release (Cheng-Xue et al. 2013; Kawai et al. 1982; Kleinman et al. 1995). By contrast, an inhibition of glucagon secretion by somatostatin can easily be detected in somatostatin knockout mice (Cheng-Xue et al. 2013).

Since glucose strongly stimulates somatostatin secretion (Vieira et al. 2007; Cheng-Xue et al. 2013; Gerber et al. 1981; Sorenson and Elde 1983), it has been suggested that somatostatin is the paracrine factor responsible for the glucagonostatic effect of glucose. This hypothesis is strongly supported by the observations that glucagon and somatostatin oscillations are antiparallel and that glucose is unable to inhibit glucagon release of islets from somatostatin knockout mice (Hauge-Evans et al. 2009). However, another study using the same mouse model showed that glucose was able to control glucagon secretion (Cheng-Xue et al. 2013) (Fig. 8b). This last observation is supported by other studies. Thus, the glucagonostatic effect of glucose was found to be preserved or even increased in the presence of a SSTR2 antagonist, a somatostatin antibody (Walker et al. 2011; Ramracheya et al. 2010; Vieira et al. 2007; de Heer et al. 2008), or after pretreatment with pertussis toxin (Cheng-Xue et al. 2013; Gopel et al. 2004) (Fig. 8a). If somatostatin is responsible for the glucagonostatic effect of glucose, it is necessary that somatostatin secretion is stimulated by the same concentrations of glucose that inhibit glucagon release. However, it is unclear whether this is really the case, since one study supports a similar sensitivity of somatostatin and glucagon secretion to glucose (Vieira et al. 2007), whereas another study reports that somatostatin is secreted at relatively high glucose concentrations, similar to those that stimulate insulin release (Hauge-Evans et al. 2009).

δ-Cells also possess K_{ATP} channels (Gopel et al. 2000b; Braun et al. 2009). As for β-cells, their closure strongly stimulates somatostatin release (Cheng-Xue et al. 2013; Braun et al. 2009; Ipp et al. 1977). Since tolbutamide, a blocker of K_{ATP} channels, inhibits glucagon release when applied to a medium containing a low glucose concentration (Fig. 8a) (Macdonald et al. 2007; Cheng-Xue et al. 2013), it is possible that this effect results from the concomitant stimulation of somatostatin release (Cheng-Xue et al. 2013). We indeed support this proposal

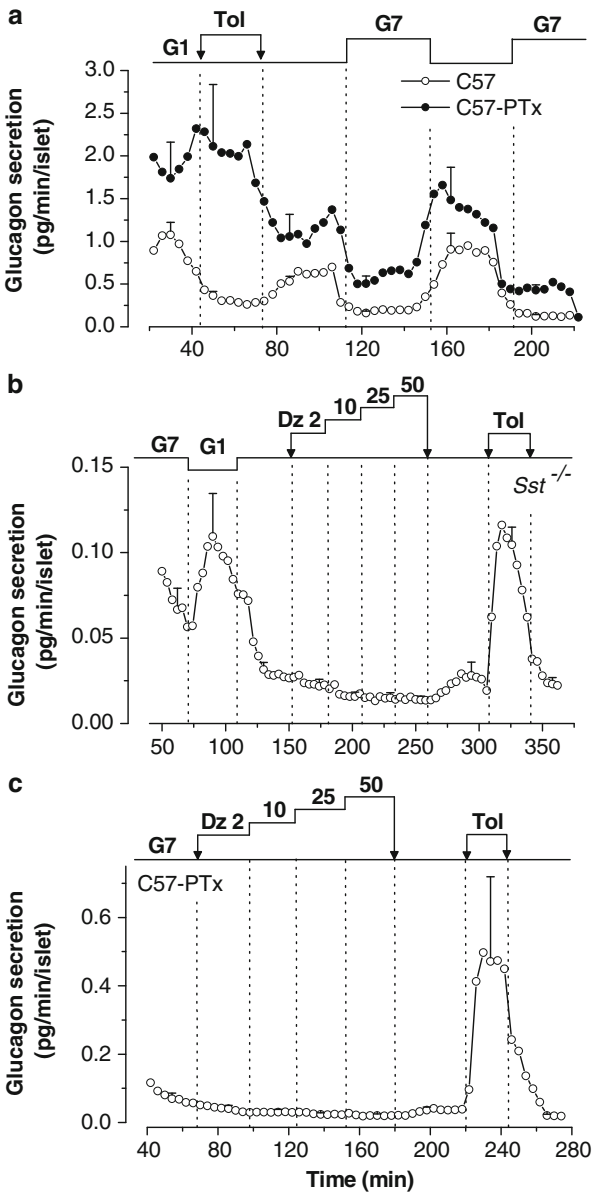

Fig. 8 (**a**). Removal of the somatostatin paracrine influence by pretreatment of islets with pertussis toxin transforms the inhibitory effect of tolbutamide into a stimulatory one but does not prevent the glucagonostatic effect of glucose (G). Islets from C57 mice were pretreated or not for 18 h with 200 ng/ml pertussis toxin (PTx). They were then perifused with a medium containing alanine, glutamine, and arginine (2 mmol/l each, mix AA). The G concentration of the medium was changed between 1 (G1) and 7 mM (G7), and 500 μM tolbutamide (Tol) was applied when indicated. (**b**–**c**). In islets devoid of any paracrine influence of somatostatin (islets from somatostatin knockout ($Sst^{-/-}$) mice (**b**) or islets of C57 mice pretreated with PTx (**c**)), opening of K_{ATP} channels with increasing concentrations of diazoxide (Dz) did not reverse the glucagonostatic effect of glucose, while closing K_{ATP} channels with 500 μM tolbutamide (Tol) stimulated glucagon release. This suggests that the glucagonostatic effect of glucose is unlikely mediated by a closure of K_{ATP} channels (Adapted with permission from Cheng-Xue et al. (2013))

because suppression of the somatostatin influence (using islets treated with pertussis toxin or islets of somatostatin knockout mice) transforms the glucagonostatic effect of tolbutamide into a strong glucagonotropic effect likely due to a direct closure of α-cell K_{ATP} channels (see below) (Fig. 8).

Direct Effects of Glucose on Glucagon Secretion

As in β-cells, an increase in $[Ca^{2+}]_c$ is the triggering signal of exocytosis of glucagon granules (Barg et al. 2000). However, it is hotly debated whether glucose exerts a direct inhibition or stimulation of α-cells. The paragraphs below will briefly summarize the hypotheses that have been suggested to explain a direct inhibition or a direct stimulation.

Inhibition by Glucose

Five models have been suggested.

The K_{ATP} Channel-Dependent Model

This model is derived from our understanding of the stimulus-secretion coupling in pancreatic β-cells and attributes a key role to K_{ATP} channels which are also expressed in α-cells (Walker et al. 2011; Rorsman et al. 2012; Macdonald et al. 2007). In β-cells, glucose metabolism increases the cytosolic ATP/ADP ratio, which closes K_{ATP} channels in the plasma membrane. The resulting decrease in K^+ conductance depolarizes the plasma membrane up to the threshold for the activation of high-threshold voltage-dependent channels (mainly L type and other types: see the chapter on ▶ Electrophysiology of Islet Cells in this book) which, by opening, leads to an increase in $[Ca^{2+}]_c$ and triggers insulin secretion. In α-cells, it is suggested that K_{ATP} channels also transduce changes in cell metabolism into changes in electrical activity, but their closure would induce an effect opposite to that found in β-cells because α-cells have two main distinct features. (a) At low glucose, K_{ATP} channels are much more closed in α- than in β-cells. It is unclear whether this results from a different metabolism and/or a different modulation of K_{ATP} channels by ATP or other factors (see above). (b) α-cells possess two types of low-threshold voltage-dependent channels (T-type Ca^{2+} channels and Na^+ channels) that are required to generate full action potential (which is not the case for β-cells) and that have the peculiarity to quickly inactivate upon sustained depolarization. The model suggests that glucagon secretion can only occur in a narrow range of K_{ATP} channel activity. It is based on several observations and in particular on the key observations that (a) closure of K_{ATP} channels by tolbutamide at low glucose inhibits glucagon secretion, (b) mild opening of the channels by low diazoxide concentrations reverses the glucagonostatic effect of high glucose, and (c) strong opening of the channels by high diazoxide concentrations inhibits glucagon release.

The detailed model proposes that, at low glucose, the K_{ATP} current in α-cells is already small (Walker et al. 2011; Rorsman et al. 2008, 2012; Huang et al. 2011a; Macdonald et al. 2007; Gopel et al. 2000a; Kanno et al. 2002; Cheng-Xue et al. 2013), so that the plasma membrane is slightly depolarized to the threshold for activation of low-threshold voltage-dependent Na^+ and T-type Ca^{2+} channels. Opening of these channels induces a further depolarization and allows the

membrane to reach the threshold for activation of high-threshold voltage-dependent Ca^{2+} channels. This sequence of events generates the upstroke phase of an action potential (Fig. 5b). The downward phase of the action potential results from the opening of voltage-dependent K^+ channels which repolarize the membrane close to the threshold for activation of low-threshold voltage-dependent Na^+ and Ca^{2+} channels, allowing the generation of a new action potential once the inactivation of low-threshold voltage-dependent channels is relieved (Fig. 5b). Only Ca^{2+} influx through high-threshold voltage-dependent Ca^{2+} channels is sufficient to trigger exocytosis of glucagon secretory granules. Hence, exocytosis depends on the frequency and amplitude of the action potentials. This model indicates that exocytosis occurs only when the activity of K_{ATP} channels, and thus the membrane potential, is maintained in a relatively narrow intermediate window. The release of glucagon is suppressed when the membrane is hyperpolarized as well as when it is too depolarized. Hence, an increase of the glucose concentration or the addition of a K_{ATP} channel blocker induces a further closure of K_{ATP} channels and a depolarization of the plasma membrane which inactivates low-threshold voltage-dependent channels. This inactivation decreases the probability of opening of high-threshold voltage-dependent Ca^{2+} channels, Ca^{2+} influx, and eventually exocytosis. Conversely, the inhibitory effect of glucose can be reversed by low concentrations of the K_{ATP} channel opener which brings back the activity of K_{ATP} channels in the optimal range of the window (corresponding to a low glucose concentration). Of course, a large increase of the K_{ATP} current (with diazoxide concentrations >10 μM) hyperpolarizes the α-cell below the threshold for activation of voltage-dependent Ca^{2+} channels and inhibits glucagon release.

This model is supported by several observations. (a) K_{ATP} channel blockers reproduce the inhibitory effect of glucose when applied in the presence of a low glucose concentration (Gromada et al. 2004; Macdonald et al. 2007; Gopel et al. 2000a). (b) By contrast, low dose of diazoxide (0.3–10 μM) reverse the inhibitory effect of glucose in static incubation experiments (Macdonald et al. 2007; Gopel et al. 2000a). (c) Glucagon secretion from islets of SUR1 knockout mice is low and displays little or no change in response to glucose (Gromada et al. 2004; Shiota et al. 2005; Munoz et al. 2005). (d) Inhibition of voltage-dependent Na^+ channels by tetrodotoxin inhibits glucagon release, consistent with the essential role of these channels for action potential generation and exocytosis (Gromada et al. 2004; Macdonald et al. 2007; Gopel et al. 2000a; Ramracheya et al. 2010). (e) Mild depolarization of islets with external K^+ inhibits glucagon secretion, consistent with the depolarization-induced inactivation of low-threshold voltage-dependent channels needed for action potential generation (Gromada et al. 2004). (f) Inhibition of voltage-dependent K^+ channels inhibits glucagon release, consistent with the decreased ability of these channels to relieve the inactivation state of low-threshold voltage-dependent channels (Macdonald et al. 2007; Spigelman et al. 2010; Ramracheya et al. 2010).

The role played by α-cell K_{ATP} channels in the glucagonostatic effect of glucose is however not unanimously admitted (Quoix et al. 2009; Vieira et al. 2007; Miki et al. 2001; Vieira et al. 2005), and the following arguments suggest that glucose

can inhibit glucagon secretion independently of K_{ATP} channel closure. (a) Glucose has a tendency to decrease and tolbutamide to increase $[Ca^{2+}]_c$ in α-cells (Quoix et al. 2009, 2007; Vieira et al. 2007; Le Marchand and Piston 2012; Wang and McDaniel 1990). (b) The K_{ATP} channel model predicts that glucose depolarizes α-cells. However, some studies reported the reverse (Allister et al. 2013; Bokvist et al. 1999; Liu et al. 2004; Hjortoe et al. 2004; Manning Fox et al. 2006). (c) Glucose decreases $[Ca^{2+}]_c$ in α-cells whose K_{ATP} channels are maximally closed with high concentrations of tolbutamide (Quoix et al. 2009; Vieira et al. 2007). (d) Glucose inhibits glucagon secretion of islets, the K_{ATP} channels of which are pharmacologically (tolbutamide) or genetically (SUR1 or Kir6.2 knockout mice) disrupted (Vieira et al. 2007; Miki et al. 2001; Cheng-Xue et al. 2013). This inhibition is much better seen in islets treated with pertussis toxin which relieves the tonic glucagonostatic effect of somatostatin (Cheng-Xue et al. 2013). (e) In the presence of an inhibitory concentration of glucose, tolbutamide stimulates glucagon release (Cheng-Xue et al. 2013). (f) No diazoxide concentration (even in the low range) reverses the glucagonostatic effect of glucose in dynamic perifusion experiments (Fig. 8; Cheng-Xue et al. 2013). (g) In the absence of paracrine influence of somatostatin (islets treated with pertussis toxin or from somatostatin knockout mice), glucagon secretion is inhibited by glucose, whereas it is strongly stimulated by tolbutamide (Fig. 8). (h) One study reported that tetrodotoxin tends to increase α-cell $[Ca^{2+}]_c$ and glucagon release of islets (Le Marchand and Piston 2012).

The reasons for all these discrepancies are unknown. They are unlikely linked to species differences since opposite conclusions were obtained using the same species, the mouse. It should be pointed out that all studies however agree that strong opening of K_{ATP} channels with large diazoxide concentrations inhibits glucagon release.

The Other Models

A second model of direct inhibition of glucagon secretion by glucose suggests that K_{ATP} channels are not involved and that the arrest of Ca^{2+} influx is mediated by a hyperpolarization of the plasma membrane resulting from glucose-induced inhibition of a depolarizing store-operated current (I_{SOC}) (Liu et al. 2004). The mechanisms would be the following. At low glucose, α-cell metabolism and ATP concentration are low. This is associated with a low activity of the sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and a low Ca^{2+} concentration in the endoplasmic reticulum which would activate a store-operated current. The nature of this current is unknown, carrying Ca^{2+} and/or other ions. The resulting depolarization causes the opening of voltage-dependent Ca^{2+} channels (VDCC), allowing Ca^{2+} influx and subsequent stimulation of glucagon secretion. Raising the glucose concentration of the medium increases the ATP concentration, which activates SERCAs. The resulting increase in the Ca^{2+} concentration of the ER inhibits I_{SOC}, bringing the membrane potential below the threshold for activation of VDCC. This lowers $[Ca^{2+}]_c$ and inhibits glucagon secretion (Vieira et al. 2007;

Liu et al. 2004). This hypothesis is based on several observations. The key ones are that pharmacological inhibition of SERCAs (by thapsigargin and cyclopiazonic acid (CPA)) increases α-cell $[Ca^{2+}]_c$ and prevents the lowering effect of glucose (Vieira et al. 2007; Liu et al. 2004). Moreover, low glucose and ER Ca^{2+} depletion trigger accumulation of STIM1 puncta in the subplasmalemmal ER where they cocluster with Orai1 in the plasma membrane to activate SOCs (Tian et al. 2012).

A third model suggests that the depolarization of the plasma membrane elicited by a low concentration of glucose results from a decrease of the activity of the hyperpolarizing Na^+/K^+ pump (Bode et al. 1999). A fourth model suggests that the glucagonostatic effect of glucose results from the inhibition of the AMP-dependent protein kinase (AMPK) (Leclerc et al. 2011). It was indeed found that inhibition of glucagon secretion by glucose was associated with inhibition of AMPK activity, while forced activation of AMPK stimulated glucagon release. In this model, AMPK stimulation by low glucose would result from the rise in AMP concomitant to the drop in ATP levels. Finally, a fifth model suggests that glucose hyperpolarizes the plasma membrane by inducing α-cell swelling (Davies et al. 2007) with subsequent activation of Cl^- influx through volume-regulated channels (Best et al. 2010) and/or CFTR (Boom et al. 2007).

Stimulation by Glucose

Another hypothesis suggests that glucose directly stimulates glucagon secretion of α-cells by a mechanism that is similar to that observed in the β-cell. Two key observations support this proposal. (a) Glucose and K_{ATP} channel closers stimulate electrical activity and increase $[Ca^{2+}]_c$ in isolated rat α-cells (Olsen et al. 2005; Franklin et al. 2005). (b) They also directly stimulate glucagon release of isolated rat and mouse α-cells (Le Marchand and Piston 2010; Olsen et al. 2005). In this situation, it is evident that the net glucagonostatic effect of glucose that is observed on islets results from an indirect paracrine influence from non-α-cells of the islet that overwhelms the direct stimulatory effect of glucose on α-cells. This interpretation is entirely compatible with elegant experiments showing that the forced acceleration of α-cell metabolism stimulates glucagon release, whereas the forced acceleration of both α- and β-cell metabolism inhibits glucagon release (Ishihara et al. 2003; Takahashi et al. 2006).

Another paradoxical stimulation of glucagon release has been documented. Several studies have tested the dose-dependent effect of glucose on glucagon secretion of whole islets or of the perfused pancreas. Most of them clearly show that the maximal inhibition of glucagon secretion is reached at around ~7 mM glucose (Gerich et al. 1974a; Walker et al. 2011; Macdonald et al. 2007; Vieira et al. 2007; Salehi et al. 2006) and that the inhibition tends to be weaker at higher glucose concentrations. One study has even documented a paradoxical stimulatory effect at glucose concentration above 20 mM, which was, unexpectedly, not prevented by the hyperpolarizing agent diazoxide (Salehi et al. 2006). The mechanisms of this effect are unknown. However, this paradoxical stimulation was not

observed by other groups using similar glucose concentrations (Gerich et al. 1974a; Cheng-Xue et al. 2013). If this stimulation is real, it might explain hyperglucagonemia in diabetes.

More than One Mechanism?

As discussed above, it is unclear whether glucose exerts a direct or indirect inhibitory effect on α-cells and whether the direct effect is stimulatory or inhibitory. However, it should be noted that these proposed mechanisms are not mutually exclusive. Thus, it is possible that their respective contributions depend on the physiological situation (such as the level of glycemia) and that, in some situations, glucagon secretion is controlled by redundant mechanisms. This complexity of control would be the price to pay to avoid that, under physiological conditions, glycemia drops below a dangerous level or, inversely, increases too high.

Therapies Targeting Glucagon Action

Anti-Glucagon Therapies in Diabetes

Rationale for Anti-Glucagon Therapies

Because the development of severe hyperglycemia requires the presence of glucagon, suppressing glucagon action in the liver has been an attractive approach to reverse the metabolic consequences of insulin deficiency (Unger and Cherrington 2012; Cho et al. 2012). The effect of reduced glucagon secretion or action on glucose homeostasis has been evaluated using several pharmacological and genetic approaches: (a) PC2 knockout mice ($Pcsk2^{-/-}$) which are unable to produce mature glucagon because they lack prohormone convertase 2 (Webb et al. 2002), (b) glucagon-GFP knockin mice in which GFP has been inserted within the pre-proglucagon gene leading to lack of glucagon production (Watanabe et al. 2012; Hayashi 2011; Hayashi et al. 2009), (c) pancreas-specific aristaless-related homeobox (ARX) knockout mice which lack glucagon-producing α-cells (Hancock et al. 2010), (d) liver-specific Gsα knockout mice in which the glucagon action in the liver is specifically blocked (Chen et al. 2005), (e) glucagon receptor knockout mice (Parker et al. 2002; Gelling et al. 2003), (f) liver-specific glucagon receptor knockout mice (Longuet et al. 2013), (g) immunoneutralization of endogenous glucagon with glucagon antibodies (Brand et al. 1994), (h) reduction of glucagon receptor expression by antisense oligonucleotide (Liang et al. 2004; Sloop et al. 2004), and (i) glucagon receptor antagonists (Cho et al. 2012; Johnson et al. 1982; Qureshi et al. 2004; Christensen et al. 2011). A vast majority of these studies concluded that anti-glucagon strategies improve glucose tolerance and attributed the improvement to a decreased glucagon action. More recent studies using glucagon receptor or glucagon knockout mice showed that other mechanisms might also contribute to the improved glucose tolerance such as a compensatory

action of incretins and particularly GLP-1, the levels of which dramatically increased when glucagon action is disrupted (Hayashi 2011; Hayashi et al. 2009; Ali et al. 2011; Gu et al. 2010; Sorensen et al. 2006; Fukami et al. 2013). Interestingly, metformin, a first-line anti-diabetic agent, has been shown to improve glucose tolerance by suppressing hepatic glucagon signaling via a decreased production of cAMP (Miller et al. 2013).

Recent observations pushed the rationale for using anti-glucagon therapies even further because it was observed that glucagon receptor knockout mice are protected against diabetes induced by streptozotocin (Lee et al. 2011, 2012; Conarello et al. 2007). These results led Unger et al. to propose a glucagonocentric view of diabetes in which glucagon is considered as the *sine qua none* condition of diabetes (Unger and Cherrington 2012).

Inhibitors of Glucagon Secretion

A suppression of glucagon secretion is therefore an attractive therapeutic possibility to treat diabetes. However, the difficulty is to find agents that act specifically on α-cells. Somatostatin receptor agonists are very powerful glucagonostatic agents but experiments on human tissue have shown that some SSTR subtypes are present both in α- and β-cells (see above).

GLP-1: glucagon-like peptide 1 (GLP-1) agonists are widely used to treat diabetes. They not only stimulate insulin secretion but also decrease glucagonemia *in vivo*. The glucagonostatic and insulinotropic effects of GLP-1 contribute equally to its glucose-lowering action (Hare et al. 2010b). The mechanisms of the decreased glucagonemia are still debated. It is indeed unclear whether it results from a direct or indirect action of GLP-1 on α-cells. It is widely accepted that β-cells strongly express GLP-1 receptors. However, it is debated whether these receptors are fully absent (Franklin et al. 2005; Tornehave et al. 2008; Moens et al. 1996), expressed in a subpopulation of α-cells (Heller et al. 1997), or only slightly expressed in α-cells (De Marinis et al. 2010). The complete lack of GLP-1 receptors in α-cells is compatible with the proposal that the glucagonostatic effect of GLP-1 is indirect, mediated by somatostatin interacting with SSTR2 on α-cells (de Heer et al. 2008) or insulin. The expression of GLP-1 receptors in a subpopulation of α-cells is supported by the observation that only a minor fraction of α-cells respond to GLP-1 with cAMP elevation (Tian et al. 2011). The low density of GLP-1 receptors in α-cells is compatible with a direct effect which has been suggested to result from a paradoxical PKA-dependent inhibition of Ca^{2+} influx, without any involvement of paracrine glucagonostatic effects of insulin or somatostatin (De Marinis et al. 2010). Inhibition of glucagon release by modulation of the autonomous nervous system by GLP-1 is also possible (Gromada et al. 2007; Balkan and Li 2000; Preitner et al. 2004). Because GLP-1 has a half-life of less than 2 min, due to rapid degradation by the enzyme dipeptidyl peptidase-4 (DDP-4), an attractive possibility is to use DDP-4 inhibitors. Although inhibition of DDP-4 is expected to increase plasma levels of GIP, the other incretin with a glucagonotropic action (Meier et al. 2003), the glucagonostatic effect of GLP-1 prevails, leading to a decrease of glucagonemia *in vivo* (Christensen et al. 2011).

Amylin: amylin or islet amyloid polypeptide (IAPP), a 37-amino acid peptide, is co-secreted with insulin by β-cells. It constitutes the main component of amyloid deposits found in the pancreas of type 2 diabetic patients and is also found in insulinomas (Westermark et al. 2011). Being present in β-cells, amylin is deficient in type 1 diabetic patients. Amylin inhibits glucagon secretion *in vivo*, particularly that elicited by amino acids. However, again, it is unclear whether this effect is direct or indirect mediated by a paracrine factor or even an action on the central nervous system (Silvestre et al. 2001; Young 2005; Panagiotidis et al. 1992; Ahren and Sorhede 2008; Akesson et al. 2003). The use of an antagonist of amylin or immunoneutralization of the endogenous amylin secretion supports a local inhibitory effect of amylin on glucagon secretion. Since the antagonist stimulated insulin, somatostatin, and glucagon release, it was suggested that endogenously produced amylin tonically inhibits stimulated secretion of the three hormones (Akesson et al. 2003; Gedulin et al. 2007). Pramlintide is a synthetic analogue of amylin which differs by replacement of three amino acids with proline. Several clinical studies performed on type 1 and type 2 diabetic patients have demonstrated robust postprandial glucagon-suppressive effects of pramlintide (Christensen et al. 2011; Nyholm et al. 1999; Levetan et al. 2003; Fineman et al. 2002a, b).

Leptin: leptin, a protein of 167 amino acids, is mainly secreted by adipocytes and plays a key role in the control of food intake, energy expenditure, and glucose homeostasis. Administration of leptin to rodents with T1DM suppresses hyperglucagonemia as effectively as somatostatin (Wang et al. 2010). The underlying mechanisms have been studied only recently. Leptin did not affect glucagon secretion from InR1G9 cells (Chen et al. 2011), but it inhibited glucagon release of mouse islets and of αTC1-9 cells (Tuduri et al. 2009; Shimizu et al. 2011). The glucagonostatic effect of leptin has been attributed to an acute decrease of the α-cell electrical activity and $[Ca^{2+}]_c$ (Tuduri et al. 2009) and to a long-term effect involving an inhibition of the glucagon gene expression via STAT3 (Marroqui et al. 2011).

Undesired Effects

Counteracting glucagon action might be more advantageous than simply prevention of hyperglycemia. Indeed, mice in which glucagon action was knockout or knockdown have reduced adiposity and circulating triglycerides and increased insulin sensitivity. However, unwanted effects have also been documented in some studies, such as frequent hypoglycemic episodes, moderate δ-cell hyperplasia, severe α-cell hyperplasia, increased fetal lethality, impaired β-cell function, upregulation of fatty acid and cholesterol biosynthesis pathways in the liver leading to excessive lipid deposition, increased bile acid production, and elevated levels of liver transaminases and LDL cholesterol (Ali and Drucker 2009; Christensen et al. 2011; Sorensen et al. 2006; Vuguin et al. 2006; Yang et al. 2011). With age, the α-cell hyperplasia can lead to neuroendocrine tumors (Yu et al. 2011). An inactivating mutation (P86S) of the glucagon receptor has also been described in human (Mahvash disease). It is characterized by a marked hyperglucagonemia and pancreatic neuroendocrine tumors (Zhou et al. 2009).

Combinatorial Therapies for the Treatment of Obesity and Diabetes

Recent work has highlighted the therapeutic potential of drugs acting simultaneously on several targets to exert additive or synergistic benefits compared to monotherapy. GLP-1 decreases food intake, inhibits gastric emptying, and lowers blood glucose by stimulating insulin secretion and decreasing glucagonemia *in vivo*. Therefore, dual-acting peptides with prolonged GLP-1 receptor agonistic and glucagon receptor antagonistic activities have been developed and were found to be beneficial for the treatment of T2DM (Claus et al. 2007). However, another strategy has recently emerged which takes advantage of several effects of glucagon which are not directly related to the control of blood glucose. As mentioned above, glucagon increases energy expenditure, thermogenesis, and lipolysis and reduces meal size by exerting a central satiety effect (Heppner et al. 2010; Habegger et al. 2010). These observations have led to the development of a new series of drugs with dual agonistic action on glucagon and GLP-1 receptors, with the idea that the hyperglycemic effect of glucagon could be counteracted by the hypoglycemic effect of GLP-1 (Day et al. 2009, 2012; Pocai et al. 2009; Tan et al. 2013). Recent observations suggest that the activation of glucagon receptor stimulates fibroblast growth factor 21 (FGF21) production and secretion by the liver and that FGF21 mediates, at least partly, the effects of glucagon on energy expenditure and lipid metabolism (Habegger et al. 2013). The beneficial effects of the combined activation of GLP-1 and glucagon receptors are also supported by reports showing that oxyntomodulin which is produced by L-cells and is a weak coagonist of glucagon and GLP-1 receptors suppresses appetite, increases energy expenditure, and reduces weight loss (Cohen et al. 2003; Wynne et al. 2006; Du et al. 2012; Kosinski et al. 2012). These combinatorial therapies, although very promising, still require careful examination for their applications to the long-term treatment of diabetes, obesity, or the metabolic syndrome. In particular, the relative ratio of GLP-1/glucagon agonistic effects of the drugs requires careful assessment to maximize weight loss and minimize hyperglycemia.

Acknowledgments PG is supported by the Fonds de la Recherche Scientifique Médicale (Brussels, grant 3.4554.10) and the Actions de Recherche Concertées (ARC 13/18-051) from the General Direction of Scientific Research of the French Community of Belgium. PG is Research Director, and AGR and HYC are postdoctoral researchers of the Fonds National de la Recherche Scientifique, Brussels.

Cross-Reference

► Electrophysiology of Islet Cells
► The Comparative Anatomy of Islets

References

Aguilar-Parada E, Eisentraut AM, Unger RH (1969) Pancreatic glucagon secretion in normal and diabetic subjects. Am J Med Sci 257:415–419

Ahren B (2000) Autonomic regulation of islet hormone secretion–implications for health and disease. Diabetologia 43:393–410

Ahren B, Sorhede WM (2008) Disturbed α-cell function in mice with β-cell specific overexpression of human islet amyloid polypeptide. Exp Diabetes Res 2008:304513

Ahren B, Veith RC, Taborsky GJ Jr (1987) Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog: 1). Effects on basal release of insulin and glucagon. Endocrinology 121:323–331

Akesson B, Panagiotidis G, Westermark P, Lundquist I (2003) Islet amyloid polypeptide inhibits glucagon release and exerts a dual action on insulin release from isolated islets. Regul Pept 111:55–60

Al-Hasani K, Pfeifer A, Courtney M, Ben-Othman N, Gjernes E, Vieira A, Druelle N, Avolio F, Ravassard P, Leuckx G, Lacas-Gervais S, Ambrosetti D, Benizri E, Hecksher-Sorensen J, Gounon P, Ferrer J, Gradwohl G, Heimberg H, Mansouri A, Collombat P (2013) Adult duct-lining cells can reprogram into β-like cells able to counter repeated cycles of toxin-induced diabetes. Dev Cell 26:86–100

Ali S, Drucker DJ (2009) Benefits and limitations of reducing glucagon action for the treatment of type 2 diabetes. Am J Physiol Endocrinol Metab 296:E415–E421

Ali S, Lamont BJ, Charron MJ, Drucker DJ (2011) Dual elimination of the glucagon and GLP-1 receptors in mice reveals plasticity in the incretin axis. J Clin Invest 121:1917–1929

Allister EM, Robson-Doucette CA, Prentice KJ, Hardy AB, Sultan S, Gaisano HY, Kong D, Gilon P, Herrera PL, Lowell BB, Wheeler MB (2013) UCP2 regulates the glucagon response to fasting and starvation. Diabetes 62:1623–1633

Alquier T, Kawashima J, Tsuji Y, Kahn BB (2007) Role of hypothalamic adenosine 5′-monophosphate-activated protein kinase in the impaired counterregulatory response induced by repetitive neuroglucopenia. Endocrinology 148:1367–1375

Alumets I, Hakanson R, Sundler F (1983) Ontogeny of endocrine cells in porcine gut and pancreas. An immunocytochemical study. Gastroenterology 85:1359

Alvina K, Ellis-Davies G, Khodakhah K (2009) T-type calcium channels mediate rebound firing in intact deep cerebellar neurons. Neuroscience 158:635–641

Andrews SS, Alfredo Lopez S, Blackard WG (1975) Effect of lipids on glucagon secretion in man. Metabolism 24:35–44

Arafat AM, Kaczmarek P, Skrzypski M, Pruszynska-Oszmalek E, Kolodziejski P, Szczepankiewicz D, Sassek M, Wojciechowicz T, Wiedenmann B, Pfeiffer AF, Nowak KW, Strowski MZ (2013) Glucagon increases circulating fibroblast growth factor 21 independently of endogenous insulin levels: a novel mechanism of glucagon-stimulated lipolysis? Diabetologia 56:588–597

Arnes L, Hill JT, Gross S, Magnuson MA, Sussel L (2012) Ghrelin expression in the mouse pancreas defines a unique multi-potent progenitor population. PLoS One 7:e52026

Aromataris EC, Roberts ML, Barritt GJ, Rychkov GY (2006) Glucagon activates Ca^{2+} and Cl^{-} channels in rat hepatocytes. J Physiol 573:611–625

Ashcroft FM, Rorsman P (2004) Molecular defects in insulin secretion in type-2 diabetes. Rev Endocr Metab Disord 5:135–142

Asplin CM, Paquette TL, Palmer JP (1981) *In vivo* inhibition of glucagon secretion by paracrine β-cell activity in man. J Clin Invest 68:314

Authier F, Desbuquois B (2008) Glucagon receptors. Cell Mol Life Sci 65:1880–1899

Bailey SJ, Ravier MA, Rutter GA (2007) Glucose-dependent regulation of γ-aminobutyric acid ($GABA_A$) receptor expression in mouse pancreatic islet α-cells. Diabetes 56:320–327

Bajorunas DR, Fortner JG, Jaspan JB (1986) Glucagon immunoreactivity and chromatographic profiles in pancreatectomized humans. Paradoxical response to oral glucose. Diabetes 35:886–893

Baldissera FG, Holst JJ (1984) Glucagon-related peptides in the human gastrointestinal mucosa. Diabetologia 26:223–228

Balkan B, Li X (2000) Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms. Am J Physiol Regul Integr Comp Physiol 279:R1449–R1454

Bansal P, Wang Q (2008) Insulin as a physiological modulator of glucagon secretion. Am J Physiol Endocrinol Metab 295:E751–E761

Barden N, Lavoie M, Dupont A, Côté J, Côté JP (1977) Stimulation of glucagon release by addition of anti-somatostatin serum to islets of Langerhans *in vitro*. Endocrinology 101:635–638

Barg S, Galvanovskis J, Göpel SO, Rorsman P, Eliasson L (2000) Tight coupling between electrical activity and exocytosis in mouse glucagon-secreting α-cells. Diabetes 49:1500–1510

Barnes AJ, Bloom SR (1976) Pancreatectomised man: a model for diabetes without glucagon. Lancet 1:219–221

Baron AD, Schaeffer L, Shragg P, Kolterman OG (1987) Role of hyperglucagonemia in maintenance of increased rates of hepatic glucose output in type II diabetics. Diabetes 36:274–283

Barthel A, Schmoll D (2003) Novel concepts in insulin regulation of hepatic gluconeogenesis. Am J Physiol Endocrinol Metab 285:E685–E692

Bataille D (2007) Pro-protein convertases in intermediary metabolism: islet hormones, brain/gut hormones and integrated physiology. J Mol Med (Berl) 85:673–684

Baum D, Porte D Jr, Ensinck J (1979) Hyperglucagonemia and α-adrenergic receptor in acute hypoxia. Am J Physiol 237:E404–E408

Berts A, Ball A, Gylfe E, Hellman B (1996) Suppression of Ca^{2+} oscillations in glucagon-producing α_2-cells by insulin glucose and amino acids. Biochim Biophys Acta Mol Cell Res 1310:212–216

Berts A, Gylfe E, Hellman B (1997) Cytoplasmic Ca^{2+} in glucagon-producing pancreatic α-cells exposed to carbachol and agents affecting Na^+ fluxes. Endocrine 6:79–83

Best L, Brown PD, Sener A, Malaisse WJ (2010) Electrical activity in pancreatic islet cells: the VRAC hypothesis. Islets 2:59–64

Bode HP, Weber S, Fehmann HC, Göke B (1999) A nutrient-regulated cytosolic calcium oscillator in endocrine pancreatic glucagon-secreting cells. Pflugers Arch 437:324–334

Bokvist K, Rorsman P, Smith PA (1990) Block of ATP-regulated and Ca^{2+}-activated K^+ channels in mouse pancreatic β-cells by external tetraethylammonium and quinine. J Physiol (Lond) 423:327–342

Bokvist K, Olsen HL, Hoy M, Gotfredsen CF, Holmes WF, Buschard K, Rorsman P, Gromada J (1999) Characterisation of sulphonylurea and ATP-regulated K^+ channels in rat pancreatic α-cells. Pflugers Arch 438:428–436

Bollheimer LC, Landauer HC, Troll S, Schweimer J, Wrede CE, Schölmerich J, Buettner R (2004) Stimulatory short-term effects of free fatty acids on glucagon secretion at low to normal glucose concentrations. Metabolism 53:1443–1448

Bolli GB, Fanelli CG (1999) Physiology of glucose counterregulation to hypoglycemia. Endocrinol Metabol Clin North Am 28:467–493

Bonner-Weir S, Orci L (1982) New perspectives on the microvasculature of the islets of Langerhans in the rat. Diabetes 31:883–889

Boom A, Lybaert P, Pollet JF, Jacobs P, Jijakli H, Golstein PE, Sener A, Malaisse WJ, Beauwens R (2007) Expression and localization of cystic fibrosis transmembrane conductance regulator in the rat endocrine pancreas. Endocrine 32:197–205

Borg WP, Sherwin RS, During MJ, Borg MA, Shulman GI (1995) Local ventromedial hypothalamus glucopenia triggers counterregulatory hormone release. Diabetes 44:180–184

Borg MA, Sherwin RS, Borg WP, Tamborlane WV, Shulman GI (1997) Local ventromedial hypothalamus glucose perfusion blocks counterregulation during systemic hypoglycemia in awake rats. J Clin Invest 99:361–365

Bosco D, Armanet M, Morel P, Niclauss N, Sgroi A, Muller YD, Giovannoni L, Parnaud G, Berney T (2010) Unique arrangement of α- and β-cells in human islets of Langerhans. Diabetes 59:1202–1210

Braaten JT, Faloona GR, Unger RH (1974) The effect of insulin on the α-cell to hyperglycemia in long-standing alloxan diabetes. J Clin Invest 53:1017–1021

Brand CL, Rolin B, Jorgensen PN, Svendsen I, Kristensen JS, Holst JJ (1994) Immunoneutralization of endogenous glucagon with monoclonal glucagon antibody normalizes hyperglycaemia in moderately streptozotocin-diabetic rats. Diabetologia 37:985–993

Braun M, Rorsman P (2010) The glucagon-producing α-cell: an electrophysiologically exceptional cell. Diabetologia 53:1827–1830

Braun M, Wendt A, Birnir B, Broman J, Eliasson L, Galvanovskis J, Gromada J, Mulder H, Rorsman P (2004a) Regulated exocytosis of GABA-containing synaptic-like microvesicles in pancreatic β-cells. J Gen Physiol 123:191–204

Braun M, Wendt A, Buschard K, Salehi A, Sewing S, Gromada J, Rorsman P (2004b) $GABA_B$ receptor activation inhibits exocytosis in rat pancreatic β-cells by G-protein-dependent activation of calcineurin. J Physiol (Lond) 559:397–409

Braun M, Wendt A, Karanauskaite J, Galvanovskis J, Clark A, Macdonald PE, Rorsman P (2007) Corelease and differential exit via the fusion pore of GABA, serotonin, and ATP from LDCV in rat pancreatic β-cells. J Gen Physiol 129:221–231

Braun M, Ramracheya R, Amisten S, Bengtsson M, Moritoh Y, Zhang Q, Johnson PR, Rorsman P (2009) Somatostatin release, electrical activity, membrane currents and exocytosis in human pancreatic δ-cells. Diabetologia 52:1566–1578

Braun M, Ramracheya R, Bengtsson M, Clark A, Walker JN, Johnson PR, Rorsman P (2010) γ-aminobutyric acid (GABA) is an autocrine excitatory transmitter in human pancreatic β-cells. Diabetes 59:1694–1701

Bringer J, Mirouze J, Marchal G, Pham TC, Luyckx A, Lefebvre P, Orsetti A (1981) Glucagon immunoreactivity and antidiabetic action of somatostatin in the totally duodenopancreatectomized and gastrectomized human. Diabetes 30:851–856

Brissova M, Fowler MJ, Nicholson WE, Chu A, Hirshberg B, Harlan DM, Powers AC (2005) Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. J Histochem Cytochem 53:1087–1097

Brunicardi FC, Stagner J, Bonner-Weir S, Wayland H, Kleinman R, Livingston E, Guth P, Menger M, McCuskey R, Intaglietta M, Charles A, Ashley S, Cheung A, Ipp E, Gilman S, Howard T, Passaro E Jr (1996) Microcirculation of the islets of Langerhans – Long Beach Veterans Administration Regional Medical Education Center Symposium. Diabetes 45:385–392

Brunicardi FC, Kleinman R, Moldovan S, Nguyen THL, Watt PC, Walsh J, Gingerich R (2001) Immunoneutralization of somatostatin, insulin, and glucagon causes alterations in islet cell secretion in the isolated perfused human pancreas. Pancreas 23:302–308

Burcelin R, Thorens B (2001) Evidence that extrapancreatic GLUT2-Dependent glucose sensors control glucagon secretion. Diabetes 50:1282–1289

Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A (2006) The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proc Natl Acad Sci USA 103:2334–2339

Cabrera O, Jacques-Silva MC, Speier S, Yang SN, Kohler M, Fachado A, Vieira E, Zierath JR, Kibbey R, Berman DM, Kenyon NS, Ricordi C, Caicedo A, Berggren PO (2008) Glutamate is a positive autocrine signal for glucagon release. Cell Metab 7:545–554

Cao W, Collins QF, Becker TC, Robidoux J, Lupo EG Jr, Xiong Y, Daniel KW, Floering L, Collins S (2005) p38 mitogen-activated protein kinase plays a stimulatory role in hepatic gluconeogenesis. J Biol Chem 280:42731–42737

Carosati E, Cruciani G, Chiarini A, Budriesi R, Ioan P, Spisani R, Spinelli D, Cosimelli B, Fusi F, Frosini M, Matucci R, Gasparrini F, Ciogli A, Stephens PJ, Devlin FJ (2006) Calcium channel antagonists discovered by a multidisciplinary approach. J Med Chem 49:5206–5216

Catterall WA (2011) Voltage-gated calcium channels. Cold Spring Harb Perspect Biol 3:a003947

Catterall WA (2012) Voltage-gated sodium channels at 60: structure, function and pathophysiology. J Physiol 590:2577–2589

Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J (2005) International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. Pharmacol Rev 57:411–425

Cejvan K, Coy DH, Efendic S (2003) Intra-islet somatostatin regulates glucagon release via type 2 somatostatin receptors in rats. Diabetes 52:1176–1181

Chen M, Gavrilova O, Zhao WQ, Nguyen A, Lorenzo J, Shen L, Nackers L, Pack S, Jou W, Weinstein LS (2005) Increased glucose tolerance and reduced adiposity in the absence of fasting hypoglycemia in mice with liver-specific $G_{S\alpha}$ deficiency. J Clin Invest 115:3217–3227

Chen L, Philippe J, Unger RH (2011) Glucagon responses of isolated α-cells to glucose, insulin, somatostatin, and leptin. Endocr Pract 17:819–825

Cheng KT, Ong HL, Liu X, Ambudkar IS (2011) Contribution of TRPC1 and Orai1 to Ca^{2+} entry activated by store depletion. Adv Exp Med Biol 704:435–449

Cheng-Xue R, Gomez-Ruiz A, Antoine N, Noel LA, Chae HY, Ravier MA, Chimienti F, Schuit FC, Gilon P (2013) Tolbutamide controls glucagon release from mouse islets differently than glucose: involvement of K_{ATP} channels from both α-cells and δ-cells. Diabetes 62:1612–1622

Chimienti F, Devergnas S, Pattou F, Schuit F, Garcia-Cuenca R, Vandewalle B, Kerr-Conte J, Van Lommel L, Grunwald D, Favier A, Seve M (2006) *In vivo* expression and functional characterization of the zinc transporter ZnT8 in glucose-induced insulin secretion. J Cell Sci 119:4199–4206

Cho YM, Merchant CE, Kieffer TJ (2012) Targeting the glucagon receptor family for diabetes and obesity therapy. Pharmacol Ther 135:247–278

Christensen M, Bagger JI, Vilsboll T, Knop FK (2011) The α-cell as target for type 2 diabetes therapy. Rev Diabet Stud 8:369–381

Claus TH, Pan CQ, Buxton JM, Yang L, Reynolds JC, Barucci N, Burns M, Ortiz AA, Roczniak S, Livingston JN, Clairmont KB, Whelan JP (2007) Dual-acting peptide with prolonged glucagon-like peptide-1 receptor agonist and glucagon receptor antagonist activity for the treatment of type 2 diabetes. J Endocrinol 192:371–380

Cohen MA, Ellis SM, Le Roux CW, Batterham RL, Park A, Patterson M, Frost GS, Ghatei MA, Bloom SR (2003) Oxyntomodulin suppresses appetite and reduces food intake in humans. J Clin Endocrinol Metab 88:4696–4701

Collins SC, Salehi A, Eliasson L, Olofsson CS, Rorsman P (2008) Long-term exposure of mouse pancreatic islets to oleate or palmitate results in reduced glucose-induced somatostatin and oversecretion of glucagon. Diabetologia 51:1689–1693

Conarello SL, Jiang G, Mu J, Li Z, Woods J, Zycband E, Ronan J, Liu F, Roy RS, Zhu L, Charron MJ, Zhang BB (2007) Glucagon receptor knockout mice are resistant to diet-induced obesity and streptozotocin-mediated β-cell loss and hyperglycaemia. Diabetologia 50:142–150

Conlon JM (1988) Proglucagon-derived peptides: nomenclature, biosynthetic relationships and physiological roles. Diabetologia 31:563–566

Consoli A, Nurjhan N, Capani F, Gerich J (1989) Predominant role of gluconeogenesis in increased hepatic glucose production in NIDDM. Diabetes 38:550–557

Cryer PE (1981) Glucose counterregulation in man. Diabetes 30:261–264

Cryer PE (1993) Glucose counterregulation: prevention and correction of hypoglycemia in humans. Am J Physiol Endocrinol Metab 264:E149–E155

Cryer PE (1996) Role of growth hormone in glucose counterregulation. Horm Res 46:192–194

Cryer PE (2002) Hypoglycaemia: the limiting factor in the glycaemic management of Type I and Type II diabetes. Diabetologia 45:937–948

Cryer PE (2012) Minireview: glucagon in the pathogenesis of hypoglycemia and hyperglycemia in diabetes. Endocrinology 153:1039–1048

Cryer PE (2013) Mechanisms of hypoglycemia-associated autonomic failure in diabetes. N Engl J Med 369:362–372

Cynober LA (2002) Plasma amino acid levels with a note on membrane transport: characteristics, regulation, and metabolic significance. Nutrition 18:761–766

D'Alessio DA, Ensinck JW (1990) Fasting and postprandial concentrations of somatostatin-28 and somatostatin-14 in type II diabetes in men. Diabetes 39:1198–1202

Dallaporta M, Perrin J, Orsini JC (2000) Involvement of adenosine triphosphate-sensitive K^+ channels in glucose-sensing in the rat solitary tract nucleus. Neurosci Lett 278:77–80

Dalle S, Fontés G, Lajoix AD, Lebrigand L, Gross R, Ribes G, Dufour M, Barry L, LeNguyen D, Bataille D (2002) Miniglucagon (glucagon 19–29). A novel regulator of the pancreatic islet physiology. Diabetes 51:406–412

Davies SL, Brown PD, Best L (2007) Glucose-induced swelling in rat pancreatic α-cells. Mol Cell Endocrinol 264:61–67

Day JW, Ottaway N, Patterson JT, Gelfanov V, Smiley D, Gidda J, Findeisen H, Bruemmer D, Drucker DJ, Chaudhary N, Holland J, Hembree J, Abplanalp W, Grant E, Ruehl J, Wilson H, Kirchner H, Lockie SH, Hofmann S, Woods SC, Nogueiras R, Pfluger PT, Perez-Tilve D, DiMarchi R, Tschop MH (2009) A new glucagon and GLP-1 co-agonist eliminates obesity in rodents. Nat Chem Biol 5:749–757

Day JW, Gelfanov V, Smiley D, Carrington PE, Eiermann G, Chicchi G, Erion MD, Gidda J, Thornberry NA, Tschop MH, Marsh DJ, SinhaRoy R, DiMarchi R, Pocai A (2012) Optimization of co-agonism at GLP-1 and glucagon receptors to safely maximize weight reduction in DIO-rodents. Biopolymers 98:443–450

de Heer J, Rasmussen C, Coy DH, Holst JJ (2008) Glucagon-like peptide-1, but not glucose-dependent insulinotropic peptide, inhibits glucagon secretion via somatostatin (receptor subtype 2) in the perfused rat pancreas. Diabetologia 51:2263–2270

De Marinis YZ, Salehi A, Ward CE, Zhang Q, Abdulkader F, Bengtsson M, Braha O, Braun M, Ramracheya R, Amisten S, Habib AM, Moritoh Y, Zhang E, Reimann F, Rosengren AH, Shibasaki T, Gribble F, Renstrom E, Seino S, Eliasson L, Rorsman P (2010) GLP-1 inhibits and adrenaline stimulates glucagon release by differential modulation of N- and L-type Ca^{2+} channel-dependent exocytosis. Cell Metab 11:543–553

de Vries MG, Arseneau LM, Lawson ME, Beverly JL (2003) Extracellular glucose in rat ventromedial hypothalamus during acute and recurrent hypoglycemia. Diabetes 52:2767–2773

Deng S, Vatamaniuk M, Huang X, Doliba N, Lian MM, Frank A, Velidedeoglu E, Desai NM, Koeberlein B, Wolf B, Barker CF, Naji A, Matschinsky FM, Markmann JF (2004) Structural and functional abnormalities in the islets isolated from type 2 diabetic subjects. Diabetes 53:624–632

Detimary P, Dejonghe S, Ling Z, Pipeleers D, Schuit F, Henquin JC (1998) The changes in adenine nucleotides measured in glucose-stimulated rodent islets occur in β-cells but not in α-cells and are also observed in human islets. J Biol Chem 273:33905–33908

Dey A, Lipkind GM, Rouille Y, Norrbom C, Stein J, Zhang C, Carroll R, Steiner DF (2005) Significance of prohormone convertase 2, PC2, mediated initial cleavage at the proglucagon interdomain site, Lys70-Arg71, to generate glucagon. Endocrinology 146:713–727

Diakogiannaki E, Gribble FM, Reimann F (2012) Nutrient detection by incretin hormone secreting cells. Physiol Behav 106:387–393

Diao J, Asghar Z, Chan CB, Wheeler MB (2005) Glucose-regulated glucagon secretion requires insulin receptor expression in pancreatic α-cells. J Biol Chem 280:33487–33496

Dinneen S, Alzaid A, Turk D, Rizza R (1995) Failure of glucagon suppression contributes to postprandial hyperglycaemia in IDDM. Diabetologia 38:337–343

Dobbins RL, Davis SN, Neal DW, Cobelli C, Cherrington AD (1994) Pulsatility does not alter the response to a physiological increment in glucagon in the conscious dog. Am J Physiol Endocrinol Metab 266:E467–E478

Dodson G, Steiner D (1998) The role of assembly in insulin's biosynthesis. Curr Opin Struct Biol 8:189–194

Drucker DJ, Asa SL (1988) Glucagon gene expression in vertebrate brain. J Biol Chem 263:13475–13478

Du X, Kosinski JR, Lao J, Shen X, Petrov A, Chicchi GG, Eiermann GJ, Pocai A (2012) Differential effects of oxyntomodulin and GLP-1 on glucose metabolism. Am J Physiol Endocrinol Metab 303:E265–E271

Dumonteil E, Ritz-Laser B, Magnan C, Grigorescu I, Ktorza A, Philippe J (1999) Chronic exposure to high glucose concentrations increases proglucagon messenger ribonucleic acid levels and glucagon release from InR1G9 cells. Endocrinology 140:4644–4650

Dumonteil E, Magnan C, Ritz-Laser B, Ktorza A, Meda P, Philippe J (2000) Glucose regulates proinsulin and prosomatostatin but not proglucagon messenger ribonucleic acid levels in rat pancreatic islets. Endocrinology 141:174–180

Dunning BE, Gerich JE (2007) The role of α-cell dysregulation in fasting and postprandial hyperglycemia in type 2 diabetes and therapeutic implications. Endocr Rev 28:253–283

Dunning BE, Foley JE, Ahren B (2005) α-cell function in health and disease: influence of glucagon-like peptide-1. Diabetologia 48:1700–1713

Duttaroy A, Zimliki CL, Gautam D, Cui Y, Mears D, Wess J (2004) Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in M_3 muscarinic acetylcholine receptor – deficient mice. Diabetes 53:1714–1720

Edwards JC, Howell SL, Taylor KW (1969) Fatty acids as regulators of glucagon secretion. Nature 224:808–809

Edwards JC, Howell SL, Taylor KW (1970) Radioimmunoassay of glucagon released from isolated guinea-pig islets of Langerhans incubated *in vitro*. Biochim Biophys Acta 215:297–309

Egefjord L, Petersen AB, Rungby J (2010) Zinc, α-cells and glucagon secretion. Curr Diabetes Rev 6:52–57

Ellingsgaard H, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT, Eppler E, Bouzakri K, Wueest S, Muller YD, Hansen AM, Reinecke M, Konrad D, Gassmann M, Reimann F, Halban PA, Gromada J, Drucker DJ, Gribble FM, Ehses JA, Donath MY (2011) Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and α-cells. Nat Med 17:1481–1489

Ensinck JW, Laschansky EC, Vogel RE, Simonowitz DA, Roos BA, Francis BH (1989) Circulating prosomatostatin-derived peptides. Differential responses to food ingestion. J Clin Invest 83:1580–1589

Erion DM, Kotas ME, McGlashon J, Yonemitsu S, Hsiao JJ, Nagai Y, Iwasaki T, Murray SF, Bhanot S, Cline GW, Samuel VT, Shulman GI, Gillum MP (2013) cAMP-responsive element-binding protein (CREB)-regulated transcription coactivator 2 (CRTC2) promotes glucagon clearance and hepatic amino acid catabolism to regulate glucose homeostasis. J Biol Chem 288:16167–16176

Esni F, Täljedal IB, Perl AK, Cremer H, Christofori G, Semb H (1999) Neural cell adhesion molecule (N-CAM) is required for cell type segregation and normal ultrastructure in pancreatic islets. J Cell Biol 144:325–337

Evans ML, McCrimmon RJ, Flanagan DE, Keshavarz T, Fan X, McNay EC, Jacob RJ, Sherwin RS (2004) Hypothalamic ATP-sensitive K^+ channels play a key role in sensing hypoglycemia and triggering counterregulatory epinephrine and glucagon responses. Diabetes 53:2542–2551

Fehmann H-C, Strowski M, Göke B (1995) Functional characterization of somatostatin receptors expressed on hamster glucagonoma cells. Am J Physiol Endocrinol Metab 268:E40–E47

Feske S (2010) CRAC channelopathies. Pflugers Arch 460:417–435

Fineman M, Weyer C, Maggs DG, Strobel S, Kolterman OG (2002a) The human amylin analog, pramlintide, reduces postprandial hyperglucagonemia in patients with type 2 diabetes mellitus. Horm Metab Res 34:504–508

Fineman MS, Koda JE, Shen LZ, Strobel SA, Maggs DG, Weyer C, Kolterman OG (2002b) The human amylin analog, pramlintide, corrects postprandial hyperglucagonemia in patients with type 1 diabetes. Metabolism 51:636–641

Franklin IK, Wollheim CB (2004) GABA in the endocrine pancreas: its putative role as an islet cell paracrine-signalling molecule. J Gen Physiol 123:185–190

Franklin I, Gromada J, Gjinovci A, Theander S, Wollheim CB (2005) β-cell secretory products activate α-cell ATP-dependent potassium channels to inhibit glucagon release. Diabetes 54:1808–1815

Fukami A, Seino Y, Ozaki N, Yamamoto M, Sugiyama C, Sakamoto-Miura E, Himeno T, Takagishi Y, Tsunekawa S, Ali S, Drucker DJ, Murata Y, Seino Y, Oiso Y, Hayashi Y (2013) Ectopic expression of GIP in pancreatic β-cells maintains enhanced insulin secretion in mice with complete absence of proglucagon-derived peptides. Diabetes 62:510–518

Galassetti P, Davis SN (2000) Effects of insulin *per se* on neuroendocrine and metabolic counterregulatory responses to hypoglycaemia. Clin Sci 99:351–362
Gao ZY, Drews G, Henquin JC (1991) Mechanisms of the stimulation of insulin release by oxytocin in normal mouse islets. Biochem J 276:169–174
Gao ZY, Gerard M, Henquin JC (1992) Glucose- and concentration-dependence of vasopressin-induced hormone release by mouse pancreatic islets. Regul Pept 38:89–98
Gaskins HR, Baldeón ME, Selassie L, Beverly JL (1995) Glucose modulates γ-aminobutyric acid release from the pancreatic βTC6 cell line. J Biol Chem 270:30286–30289
Gedulin BR, Jodka CM, Herrmann K, Young AA (2007) Role of endogenous amylin in glucagon secretion and gastric emptying in rats demonstrated with the selective antagonist, AC187. Regul Pept 137:121–127
Gelling RW, Du XQ, Dichmann DS, Romer J, Huang H, Cui L, Obici S, Tang B, Holst JJ, Fledelius C, Johansen PB, Rossetti L, Jelicks LA, Serup P, Nishimura E, Charron MJ (2003) Lower blood glucose, hyperglucagonemia, and pancreatic α-cell hyperplasia in glucagon receptor knockout mice. Proc Natl Acad Sci USA 100:1438–1443
Gerber PPG, Trimble ER, Wollheim CB, Renold AE, Miller RE (1981) Glucose and cyclic AMP as stimulators of somatostatin and insulin secretion from the isolated rat pancreas: a quantitative study. Diabetes 30:40–44
Gerich JE (1988) Lilly lecture 1988. Glucose counterregulation and its impact on diabetes mellitus. Diabetes 37:1608–1617
Gerich JE, Langlois M, Noacco C, Karam JH, Forsham PH (1973) Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic α-cell defect. Science 182:171–173
Gerich JE, Charles MA, Grodsky GM (1974a) Characterization of effects of arginine and glucose on glucagon and insulin release from perfused rat pancreas. J Clin Invest 54:833–841
Gerich JE, Langlois M, Schneider V, Karam JH, Noacco C (1974b) Effects of alterations of plasma free fatty acid levels on pancreatic glucagon secretion in man. J Clin Invest 53:1284–1289
Gerich JE, Lorenzi M, Schneider V, Karam JH, Rivier J, Guillemin R, Forsham PH (1974c) Effects of somatostatin on plasma glucose and glucagon levels in human diabetes mellitus. N Engl J Med 297:544–547
Gerich JE, Lorenzi M, Schneider V, Kwan CW, Karam JH, Guillemin R, Forsham PH (1974d) Inhibition of pancreatic glucagon responses to arginine by somatostatin in normal man and in insulin-dependent diabetics. Diabetes 23:876–880
Gerich JE, Lorenzi M, Bier DM, Schneider V, Tsalikian E, Karam JH, Forsham PH (1975a) Prevention of human diabetic ketoacidosis by somatostatin: role of glucagon. N Engl J Med 292:985–989
Gerich JE, Tsalikian E, Lorenzi M, Schneider V, Bohannon NV, Gustafson G, Karam JH (1975b) Normalization of fasting hyperglucagonemia and excessive glucagon responses to intravenous arginine in human diabetes mellitus by prolonged infusion of insulin. J Clin Endocrinol Metab 41:1178
Gerich JE, Langlois M, Noacco C, Lorenzi M, Karam JH, Forsham PH (1976a) Comparison of suppressive effects of elevated plasma glucose and free fatty acid levels on glucagon secretion in normal and insulin-dependent diabetic subjects. Evidence for selective α-cell insensitivity to glucose in diabetes mellitus. J Clin Invest 58:320–325
Gerich JE, Charles MA, Grodsky GM (1976b) Regulation of pancreatic insulin and glucagon. Annu Rev Physiol 38:353–388
Gersell DJ, Gingerich RL, Greider MH (1979) Regional distribution and concentration of pancreatic polypeptide in the human and canine pancreas. Diabetes 28:11–15
Gilon P, Henquin JC (2001) Mechanisms and physiological significance of the cholinergic control of pancreatic β-cell function. Endocr Rev 22:565–604
Gilon P, Campistron G, Geffard M, Remacle C (1988) Immunocytochemical localisation of GABA in endocrine cells of the rat entero-pancreatic system. Biol Cell 62:265–273
Gilon P, Tappaz M, Remacle C (1991a) Localization of GAD-like immunoreactivity in the pancreas and stomach of the rat and mouse. Histochemistry 96:355–365

Gilon P, Bertrand G, Loubatières-Mariani MM, Remacle C, Henquin JC (1991b) The influence of γ-aminobutyric acid on hormone release by the mouse and rat endocrine pancreas. Endocrinology 129:2521–2529

Gilon P, Ravier MA, Jonas JC, Henquin JC (2002) Control mechanisms of the oscillations of insulin secretion *in vitro* and *in vivo*. Diabetes 51(Suppl 1):S144–S151

Goodner CJ, Walike BC, Koerker DJ, Ensinck JW, Brown AC, Chideckel EW, Palmer J, Kalnasy L (1977) Insulin, glucagon, and glucose exhibit synchronous, sustained oscillations in fasting monkeys. Science 195:177–179

Goodner CJ, Hom FG, Koerker DJ (1982) Hepatic glucose production oscillates in synchrony with the islet secretory cycle in pasting rhesus monkeys. Science 215:1257–1259

Goodner CJ, Koerker DJ, Stagner JI, Samols E (1991) *In vitro* pancreatic hormonal pulses are less regular and more frequent than *in vivo*. Am J Physiol Endocrinol Metab 260:E422–E429

Gopel SO, Kanno T, Barg S, Weng XG, Gromada J, Rorsman P (2000a) Regulation of glucagon release in mouse α-cells by K_{ATP} channels and inactivation of TTX-sensitive Na^+ channels. J Physiol 528:509–520

Gopel SO, Kanno T, Barg S, Rorsman P (2000b) Patch-clamp characterisation of somatostatin-secreting-cells in intact mouse pancreatic islets. J Physiol 528:497–507

Gopel S, Zhang Q, Eliasson L, Ma XS, Galvanovskis J, Kanno T, Salehi A, Rorsman P (2004) Capacitance measurements of exocytosis in mouse pancreatic α-, β- and δ-cells within intact islets of Langerhans. J Physiol 556:711–726

Gorus FK, Malaisse WJ, Pipeleers DG (1984) Differences in glucose handling by pancreatic α- and β-cells. J Biol Chem 259:1196–1200

Grapengiesser E, Salehi A, Qader SS, Hellman B (2006) Glucose induces glucagon release pulses antisynchronous with insulin and sensitive to purinoceptor inhibition. Endocrinology 147:3472–3477

Gravholt CH, Moller N, Jensen MD, Christiansen JS, Schmitz O (2001) Physiological levels of glucagon do not influence lipolysis in abdominal adipose tissue as assessed by microdialysis. J Clin Endocrinol Metab 86:2085–2089

Greenbaum CJ, Havel PJ, Taborsky GJ Jr, Klaff LJ (1991) Intra-islet insulin permits glucose to directly suppress pancreatic A cell function. J Clin Invest 88:767–773

Grimelius L, Capella C, Buffa R, Polak JM, Pearse AG, Solcia E (1976) Cytochemical and ultrastructural differentiation of enteroglucagon and pancreatic-type glucagon cells of the gastrointestinal tract. Virchows Arch B Cell Pathol 20:217–228

Gromada J, Bokvist K, Ding WG, Barg S, Buschard K, Renström E, Rorsman P (1997) Adrenaline stimulates glucagon secretion in pancreatic α-cells by increasing the Ca^{2+} current and the number of granules close to the L-type Ca^{2+} channels. J Gen Physiol 110:217–228

Gromada J, Hoy M, Olsen HL, Gotfredsen CF, Buschard K, Rorsman P, Bokvist K (2001a) G_{i2} proteins couple somatostatin receptors to low-conductance K^+ channels in rat pancreatic α-cells. Pflugers Arch 442:19–26

Gromada J, Hoy M, Buschard K, Salehi A, Rorsman P (2001b) Somatostatin inhibits exocytosis in rat pancreatic α-cells by G_{i2}-dependent activation of calcineurin and depriming of secretory granules. J Physiol (Lond) 535:519–532

Gromada J, Ma XHM, Bokvist K, Salehi A, Berggren PO, Rorsman P (2004) ATP-sensitive K^+ channel-dependent regulation of glucagon release and electrical activity by glucose in wild-type and $SUR1^{-/-}$ mouse α-cells. Diabetes 53:S181–S189

Gromada J, Franklin I, Wollheim CB (2007) α-cells of the endocrine pancreas: 35 years of research but the enigma remains. Endocr Rev 28:84–116

Gromada J, Duttaroy A, Rorsman P (2009) The insulin receptor talks to glucagon? Cell Metab 9:303–305

Gross R, Mialhe P (1974) Free fatty acid-glucagon feed-back mechanism. Diabetologia 10:277–283

Gu W, Winters KA, Motani AS, Komorowski R, Zhang Y, Liu Q, Wu X, Rulifson IC, Sivits G Jr, Graham M, Yan H, Wang P, Moore S, Meng T, Lindberg RA, Veniant MM (2010) Glucagon

receptor antagonist-mediated improvements in glycemic control are dependent on functional pancreatic GLP-1 receptor. Am J Physiol Endocrinol Metab 299:E624–E632
Gutniak M, Grill V, Wiechel KL, Efendic S (1987) Basal and meal-induced somatostatin-like immunoreactivity in healthy subjects and in IDDM and totally pancreatectomized patients. Effects of acute blood glucose normalization. Diabetes 36:802–807
Gyulkhandanyan AV, Lu H, Lee SC, Bhattacharjee A, Wijesekara N, Fox JE, MacDonald PE, Chimienti F, Dai FF, Wheeler MB (2008) Investigation of transport mechanisms and regulation of intracellular Zn^{2+} in pancreatic α-cells. J Biol Chem 283:10184–10197
Habegger KM, Heppner KM, Geary N, Bartness TJ, DiMarchi R, Tschop MH (2010) The metabolic actions of glucagon revisited. Nat Rev Endocrinol 6:689–697
Habegger KM, Stemmer K, Cheng C, Muller TD, Heppner KM, Ottaway N, Holland J, Hembree JL, Smiley D, Gelfanov V, Krishna R, Arafat AM, Konkar A, Belli S, Kapps M, Woods SC, Hofmann SM, D'Alessio D, Pfluger PT, Perez-Tilve D, Seeley RJ, Konishi M, Itoh N, Kharitonenkov A, Spranger J, DiMarchi RD, Tschop MH (2013) Fibroblast growth factor 21 mediates specific glucagon actions. Diabetes 62:1453–1463
Hahn HJ, Gottschling HD, Woltanski P (1978) Effect of somatostatin on insulin secretion and cAMP content of isolated pancreatic rat islets. Metabolism 27:1291–1294
Han SM, Namkoong C, Jang PG, Park IS, Hong SW, Katakami H, Chun S, Kim SW, Park JY, Lee KU, Kim MS (2005) Hypothalamic AMP-activated protein kinase mediates counter-regulatory responses to hypoglycaemia in rats. Diabetologia 48:2170–2178
Hancock AS, Du A, Liu J, Miller M, May CL (2010) Glucagon deficiency reduces hepatic glucose production and improves glucose tolerance in adult mice. Mol Endocrinol 24:1605–1614
Hansen BC, Jen KLC, Pek SB, Wolfe RA (1982) Rapid oscillations in plasma insulin, glucagon, and glucose in obese and normal weight humans. J Clin Endocrinol Metab 54:785–792
Hansen LH, Gromada J, Bouchelouche P, Whitmore T, Jelinek L, Kindsvogel W, Nishimura E (1998) Glucagon-mediated Ca^{2+} signaling in BHK cells expressing cloned human glucagon receptors. Am J Physiol Cell Physiol 274:C1552–C1562
Hansen AM, Bodvarsdottir TB, Nordestgaard DN, Heller RS, Gotfredsen CF, Maedler K, Fels JJ, Holst JJ, Karlsen AE (2011) Upregulation of α-cell glucagon-like peptide 1 (GLP-1) in *Psammomys obesus*–an adaptive response to hyperglycaemia? Diabetologia 54:1379–1387
Hardy AB, Fox JE, Giglou PR, Wijesekara N, Bhattacharjee A, Sultan S, Gyulkhandanyan AV, Gaisano HY, MacDonald PE, Wheeler MB (2009) Characterization of Erg K^+ channels in α- and β-cells of mouse and human islets. J Biol Chem 284:30441–30452
Hardy AB, Serino AS, Wijesekara N, Chimienti F, Wheeler MB (2011) Regulation of glucagon secretion by zinc: lessons from the β cell-specific *Znt8* knockout mouse model. Diabetes Obes Metab 13(Suppl 1):112–117
Hare KJ, Vilsboll T, Holst JJ, Knop FK (2010a) Inappropriate glucagon response after oral compared with isoglycemic intravenous glucose administration in patients with type 1 diabetes. Am J Physiol Endocrinol Metab 298:E832–E837
Hare KJ, Vilsboll T, Asmar M, Deacon CF, Knop FK, Holst JJ (2010b) The glucagonostatic and insulinotropic effects of glucagon-like peptide 1 contribute equally to its glucose-lowering action. Diabetes 59:1765–1770
Hatton TW, Yip CC, Vranic M (1985) Biosynthesis of glucagon (IRG3500) in canine gastric mucosa. Diabetes 34:38–46
Hauge-Evans AC, King AJ, Carmignac D, Richardson CC, Robinson IC, Low MJ, Christie MR, Persaud SJ, Jones PM (2009) Somatostatin secreted by islet δ-cells fulfills multiple roles as a paracrine regulator of islet function. Diabetes 58:403–411
Havel PJ, Taborsky GJ Jr (1994) The contribution of the autonomic nervous system to increased glucagon secretion during hypoglycemic stress: update 1994. Endocr Rev 2:201–204
Havel PJ, Valverde C (1996) Autonomic mediation of glucagon secretion during insulin- induced hypoglycemia in rhesus monkeys. Diabetes 45:960–966

Havel PJ, Veith RC, Dunning BE, Taborsky GJ Jr (1991) Role for autonomic nervous system to increase pancreatic glucagon secretion during marked insulin-induced hypoglycemia in dogs. Diabetes 40:1107–1114

Havel PJ, Akpan JO, Curry DL, Stern JS, Gingerich RL, Ahrén B (1993) Autonomic control of pancreatic polypeptide and glucagon secretion during neuroglucopenia and hypoglycemia in mice. Am J Physiol Regul Integr Comp Physiol 265:R246–R254

Hayashi Y (2011) Metabolic impact of glucagon deficiency. Diabetes Obes Metab 13(Suppl 1):151–157

Hayashi Y, Yamamoto M, Mizoguchi H, Watanabe C, Ito R, Yamamoto S, Sun XY, Murata Y (2009) Mice deficient for glucagon gene-derived peptides display normoglycemia and hyperplasia of islet α-cells but not of intestinal L-cells. Mol Endocrinol 23:1990–1999

Heimberg H, De Vos A, Pipeleers DG, Thorens B, Schuit F (1995) Differences in glucose transporter gene expression between rat pancreatic α- and β-cells are correlated to differences in glucose transport but not in glucose utilization. J Biol Chem 270:8971–8975

Heimberg H, De Vos A, Moens K, Quartier E, Bouwens L, Pipeleers DG, Van Schaftingen E, Madsen O, Schuit F (1996) The glucose sensor protein glucokinase is expressed in glucagon-producing α-cells. Proc Natl Acad Sci USA 93:7036–7041

Heller SR, Cryer PE (1991) Hypoinsulinemia is not critical to glucose recovery from hypoglycemia in humans. Am J Physiol 261:E41–E48

Heller RS, Kieffer TJ, Habener JF (1997) Insulinotropic glucagon-like peptide 1 receptor expression in glucagon-producing α-cells of the rat endocrine pancreas. Diabetes 46:785–791

Hellman B, Salehi A, Gylfe E, Dansk H, Grapengiesser E (2009) Glucose generates coincident insulin and somatostatin pulses and antisynchronous glucagon pulses from human pancreatic islets. Endocrinology 150:5334–5340

Hellman B, Salehi A, Grapengiesser E, Gylfe E (2012) Isolated mouse islets respond to glucose with an initial peak of glucagon release followed by pulses of insulin and somatostatin in antisynchrony with glucagon. Biochem Biophys Res Commun 417:1219–1223

Henningsson R, Lundquist I (1998) Arginine-induced insulin release is decreased and glucagon increased in parallel with islet NO production. Am J Physiol Endocrinol Metab 275:E500–E506

Henquin JC, Rahier J (2011) Pancreatic α-cell mass in European subjects with type 2 diabetes. Diabetologia 54:1720–1725

Heppner KM, Habegger KM, Day J, Pfluger PT, Perez-Tilve D, Ward B, Gelfanov V, Woods SC, DiMarchi R, Tschop M (2010) Glucagon regulation of energy metabolism. Physiol Behav 100:545–548

Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C, Puigserver P, Spiegelman B, Montminy M (2001) CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. Nature 413:179–183

Hjortoe GM, Hagel GM, Terry BR, Thastrup O, Arkhammar POG (2004) Functional identification and monitoring of individual α- and β-cells in cultured mouse islets of Langerhans. Acta Diabetol 41:185–193

Holst JJ, Aggestrup S, Loud FB, Olesen M (1983a) Content and gel filtration profiles of glucagon-like and somatostatin-like immunoreactivity in human fundic mucosa. J Clin Endocrinol Metab 56:729–732

Holst JJ, Pedersen JH, Baldissera F, Stadil F (1983b) Circulating glucagon after total pancreatectomy in man. Diabetologia 25:396–399

Holst JJ, Bersani M, Johnsen AH, Kofod H, Hartmann B, Orskov C (1994) Proglucagon processing in porcine and human pancreas. J Biol Chem 269:18827–18833

Holst JJ, Vilsboll T, Deacon CF (2009) The incretin system and its role in type 2 diabetes mellitus. Mol Cell Endocrinol 297:127–136

Hong J, Abudula R, Chen J, Jeppesen PB, Dyrskog SE, Xiao J, Colombo M, Hermansen K (2005) The short-term effect of fatty acids on glucagon secretion is influenced by their chain length, spatial configuration, and degree of unsaturation: studies *in vitro*. Metabolism 54:1329–1336

Hong J, Chen L, Jeppesen PB, Nordentoft I, Hermansen K (2006) Stevioside counteracts the α-cell hypersecretion caused by long-term palmitate exposure. Am J Physiol Endocrinol Metab 290: E416–E422

Hong J, Jeppesen PB, Nordentoft I, Hermansen K (2007) Fatty acid-induced effect on glucagon secretion is mediated via fatty acid oxidation. Diabetes Metab Res Rev 23:202–210

Hope KM, Tran POT, Zhou H, Oseid E, Leroy E, Robertson RP (2004) Regulation of α-cell function by the β-cell in isolated human and rat islets deprived of glucose: the "Switch-off" hypothesis. Diabetes 53:1488–1495

Huang L, Shen H, Atkinson MA, Kennedy RT (1995) Detection of exocytosis at individual pancreatic β-cells by amperometry at a chemically modified microelectrode. Proc Natl Acad Sci USA 92:9608–9612

Huang YC, Gaisano HY, Leung YM (2011a) Electrophysiological identification of mouse islet α-cells: from isolated single α-cells to *in situ* assessment within pancreas slices. Islets 3:139–143

Huang YC, Rupnik M, Gaisano HY (2011b) Unperturbed islet α-cell function examined in mouse pancreas tissue slices. J Physiol 589:395–408

Huang YC, Rupnik MS, Karimian N, Herrera PL, Gilon P, Feng ZP, Gaisano HY (2013) *In situ* electrophysiological examination of pancreatic α-cells in the streptozotocin-induced diabetes model, revealing the cellular basis of glucagon hypersecretion. Diabetes 62:519–530

Hunyady B, Hipkin RW, Schonbrunn A, Mezey E (1997) Immunohistochemical localization of somatostatin receptor SST2A in the rat pancreas. Endocrinology 138:2632–2635

Ikeda T, Yoshida T, Ito Y, Murakami I, Mokuda O, Tominaga M, Mashiba H (1987) Effect of β-hydroxybutyrate and acetoacetate on insulin and glucagon secretion from perfused rat pancreas. Arch Biochem Biophys 257:140–143

Ipp E, Dobbs RE, Arimura A, Vale W, Harris V, Unger RH (1977) Release of immunoreactive somatostatin from the pancreas in response to glucose, amino acids, pancreozymin-cholecystokinin, and tolbutamide. J Clin Invest 60:760–765

Ishihara H, Maechler P, Gjinovci A, Herrera PL, Wollheim CB (2003) Islet β-cell secretion determines glucagon release from neighbouring α-cells. Nat Cell Biol 5:330–335

Jamison RA, Stark R, Dong J, Yonemitsu S, Zhang D, Shulman GI, Kibbey RG (2011) Hyperglucagonemia precedes a decline in insulin secretion and causes hyperglycemia in chronically glucose-infused rats. Am J Physiol Endocrinol Metab 301:E1174–E1183

Jaspan JB, Lever E, Polonsky KS, Van Cauter E (1986) *In vivo* pulsatility of pancreatic islet peptides. Am J Physiol 251:E215–E226

Jelinek LJ, Lok S, Rosenberg GB, Smith RA, Grant FJ, Biggs SH, Bensch PA, Kuijper JL, Sheppard PO, Sprecher CA, O'Hara PJ, Foster D, Walker KM, Chen LHJ, McKernan PA, Kindsvogel W (1993) Expression cloning and signaling properties of the rat glucagon receptor. Science 259:1614–1616

Jijakli H, Rasschaert J, Nadi AB, Leclercq-Meyer V, Sener A, Malaisse WJ (1996) Relevance of lactate dehydrogenase activity to the control of oxidative glycolysis in pancreatic islet β-cell. Arch Biochem Biophys 327:260–264

Jin Y, Korol SV, Jin Z, Barg S, Birnir B (2013) In intact islets interstitial GABA activates $GABA_A$ receptors that generate tonic currents in α-cells. PLoS One 8:e67228

Jing X, Li DQ, Olofsson CS, Salehi A, Surve VV, Caballero J, Ivarsson R, Lundquist I, Pereverzev A, Schneider T, Rorsman P, Renström E (2005) $Ca_v2.3$ calcium channels control second-phase insulin release. J Clin Invest 115:146–154

Johnson D, Bennett ES (2006) Isoform-specific effects of the β_2 subunit on voltage-gated sodium channel gating. J Biol Chem 281:25875–25881

Johnson DG, Goebel CU, Hruby VJ, Bregman MD, Trivedi D (1982) Hyperglycemia of diabetic rats decreased by a glucagon receptor antagonist. Science 215:1115–1116

Jones BJ, Tan T, Bloom SR (2012) Minireview: glucagon in stress and energy homeostasis. Endocrinology 153:1049–1054

Jorgensen NB, Dirksen C, Bojsen-Moller KN, Jacobsen SH, Worm D, Hansen DL, Kristiansen VB, Naver L, Madsbad S, Holst JJ (2013) Exaggerated glucagon-like peptide 1 response is important

for improved β-cell function and glucose tolerance after Roux-en-Y gastric bypass in patients with type 2 diabetes. Diabetes 62:3044–3052

Kailey B, van de Bunt M, Cheley S, Johnson PR, MacDonald PE, Gloyn AL, Rorsman P, Braun M (2012) SSTR2 is the functionally dominant somatostatin receptor in human pancreatic β- and α-cells. Am J Physiol Endocrinol Metab 303:E1107–E1116

Kang L, Routh VH, Kuzhikandathil EV, Gaspers LD, Levin BE (2004) Physiological and molecular characteristics of rat hypothalamic ventromedial nucleus glucosensing neurons. Diabetes 53:549–559

Kanno T, Gopel SO, Rorsman P, Wakui M (2002) Cellular function in multicellular system for hormone-secretion: electrophysiological aspect of studies on α-, β- and δ-cells of the pancreatic islet. Neurosci Res 42:79–90

Karimian N, Qin T, Liang T, Osundiji M, Huang Y, Teich T, Riddell MC, Cattral MS, Coy DH, Vranic M, Gaisano HY (2013) Somatostatin receptor type 2 antagonism improves glucagon counterregulation in biobreeding diabetic rats. Diabetes 62:2968–2977

Karschin C, Ecke C, Ashcroft FM, Karschin A (1997) Overlapping distribution of K_{ATP} channel-forming Kir6.2 subunit and the sulfonylurea receptor SUR1 in rodent brain. FEBS Lett 401:59–64

Kawai K, Ipp E, Orci L, Perrelet A, Unger RH (1982) Circulating somatostatin acts on the islets of Langerhans by way of a somatostatin-poor compartment. Science 218:477–478

Kawamori D, Kulkarni RN (2009) Insulin modulation of glucagon secretion: the role of insulin and other factors in the regulation of glucagon secretion. Islets 1:276–279

Kawamori D, Kurpad AJ, Hu J, Liew CW, Shih JL, Ford EL, Herrera PL, Polonsky KS, McGuinness OP, Kulkarni RN (2009) Insulin signaling in α-cells modulates glucagon secretion *in vivo*. Cell Metab 9:350–361

Kawamori D, Welters HJ, Kulkarni RN (2010) Molecular pathways underlying the pathogenesis of pancreatic α-cell dysfunction. Adv Exp Med Biol 654:421–445

Kawamori D, Akiyama M, Hu J, Hambro B, Kulkarni RN (2011) Growth factor signalling in the regulation of α-cell fate. Diabetes Obes Metab 13(Suppl 1):21–30

Kendall DM, Poitout V, Olson LK, Sorenson RL, Robertson RP (1995) Somatostatin coordinately regulates glucagon gene expression and exocytosis in HIT-T15 cells. J Clin Invest 96:2496–2502

Kern W, Offenheuser S, Born J, Fehm HL (1996) Entrainment of ultradian oscillations in the secretion of insulin and glucagon to the nonrapid eye movement rapid eye movement sleep rhythm in humans. J Clin Endocrinol Metab 81:1541–1547

Kieffer TJ, Habener JL (1999) The glucagon-like peptides. Endocr Rev 20:876–913

Kilimnik G, Kim A, Jo J, Miller K, Hara M (2009) Quantification of pancreatic islet distribution *in situ* in mice. Am J Physiol Endocrinol Metab 297:E1331–E1338

Kimball SR, Siegfried BA, Jefferson LS (2004) Glucagon represses signaling through the mammalian target of rapamycin in rat liver by activating AMP-activated protein kinase. J Biol Chem 279:54103–54109

Kleinman R, Gingerich R, Ohning G, Wong H, Olthoff K, Walsh J, Brunicardi FC (1995) The influence of somatostatin on glucagon and pancreatic polypeptide secretion in the isolated perfused human pancreas. Int J Pancreatol 18:51–57

Knop FK, Vilsboll T, Madsbad S, Holst JJ, Krarup T (2007a) Inappropriate suppression of glucagon during OGTT but not during isoglycaemic i.v. glucose infusion contributes to the reduced incretin effect in type 2 diabetes mellitus. Diabetologia 50:797–805

Knop FK, Vilsboll T, Hojberg PV, Larsen S, Madsbad S, Volund A, Holst JJ, Krarup T (2007b) Reduced incretin effect in type 2 diabetes: cause or consequence of the diabetic state? Diabetes 56:1951–1959

Knop FK, Hare KJ, Pedersen J, Hendel JW, Poulsen SS, Holst JJ, Vilsboll T (2011) Prohormone convertase 2 positive enteroendocrine cells are more abundant in patients with type 2 diabetes – a potential source of gut-derived glucagon. Diabetes 60:A478

Komjati M, Bratusch-Marrain P, Waldhausl W (1986) Superior efficacy of pulsatile versus continuous hormone exposure on hepatic glucose production *in vitro*. Endocrinology 118:312–319

Koo SH, Flechner L, Qi L, Zhang X, Screaton RA, Jeffries S, Hedrick S, Xu W, Boussouar F, Brindle P, Takemori H, Montminy M (2005) The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. Nature 437:1109–1111

Kosinski JR, Hubert J, Carrington PE, Chicchi GG, Mu J, Miller C, Cao J, Bianchi E, Pessi A, SinhaRoy R, Marsh DJ, Pocai A (2012) The glucagon receptor is involved in mediating the body weight-lowering effects of oxyntomodulin. Obesity (Silver Spring) 20:1566–1571

Kumar U, Sasi R, Suresh S, Patel A, Thangaraju M, Metrakos P, Patel SC, Patel YC (1999) Subtype-selective expression of the five somatostatin receptors (hSSTR1-5) in human pancreatic islet cells – a quantitative double-label immunohistochemical analysis. Diabetes 48:77–85

Lacerda AE, Kim HS, Ruth P, Perez-Reyes E, Flockerzi V, Hofmann F, Birnbaumer L, Brown AM (1991) Normalization of current kinetics by interaction between the α_1 and β subunits of the skeletal muscle dihydropyridine-sensitive Ca^{2+} channel. Nature 352:527–530

Lang DA, Matthews DR, Peto J, Turner RC (1979) Cyclic oscillations of basal plasma glucose and insulin concentrations in human beings. N Engl J Med 301:1023–1027

Lang DA, Matthews DR, Burnett M, Ward GM, Turner RC (1982) Pulsatile synchronous basal insulin and glucagon secretion in man. Diabetes 31:22–26

Larsen PJ, Tang-Christensen M, Holst JJ, Orskov C (1997) Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. Neuroscience 77:257–270

Larsson H, Ahrén B (2000) Glucose intolerance is predicted by low insulin secretion and high glucagon secretion: outcome of a prospective study in postmenopausal Caucasian women. Diabetologia 43:194–202

Larsson LI, Holst J, Hakanson R, Sundler F (1975) Distribution and properties of glucagon immunoreactivity in the digestive tract of various mammals: an immunohistochemical and immunochemical study. Histochemistry 44:281–290

Le Marchand SJ, Piston DW (2010) Glucose suppression of glucagon secretion: metabolic and calcium responses from α-cells in intact mouse pancreatic islets. J Biol Chem 285:14389–14398

Le Marchand SJ, Piston DW (2012) Glucose decouples intracellular Ca^{2+} activity from glucagon secretion in mouse pancreatic islet α-cells. PLoS One 7:e47084

Leclerc I, Sun G, Morris C, Fernandez-Millan E, Nyirenda M, Rutter GA (2011) AMP-activated protein kinase regulates glucagon secretion from mouse pancreatic α-cells. Diabetologia 54:125–134

Leclercq-Meyer V, Marchand J, Woussen Colle MC, Giroix MH, Malaisse WJ (1985) Multiple effects of leucine on glucagon, insulin, and somatostatin secretion from the perfused rat pancreas. Endocrinology 116:1168–1174

Lee YC, Asa SL, Drucker DJ (1992) Glucagon gene 5′-flanking sequences direct expression of simian virus 40 large T antigen to the intestine, producing carcinoma of the large bowel in transgenic mice. J Biol Chem 267:10705–10708

Lee Y, Wang MY, Du XQ, Charron MJ, Unger RH (2011) Glucagon receptor knockout prevents insulin-deficient type 1 diabetes in mice. Diabetes 60:391–397

Lee Y, Berglund ED, Wang MY, Fu X, Yu X, Charron MJ, Burgess SC, Unger RH (2012) Metabolic manifestations of insulin deficiency do not occur without glucagon action. Proc Natl Acad Sci USA 109:14972–14976

Lefebvre PJ (2011) Early milestones in glucagon research. Diabetes Obes Metab 13(Suppl 1):1–4

Lefebvre PJ, Luyckx AS (1980) Neurotransmitters and glucagon release from the isolated, perfused canine stomach. Diabetes 29:697–701

Lefèbvre PJ, Paolisso G, Scheen AJ, Henquin JC (1987) Pulsatility of insulin and glucagon release: physiological significance and pharmacological implications. Diabetologia 30:443–452

Lefebvre P, Paolisso G, Scheen AJ (1996) Pulsatility of glucagon. Handb Exp Pharmacol 123:105–113

Lemaire K, Ravier MA, Schraenen A, Creemers JW, Van de Plas R, Granvik M, Van LL, Waelkens E, Chimienti F, Rutter GA, Gilon P, in't Veld PA, Schuit FC (2009) Insulin

crystallization depends on zinc transporter ZnT8 expression, but is not required for normal glucose homeostasis in mice. Proc Natl Acad Sci USA 106:14872–14877

Leung YM, Ahmed I, Sheu L, Tsushima RG, Diamant NE, Hara M, Gaisano HY (2005) Electrophysiological characterization of pancreatic islet cells in the mouse insulin promoter-green fluorescent protein mouse. Endocrinology 146:4766–4775

Leung YM, Ahmed I, Sheu L, Gao X, Hara M, Tsushima RG, Diamant NE, Gaisano HY (2006a) Insulin regulates islet α-cell function by reducing K_{ATP} channel sensitivity to adenosine 5′-triphosphate inhibition. Endocrinology 147:2155–2162

Leung YM, Ahmed I, Sheu L, Tsushima RG, Diamant NE, Gaisano HY (2006b) Two populations of pancreatic islet α-cells displaying distinct Ca^{2+} channel properties. Biochem Biophys Res Commun 345:340–344

Levetan C, Want LL, Weyer C, Strobel SA, Crean J, Wang Y, Maggs DG, Kolterman OG, Chandran M, Mudaliar SR, Henry RR (2003) Impact of pramlintide on glucose fluctuations and postprandial glucose, glucagon, and triglyceride excursions among patients with type 1 diabetes intensively treated with insulin pumps. Diabetes Care 26:1–8

Li C, Liu C, Nissim I, Chen J, Chen P, Doliba N, Zhang T, Nissim I, Daikhin Y, Stokes D, Yudkoff M, Bennett MJ, Stanley CA, Matschinsky FM, Naji A (2013) Regulation of glucagon secretion in normal and diabetic human islets by γ-hydroxybutyrate and glycine. J Biol Chem 288:3938–3951

Liang Y, Osborne MC, Monia BP, Bhanot S, Gaarde WA, Reed C, She P, Jetton TL, Demarest KT (2004) Reduction in glucagon receptor expression by an antisense oligonucleotide ameliorates diabetic syndrome in *db*/*db* mice. Diabetes 53:410–417

Liu YJ, Vieira E, Gylfe E (2004) A store-operated mechanism determines the activity of the electrically excitable glucagon-secreting pancreatic α-cell. Cell Calcium 35:357–365

Longuet C, Robledo AM, Dean ED, Dai C, Ali S, McGuinness I, de Chavez V, Vuguin PM, Charron MJ, Powers AC, Drucker DJ (2013) Liver-specific disruption of the murine glucagon receptor produces α-cell hyperplasia: evidence for a circulating α-cell growth factor. Diabetes 62:1196–1205

Louw J, Woodroof CW, Wolfe-Coote SA (1997) Distribution of endocrine cells displaying immunoreactivity for one or more peptides in the pancreas of the adult vervet monkey (*Cercopithecus aethiops*). Anat Rec 247:405–412

Ludvigsen E (2007) Somatostatin receptor expression and biological functions in endocrine pancreatic cells: review based on a doctoral thesis. Ups J Med Sci 112:1–20

Ludvigsen E, Olsson R, Stridsberg M, Janson ET, Sandler S (2004) Expression and distribution of somatostatin receptor subtypes in the pancreatic islets of mice and rats. J Histochem Cytochem 52:391–400

Ludvigsen E, Stridsberg M, Taylor JE, Culler MD, Oberg K, Janson ET, Sandler S (2007) Regulation of insulin and glucagon secretion from rat pancreatic islets *in vitro* by somatostatin analogues. Regul Pept 138:1–9

Lui EY, Asa SL, Drucker DJ, Lee YC, Brubaker PL (1990) Glucagon and related peptides in fetal rat hypothalamus *in vivo* and *in vitro*. Endocrinology 126:110–117

Luyckx AS, Gerard J, Gaspard U, Lefebvre PJ (1975) Plasma glucagon levels in normal women during pregnancy. Diabetologia 11:549–554

MacDonald PE, Obermuller S, Vikman J, Galvanovskis J, Rorsman P, Eliasson L (2005) Regulated exocytosis and kiss-and-run of synaptic-like microvesicles in INS-1 and primary rat β-cells. Diabetes 54:736–743

Macdonald PE, Marinis YZ, Ramracheya R, Salehi A, Ma X, Johnson PR, Cox R, Eliasson L, Rorsman P (2007) A K_{ATP} channel-dependent pathway within α-cells regulates glucagon release from both rodent and human islets of Langerhans. PLoS Biol 5:e143

Manning Fox JE, Gyulkhandanyan AV, Satin LS, Wheeler MB (2006) Oscillatory membrane potential response to glucose in islet β-cells: a comparison of islet-cell electrical activity in mouse and rat. Endocrinology 147:4655–4663

Marhfour I, Moulin P, Marchandise J, Rahier J, Sempoux C, Guiot Y (2009) Impact of Sur1 gene inactivation on the morphology of mouse pancreatic endocrine tissue. Cell Tissue Res 335:505–515
Marroqui L, Vieira E, Gonzalez A, Nadal A, Quesada I (2011) Leptin downregulates expression of the gene encoding glucagon in αTC1-9 cells and mouse islets. Diabetologia 54:843–851
Marty N, Dallaporta M, Foretz M, Emery M, Tarussio D, Bady I, Binnert C, Beermann F, Thorens B (2005) Regulation of glucagon secretion by glucose transporter type 2 (glut2) and astrocyte-dependent glucose sensors. J Clin Invest 115:3545–3553
Maruyama H, Hisatomi A, Orci L, Grodsky GM, Unger RH (1984) Insulin within islets is a physiologic glucagon release inhibitor. J Clin Invest 74:2296–2299
Masur K, Tibaduiza EC, Chen C, Ligon B, Beinborn M (2005) Basal receptor activation by locally produced glucagon-like peptide-1 contributes to maintaining β-cell function. Mol Endocrinol 19:1373–1382
Mayo KE, Miller LJ, Bataille D, Dalle S, Goke B, Thorens B, Drucker DJ (2003) International Union of Pharmacology. XXXV. The glucagon receptor family. Pharmacol Rev 55:167–194
McCrimmon RJ, Fan X, Ding Y, Zhu W, Jacob RJ, Sherwin RS (2004) Potential role for AMP-activated protein kinase in hypoglycemia sensing in the ventromedial hypothalamus. Diabetes 53:1953–1958
McCrimmon RJ, Evans ML, Fan X, McNay EC, Chan O, Ding Y, Zhu W, Gram DX, Sherwin RS (2005) Activation of ATP-sensitive K^+ channels in the ventromedial hypothalamus amplifies counterregulatory hormone responses to hypoglycemia in normal and recurrently hypoglycemic rats. Diabetes 54:3169–3174
McGuinness OP (2005) Defective glucose homeostasis during infection. Annu Rev Nutr 25:9–35
Meier JJ, Gallwitz B, Siepmann N, Holst JJ, Deacon CF, Schmidt WE, Nauck MA (2003) Gastric inhibitory polypeptide (GIP) dose-dependently stimulates glucagon secretion in healthy human subjects at euglycaemia. Diabetologia 46:798–801
Meier JJ, Kjems LL, Veldhuis JD, Lefèbvre P, Butler PC (2006) Postprandial suppression of glucagon secretion depends on intact pulsatile insulin secretion. Further evidence for the intraislet insulin hypothesis. Diabetes 55:1051–1056
Menge BA, Gruber L, Jorgensen SM, Deacon CF, Schmidt WE, Veldhuis JD, Holst JJ, Meier JJ (2011) Loss of inverse relationship between pulsatile insulin and glucagon secretion in patients with type 2 diabetes. Diabetes 60:2160–2168
Mercan D, Delville J-P, Leclercq-Meyer V, Malaisse WJ (1993) Preferential stimulation by D-glucose of oxidative glycolysis in pancreatic islets: comparison between B and non-B cells. Biochem Int 29:475–481
Mercan D, Kadiata MM, Malaisse WJ (1999) Differences in the time course of the metabolic response of B and non-B pancreatic islet cells to D-glucose and metabolized or non-metabolized hexose esters. Biochem Biophys Res Commun 262:346–349
Mighiu PI, Yue JT, Filippi BM, Abraham MA, Chari M, Lam CK, Yang CS, Christian NR, Charron MJ, Lam TK (2013) Hypothalamic glucagon signaling inhibits hepatic glucose production. Nat Med 19:766–772
Miki T, Liss B, Minami K, Shiuchi T, Saraya A, Kashima Y, Horiuchi M, Ashcroft F, Minokoshi Y, Roeper J, Seino S (2001) ATP-sensitive K^+ channels in the hypothalamus are essential for the maintenance of glucose homeostasis. Nat Neurosci 4:507–512
Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ (2013) Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature 494:256–260
Minami K, Miki T, Kadowaki T, Seino S (2004) Roles of ATP-sensitive K^+ channels as metabolic sensors: studies of Kir6.x null mice. Diabetes 53:S176–S180
Moens K, Heimberg H, Flamez D, Huypens P, Quartier E, Ling ZD, Pipeleers DG, Gremlich S, Thorens B, Schuit F (1996) Expression and functional activity of glucagon, glucagon-like peptide I, and glucose-dependent insulinotropic peptide receptors in rat pancreatic islet cells. Diabetes 45:257–261
Moller N, Beckwith R, Butler PC, Christensen NJ, Orskov H, Alberti KG (1989) Metabolic and hormonal responses to exogenous hyperthermia in man. Clin Endocrinol (Oxf) 30:651–660

Muller WA, Faloona GR, Unger RH (1973) Hyperglucagonemia in diabetic ketoacidosis. Its prevalence and significance. Am J Med 54:52–57

Munoz A, Hu M, Hussain K, Bryan J, Aguilar-Bryan L, Rajan AS (2005) Regulation of glucagon secretion at low glucose concentrations: evidence for adenosine triphosphate-sensitive potassium channel involvement. Endocrinology 146:5514–5521

Muscogiuri G, Mezza T, Prioletta A, Sorice GP, Clemente G, Sarno G, Nuzzo G, Pontecorvi A, Holst JJ, Giaccari A (2013) Removal of duodenum elicits GLP-1 secretion. Diabetes Care 36:1641–1646

Nadal A, Quesada I, Soria B (1999) Homologous and heterologous asynchronicity between identified α-, β- and δ-cells within intact islets of Langerhans in the mouse. J Physiol (Lond) 517:85–93

Nagamatsu S, Watanabe T, Nakamichi Y, Yamamura C, Tsuzuki K, Matsushima S (1999) A-soluble *N*-ethylmaleimide-sensitive factor attachment protein is expressed in pancreatic β-cells and functions in insulin but not γ-aminobutyric acid secretion. J Biol Chem 274:8053–8060

Nian M, Drucker DJ, Irwin D (1999) Divergent regulation of human and rat proglucagon gene promoters *in vivo*. Am J Physiol 277:G829–G837

Nian M, Gu J, Irwin DM, Drucker DJ (2002) Human glucagon gene promoter sequences regulating tissue-specific versus nutrient-regulated gene expression. Am J Physiol Regul Integr Comp Physiol 282:R173–R183

Nicolson TJ, Bellomo EA, Wijesekara N, Loder MK, Baldwin JM, Gyulkhandanyan AV, Koshkin V, Tarasov AI, Carzaniga R, Kronenberger K, Taneja TK, da Silva X, Libert S, Froguel P, Scharfmann R, Stetsyuk V, Ravassard P, Parker H, Gribble FM, Reimann F, Sladek R, Hughes SJ, Johnson PR, Masseboeuf M, Burcelin R, Baldwin SA, Liu M, Lara-Lemus R, Arvan P, Schuit FC, Wheeler MB, Chimienti F, Rutter GA (2009) Insulin storage and glucose homeostasis in mice null for the granule zinc transporter ZnT8 and studies of the type 2 diabetes-associated variants. Diabetes 58:2070–2083

Nie Y, Nakashima M, Brubaker PL, Li QL, Perfetti R, Jansen E, Zambre Y, Pipeleers D, Friedman TC (2000) Regulation of pancreatic PC1 and PC2 associated with increased glucagon-like peptide 1 in diabetic rats. J Clin Invest 105:955–965

Nieto FR, Cobos EJ, Tejada MA, Sanchez-Fernandez C, Gonzalez-Cano R, Cendan CM (2012) Tetrodotoxin (TTX) as a therapeutic agent for pain. Mar Drugs 10:281–305

Novak U, Wilks A, Buell G, McEwen S (1987) Identical mRNA for preproglucagon in pancreas and gut. Eur J Biochem 164:553–558

Nyholm B, Orskov L, Hove KY, Gravholt CH, Moller N, Alberti KGMM, Moyses C, Kolterman O, Schmitz O (1999) The amylin analog pramlintide improves glycemic control and reduces postprandial glucagon concentrations in patients with type 1 diabetes mellitus. Metabolism 48:935–941

Ohneda A, Watanabe K, Horigome K, Sakai T, Kai Y, Oikawa S (1978) Abnormal response of pancreatic glucagon to glycemic changes in diabetes mellitus. J Clin Endocrinol Metab 46:504–510

Ohneda A, Kobayashi T, Nihei J (1984) Response of extrapancreatic immunoreactive glucagon to intraluminal nutrients in pancreatectomized dogs. Horm Metab Res 16:344–348

Ohtsuka K, Nimura Y, Yasui K (1986) Paradoxical elevations of plasma glucagon levels in patients after pancreatectomy or gastrectomy. Jpn J Surg 16:1–7

Olofsson CS, Salehi A, Gopel SO, Holm C, Rorsman P (2004) Palmitate stimulation of glucagon secretion in mouse pancreatic α-cells results from activation of L-type calcium channels and elevation of cytoplasmic calcium. Diabetes 53:2836–2843

Olsen HL, Theander S, Bokvist K, Buschard K, Wollheim CB, Gromada J (2005) Glucose stimulates glucagon release in single rat α-cells by mechanisms that mirror the stimulus-secretion coupling in β-cells. Endocrinology 146:4861–4870

Orci L, Baetens D, Rufener C, Amherdt M, Ravazzola M, Studer P, Malaisse-Lagae F, Unger RH (1976a) Hypertrophy and hyperplasia of somatostatin-containing δ-cells in diabetes. Proc Natl Acad Sci USA 73:1338–1342

Orci L, Baetens D, Ravazzola M, Stefan Y, Malaisse-Lagae F (1976b) Pancreatic polypeptide and glucagon: non-random distribution in pancreatic islets. Life Sci 19:1811–1815

Orskov C, Holst JJ, Poulsen SS, Kirkegaard P (1987) Pancreatic and intestinal processing of proglucagon in man. Diabetologia 30:874–881

Orskov C, Rabenhoj L, Wettergren A, Kofod H, Holst JJ (1994) Tissue and plasma concentrations of amidated and glycine- extended glucagon-like peptide 1 in humans. Diabetes 43:535–539

Ostenson CG, Grebing C (1985) Evidence for metabolic regulation of pancreatic glucagon secretion by L-glutamine. Acta Endocrinol (Copenh) 108:386–391

Panagiotidis G, Salehi AA, Westermark P, Lundquist I (1992) Homologous islet amyloid polypeptide: effects on plasma levels of glucagon, insulin and glucose in the mouse. Diabetes Res Clin Pract 18:167–171

Paolisso G, Buonocore S, Gentile S, Sgambato S, Varricchio M, Scheen A, D'Onofrio F, Lefebvre PJ (1990) Pulsatile glucagon has greater hyperglycaemic, lipolytic and ketogenic effects than continuous hormone delivery in man: effect of age. Diabetologia 33:272–277

Parekh AB, Putney JW Jr (2005) Store-operated calcium channels. Physiol Rev 85:757–810

Parker JC, Andrews KM, Allen MR, Stock JL, McNeish JD (2002) Glycemic control in mice with targeted disruption of the glucagon receptor gene. Biochem Biophys Res Commun 290:839–843

Parker HE, Reimann F, Gribble FM (2010) Molecular mechanisms underlying nutrient-stimulated incretin secretion. Expert Rev Mol Med 12:e1

Patel YC, Wheatley T, Ning C (1981) Multiple forms of immunoreactive somatostatin: comparison of distribution in neural and nonneural tissues and portal plasma of the rat. Endocrinology 109:1943–1949

Patel YC, Amherdt M, Orci L (1982) Quantitative electron microscopic autoradiography of insulin, glucagon, and somatostatin binding sites on islets. Science 217:1155–1156

Pecker F, Pavoine C (1996) Mode of action of glucagon revisited. Handb Exp Pharmacol 123:75–104

Peng IC, Chen Z, Sun W, Li YS, Marin TL, Hsu PH, Su MI, Cui X, Pan S, Lytle CY, Johnson DA, Blaeser F, Chatila T, Shyy JY (2012) Glucagon regulates ACC activity in adipocytes through the CAMKKβ/AMPK pathway. Am J Physiol Endocrinol Metab 302:E1560–E1568

Perez-Reyes E, Kim HS, Lacerda AE, Horne W, Wei XY, Rampe D, Campbell KP, Brown AM, Birnbaumer L (1989) Induction of calcium currents by the expression of the α_1-subunit of the dihydropyridine receptor from skeletal muscle. Nature 340:233–236

Philippe J (1991) Insulin regulation of the glucagon gene is mediated by an insulin-responsive DNA element. Proc Natl Acad Sci USA 88:7224–7227

Philippe J (1996) The glucagon gene and its expression. Handb Exp Pharmacol 123:11–30

Pipeleers DG, in't Veld PA, Van de Winkel M, Maes E, Schuit FC, Gepts W (1985a) A new *in vitro* model for the study of pancreatic A and B cells. Endocrinology 117:806–816

Pipeleers DG, Schuit FC, In't Veld PA, Maes E, Hooghe Peters EL, Van de Winkel M, Gepts W (1985b) Interplay of nutrients and hormones in the regulation of insulin release. Endocrinology 117:824–833

Pipeleers DG, Schuit FC, Van Schravendijk CF, Van de Winkel M (1985c) Interplay of nutrients and hormones in the regulation of glucagon release. Endocrinology 117:817–823

Pizarro-Delgado J, Braun M, Hernandez-Fisac I, Martin-Del-Rio R, Tamarit-Rodriguez J (2010) Glucose promotion of GABA metabolism contributes to the stimulation of insulin secretion in β-cells. Biochem J 431:381–389

Plant TD (1988) Na^+ currents in cultured mouse pancreatic β-cells. Pflugers Arch 411:429–435

Pocai A, Carrington PE, Adams JR, Wright M, Eiermann G, Zhu L, Du X, Petrov A, Lassman ME, Jiang G, Liu F, Miller C, Tota LM, Zhou G, Zhang X, Sountis MM, Santoprete A, Capito' E, Chicchi GG, Thornberry N, Bianchi E, Pessi A, Marsh DJ, SinhaRoy R (2009) Glucagon-like peptide 1/glucagon receptor dual agonism reverses obesity in mice. Diabetes 58:2258–2266

Preitner F, Ibberson M, Franklin I, Binnert C, Pende M, Gjinovci A, Hansotia T, Drucker DJ, Wollheim C, Burcelin R, Thorens B (2004) Gluco-incretins control insulin secretion at multiple levels as revealed in mice lacking GLP-1 and GIP receptors. J Clin Invest 113:635–645

Quesada I, Todorova MG, Alonso-Magdalena P, Beltra M, Carneiro EM, Martin F, Nadal A, Soria B (2006a) Glucose induces opposite intracellular Ca^{2+} concentration oscillatory patterns in identified α- and β-cells within intact human islets of Langerhans. Diabetes 55:2463–2469

Quesada I, Todorova MG, Soria B (2006b) Different metabolic responses in α-, β-, and δ-cells of the islet of Langerhans monitored by redox confocal microscopy. Biophys J 90:2641–2650

Quesada I, Tuduri E, Ripoll C, Nadal A (2008) Physiology of the pancreatic α-cell and glucagon secretion: role in glucose homeostasis and diabetes. J Endocrinol 199:5–19

Quoix N, Cheng-Xue R, Guiot Y, Herrera PL, Henquin JC, Gilon P (2007) The GluCre-ROSA26EYFP mouse: a new model for easy identification of living pancreatic α-cells. FEBS Lett 581:4235–4240

Quoix N, Cheng-Xue R, Mattart L, Zeinoun Z, Guiot Y, Beauvois MC, Henquin JC, Gilon P (2009) Glucose and pharmacological modulators of ATP-sensitive K^+ channels control $[Ca^{2+}]_c$ by different mechanisms in isolated mouse α-cells. Diabetes 58:412–421

Qureshi SA, Candelore MR, Xie D, Yang X, Tota LM, Ding VDH, Li Z, Bansal A, Miller C, Cohen SM, Jiang G, Brady E, Saperstein R, Duffy JL, Tata JR, Chapman KT, Moller DE, Zhang BB (2004) A novel glucagon receptor antagonist inhibits glucagon-mediated biological effects. Diabetes 53:3267–3273

Rahier J, Goebbels RM, Henquin JC (1983) Cellular composition of the human diabetic pancreas. Diabetologia 24:366–371

Rajan AS, Aguilar-Bryan L, Nelson DA, Nichols CG, Wechsler SW, Lechago J, Bryan J (1993) Sulfonylurea receptors and ATP-sensitive K^+ channels in clonal pancreatic α-cells. Evidence for two high affinity sulfonylurea receptors. J Biol Chem 268:15221–15228

Raju B, Cryer PE (2005) Maintenance of the postabsorptive plasma glucose concentration: insulin or insulin plus glucagon? Am J Physiol Endocrinol Metab 289:E181–E186

Ramnanan CJ, Edgerton DS, Kraft G, Cherrington AD (2011) Physiologic action of glucagon on liver glucose metabolism. Diabetes Obes Metab 13(Suppl 1):118–125

Ramracheya R, Ward C, Shigeto M, Walker JN, Amisten S, Zhang Q, Johnson PR, Rorsman P, Braun M (2010) Membrane potential-dependent inactivation of voltage-gated ion channels in α-cells inhibits glucagon secretion from human islets. Diabetes 59:2198–2208

Ranganath L, Schaper F, Gama R, Morgan L (2001) Does glucagon have a lipolytic effect? Clin Endocrinol (Oxf) 54:125–126

Raskin P, Unger RH (1978a) Hyperglucagonemia and its suppression. Importance in the metabolic control of diabetes. N Engl J Med 299:433–436

Raskin P, Unger RH (1978b) Effect of insulin therapy on the profiles of plasma immunoreactive glucagon in juvenile-type and adult-type diabetics. Diabetes 27:411–419

Ravazzola M, Unger RH, Orci L (1981) Demonstration of glucagon in the stomach of human fetuses. Diabetes 30:879–882

Ravier MA, Rutter GA (2005) Glucose or insulin, but not zinc ions, inhibit glucagon secretion from mouse pancreatic α-cells. Diabetes 54:1789–1797

Reaven GM, Chen YD, Golay A, Swislocki AL, Jaspan JB (1987) Documentation of hyperglucagonemia throughout the day in nonobese and obese patients with noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 64:106–110

Reetz A, Solimena M, Matteoli M, Folli F, Takei K, De Camilli P (1991) GABA and pancreatic β-cells: colocalization of glutamic acid decarboxylase (GAD) and GABA with synaptic-like microvesicles suggests their role in GABA storage and secretion. EMBO J 10:1275–1284

Reichlin S (1983a) Somatostatin. N Engl J Med 309:1495–1501

Reichlin S (1983b) Somatostatin (second of two parts). N Engl J Med 309:1556–1563

Robertson RP, Zhou H, Slucca M (2011) A role for zinc in pancreatic islet β-cell cross-talk with the α-cell during hypoglycaemia. Diabetes Obes Metab 13(Suppl 1):106–111

Rocha DM, Faloona GR, Unger RH (1972) Glucagon-stimulating activity of 20 amino acids in dogs. J Clin Invest 51:2346–2351

Rodgers RL (2012) Glucagon and cyclic AMP: time to turn the page? Curr Diabetes Rev 8:362–381

Rodriguez-Diaz R, Abdulreda MH, Formoso AL, Gans I, Ricordi C, Berggren PO, Caicedo A (2011a) Innervation patterns of autonomic axons in the human endocrine pancreas. Cell Metab 14:45–54

Rodriguez-Diaz R, Dando R, Jacques-Silva MC, Fachado A, Molina J, Abdulreda MH, Ricordi C, Roper SD, Berggren PO, Caicedo A (2011b) α-cells secrete acetylcholine as a non-neuronal paracrine signal priming β-cell function in humans. Nat Med 17:888–892

Rohrer S, Menge BA, Gruber L, Deacon CF, Schmidt WE, Veldhuis JD, Holst JJ, Meier JJ (2012) Impaired crosstalk between pulsatile insulin and glucagon secretion in prediabetic individuals. J Clin Endocrinol Metab 97:E791–E795

Ronner P, Matschinsky FM, Hang TL, Epstein AJ, Buettger C (1993) Sulfonylurea-binding sites and ATP-sensitive K^+ channels in α-TC glucagonoma and β-TC insulinoma cells. Diabetes 42:1760–1772

Rorsman P, Hellman B (1988) Voltage-activated currents in guinea pig pancreatic α_2 cells. Evidence for Ca^{2+}-dependent action potentials. J Gen Physiol 91:223–242

Rorsman P, Berggren PO, Bokvist K, Ericson H, Mohler H, Ostenson CG, Smith PA (1989) Glucose-inhibition of glucagon secretion involves activation of $GABA_A$-receptor chloride channels. Nature 341:233–236

Rorsman P, Ashcroft FM, Berggren PO (1991) Regulation of glucagon release from pancreatic α-cells. Biochem Pharmacol 41:1783–1790

Rorsman P, Salehi SA, Abdulkader F, Braun M, MacDonald PE (2008) K_{ATP}-channels and glucose-regulated glucagon secretion. Trends Endocrinol Metab 19:277–284

Rorsman P, Braun M, Zhang Q (2012) Regulation of calcium in pancreatic α- and β-cells in health and disease. Cell Calcium 51(3–4):300–308

Rossowski WJ, Coy DH (1994) Specific inhibition of rat pancreatic insulin or glucagon release by receptor-selective somatostatin analogs. Biochem Biophys Res Commun 205:341–346

Rouille Y, Westermark G, Martin SK, Steiner DF (1994) Proglucagon is processed to glucagon by prohormone convertase PC2 in αTC1-6 cells. Proc Natl Acad Sci USA 91:3242–3246

Rutter GA (2010) Think zinc: new roles for zinc in the control of insulin secretion. Islets 2:49–50

Salehi A, Vieira E, Gylfe E (2006) Paradoxical stimulation of glucagon secretion by high glucose concentrations. Diabetes 55:2318–2323

Salehi A, Qader SS, Grapengiesser E, Hellman B (2007) Pulses of somatostatin release are slightly delayed compared with insulin and antisynchronous to glucagon. Regul Pept 144:43–49

Salehi A, Parandeh F, Fredholm BB, Grapengiesser E, Hellman B (2009) Absence of adenosine A1 receptors unmasks pulses of insulin release and prolongs those of glucagon and somatostatin. Life Sci 85:470–476

Samols E, Stagner JI, Ewart RB, Marks V (1988) The order of islet microvascular cellular perfusion is B–A–D in the perfused rat pancreas. J Clin Invest 82:350–353

Schafer MK, Day R, Cullinan WE, Chretien M, Seidah NG, Watson SJ (1993) Gene expression of prohormone and proprotein convertases in the rat CNS: a comparative *in situ* hybridization analysis. J Neurosci 13:1258–1279

Schuit FC, Derde MP, Pipeleers DG (1989) Sensitivity of rat pancreatic A and B cells to somatostatin. Diabetologia 32:207–212

Schuit F, De Vos A, Farfari S, Moens K, Pipeleers D, Brun T, Prentki M (1997) Metabolic fate of glucose in purified islet cells. Glucose-regulated anaplerosis in β-cells. J Biol Chem 272:18572–18579

Seino S, Iwanaga T, Nagashima K, Miki T (2000) Diverse roles of K_{ATP} channels learned from Kir6.2 genetically engineered mice. Diabetes 49:311–318

Sekine N, Cirulli V, Regazzi R, Brown LJ, Gine E, Tamarit-Rodriguez J, Girotti M, Marie S, MacDonald MJ, Wollheim CB, Rutter GA (1994) Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogenase in pancreatic β-cells. Potential role in nutrient sensing. J Biol Chem 269:4895–4902

Seyffert WA Jr, Madison LL (1967) Physiologic effects of metabolic fuels carbohydrate metabolism. I. Acute effect of elevation of plasma free fatty acids on hepatic glucose output, peripheral glucose utilization, serum insulin, and plasma glucagon levels. Diabetes 16:765–776

Shah P, Basu A, Basu R, Rizza R (1999) Impact of lack of suppression of glucagon on glucose tolerance in humans. Am J Physiol Endocrinol Metab 277:E283–E290

Shah P, Vella A, Basu A, Basu R, Schwenk WF, Rizza RA (2000) Lack of suppression of glucagon contributes to postprandial hyperglycemia in subjects with type 2 diabetes mellitus. J Clin Endocrinol Metab 85:4053–4059

Shimizu H, Tsuchiya T, Ohtani K, Shimomura K, Oh I, Ariyama Y, Okada S, Kishi M, Mori M (2011) Glucagon plays an important role in the modification of insulin secretion by leptin. Islets 3:150–154

Shiota C, Rocheleau JV, Shiota M, Piston DW, Magnuson MA (2005) Impaired glucagon secretory responses in mice lacking the type 1 sulfonylurea receptor. Am J Physiol Endocrinol Metab 289:E570–E577

Shyng SL, Nichols CG (1997) Octameric stoichiometry of the K_{ATP} channel complex. J Gen Physiol 110:655–664

Silvestre RA, Rodríguez-Gallardo J, Jodka C, Parkes DG, Pittner RA, Young AA, Marco J (2001) Selective amylin inhibition of the glucagon response to arginine is extrinsic to the pancreas. Am J Physiol Endocrinol Metab 280:E443–E449

Singer D, Biel M, Lotan I, Flockerzi V, Hofmann F, Dascal N (1991) The roles of the subunits in the function of the calcium channel. Science 253:1553–1557

Singh V, Brendel MD, Zacharias S, Mergler S, Jahr H, Wiedenmann B, Bretzel RG, Plockinger U, Strowski MZ (2007) Characterization of somatostatin receptor subtype-specific regulation of insulin and glucagon secretion: an *in vitro* study on isolated human pancreatic islets. J Clin Endocrinol Metab 92:673–680

Siu FY, He M, de Graff C, Han GW, Yang D, Zhang Z, Zhou C, Xu Q, Wacker D, Joseph JS, Liu W, Lau J, Cherezov V, Katritch V, Wang MW, Stevens RC (2012) Structure of the human glucagon class B G-protein-coupled receptor. Nature 499:444–449

Sivarajah P, Wheeler MB, Irwin DM (2001) Evolution of receptors for proglucagon-derived peptides: isolation of frog glucagon receptors. Comp Biochem Physiol B Biochem Mol Biol 128:517–527

Sloop KW, Cao JX, Siesky AM, Zhang HY, Bodenmiller DM, Cox AL, Jacobs SJ, Moyers JS, Owens RA, Showalter AD, Brenner MB, Raap A, Gromada J, Berridge BR, Monteith DK, Porksen N, McKay RA, Monia BP, Bhanot S, Watts LM, Michael MD (2004) Hepatic and glucagon-like peptide-1-mediated reversal of diabetes by glucagon receptor antisense oligonucleotide inhibitors. J Clin Invest 113:1571–1581

Smismans A, Schuit F, Pipeleers D (1997) Nutrient regulation of γ-aminobutyric acid release from islet β-cells. Diabetologia 40:1411–1415

Solomon TP, Knudsen SH, Karstoft K, Winding K, Holst JJ, Pedersen BK (2012) Examining the effects of hyperglycemia on pancreatic endocrine function in humans: evidence for *in vivo* glucotoxicity. J Clin Endocrinol Metab 97:4682–4691

Sorensen H, Winzell MS, Brand CL, Fosgerau K, Gelling RW, Nishimura E, Ahren B (2006) Glucagon receptor knockout mice display increased insulin sensitivity and impaired β-cell function. Diabetes 55:3463–3469

Sorenson RL, Elde RP (1983) Dissociation of glucose stimulation of somatostatin and insulin release from glucose inhibition of glucagon release in the isolated perfused rat pancreas. Diabetes 32:561–567

Spigelman AF, Dai X, MacDonald PE (2010) Voltage-dependent K^+ channels are positive regulators of α-cell action potential generation and glucagon secretion in mice and humans. Diabetologia 53:1917–1926

Stagner JI, Samols E (1986) Retrograde perfusion as a model for testing the relative effects of glucose versus insulin on the A cell. J Clin Invest 77:1034–1037

Stagner JI, Samols E (1992) The vascular order of islet cellular perfusion in the human pancreas. Diabetes 41:93–97

Stagner JI, Samols E, Weir GC (1980) Sustained oscillations of insulin, glucagon and somatostatin from the isolated canine pancreas during exposure to a constant glucose concentration. J Clin Invest 65:939–942

Stagner JI, Samols E, Bonner-Weir S (1988) β–α–δ pancreatic islet cellular perfusion in dogs. Diabetes 37:1715–1721

Stagner JI, Samols E, Marks V (1989) The anterograde and retrograde infusion of glucagon antibodies suggests that A cells are vascularly perfused before D cells within the rat islet. Diabetologia 32:203–206

Starke A, Imamura T, Unger RH (1987) Relationship of glucagon suppression by insulin and somatostatin to the ambient glucose concentration. J Clin Invest 79:20–24

Steiner DJ, Kim A, Miller K, Hara M (2010) Pancreatic islet plasticity: interspecies comparison of islet architecture and composition. Islets 2:135–145

Strowski MZ, Blake AD (2008) Function and expression of somatostatin receptors of the endocrine pancreas. Mol Cell Endocrinol 286:169–179

Strowski MZ, Parmar RM, Blake AD, Schaeffer JM (2000) Somatostatin inhibits insulin and glucagon secretion via two receptor subtypes: an *in vitro* study of pancreatic islets from somatostatin receptor 2 knockout mice. Endocrinology 141:111–117

Strowski MZ, Cashen DE, Birzin ET, Yang L, Singh V, Jacks TM, Nowak KW, Rohrer SP, Patchett AA, Smith RG, Schaeffer JM (2006) Antidiabetic activity of a highly potent and selective nonpeptide somatostatin receptor subtype-2 agonist. Endocrinology 147:4664–4673

Sundler F, Alumets J, Holst J, Larsson LI, Hakanson R (1976) Ultrastructural identification of cells storing pancreatic-type glucagon in dog stomach. Histochemistry 50:33–37

Suzuki M, Fujikura K, Inagaki N, Seino S, Takata K (1997) Localization of the ATP-sensitive K^+ channel subunit Kir6.2 in mouse pancreas. Diabetes 46:1440–1444

Svoboda M, Tastenoy M, Vertongen P, Robberecht P (1994) Relative quantitative analysis of glucagon receptor mRNA in rat tissues. Mol Cell Endocrinol 105:131–137

Taborsky GJ Jr, Mundinger TO (2012) Minireview: the role of the autonomic nervous system in mediating the glucagon response to hypoglycemia. Endocrinology 153:1055–1062

Taborsky GJ, Ahrén B, Havel PJ (1998) Autonomic mediation of glucagon secretion during hypoglycemia – implications for impaired α-cell responses in type 1 diabetes. Diabetes 47:995–1005

Takahashi R, Ishihara H, Tamura A, Yamaguchi S, Yamada T, Takei D, Katagiri H, Endou H, Ola Y (2006) Cell type-specific activation of metabolism reveals that β-cell secretion suppresses glucagon release from α-cells in rat pancreatic islets. Am J Physiol Endocrinol Metab 290:E308–E316

Tan TM, Field BC, McCullough KA, Troke RC, Chambers ES, Salem V, Gonzalez MJ, Baynes KC, De SA, Viardot A, Alsafi A, Frost GS, Ghatei MA, Bloom SR (2013) Coadministration of glucagon-like peptide-1 during glucagon infusion in humans results in increased energy expenditure and amelioration of hyperglycemia. Diabetes 62:1131–1138

Taneera J, Jin Z, Jin Y, Muhammed SJ, Zhang E, Lang S, Salehi A, Korsgren O, Renstrom E, Groop L, Birnir B (2012) γ-Aminobutyric acid (GABA) signalling in human pancreatic islets is altered in type 2 diabetes. Diabetologia 55:1985–1994

Taniguchi H, Okada Y, Seguchi H, Shimada C, Seki M, Tsutou A, Baba S (1979) High concentration of γ-aminobutyric acid in pancreatic β-cells. Diabetes 28:629–633

Thomas-Reetz AC, Hell JW, During MJ, Walch-Solimena C, Jahn R, De Camilli P (1993) A γ-aminobutyric acid transporter driven by a proton pump is present in synaptic-like microvesicles of pancreatic β-cells. Proc Natl Acad Sci USA 90:5317–5321

Thorel F, Nepote V, Avril I, Kohno K, Desgraz R, Chera S, Herrera PL (2010) Conversion of adult pancreatic α-cells to β-cells after extreme β-cell loss. Nature 464:1149–1154

Thorel F, Damond N, Chera S, Wiederkehr A, Thorens B, Meda P, Wollheim CB, Herrera PL (2011) Normal glucagon signaling and β-cell function after near-total α-cell ablation in adult mice. Diabetes 60:2872–2882

Thorens B (2011) Brain glucose sensing and neural regulation of insulin and glucagon secretion. Diabetes Obes Metab 13(Suppl 1):82–88

Thorens B (2012) Sensing of glucose in the brain. Handb Exp Pharmacol 209:277–294

Tian G, Sandler S, Gylfe E, Tengholm A (2011) Glucose- and hormone-induced cAMP oscillations in α- and β-cells within intact pancreatic islets. Diabetes 60:1535–1543

Tian G, Tepikin AV, Tengholm A, Gylfe E (2012) cAMP induces stromal interaction molecule 1 (STIM1) puncta but neither Orai1 protein clustering nor store-operated Ca^{2+} entry (SOCE) in islet cells. J Biol Chem 287:9862–9872

Tornehave D, Kristensen P, Romer J, Knudsen LB, Heller RS (2008) Expression of the GLP-1 receptor in mouse, rat, and human pancreas. J Histochem Cytochem 56:841–851

Tottene A, Moretti A, Pietrobon D (1996) Functional diversity of P-type and R-type calcium channels in rat cerebellar neurons. J Neurosci 16:6353–6363

Trapp S, Ballanyi K (1995) K_{ATP} channel mediation of anoxia-induced outward current in rat dorsal vagal neurons *in vitro*. J Physiol 487(Pt 1):37–50

Tsutsumi Y (1984) Immunohistochemical studies on glucagon, glicentin and pancreatic polypeptide in human stomach: normal and pathological conditions. Histochem J 16:869–883

Tu JA, Tuch BE, Si ZY (1999) Expression and regulation of glucokinase in rat islet β- and α-cells during development. Endocrinology 140:3762–3766

Tuduri E, Marroqui L, Soriano S, Ropero AB, Batista TM, Piquer S, Lopez-Boado MA, Carneiro EM, Gomis R, Nadal A, Quesada I (2009) Inhibitory effects of leptin on pancreatic α-cell function. Diabetes 58:1616–1624

Ugleholdt R, Zhu X, Deacon CF, Orskov C, Steiner DF, Holst JJ (2004) Impaired intestinal proglucagon processing in mice lacking prohormone convertase 1. Endocrinology 145:1349–1355

Unger RH (1985) Glucagon physiology and pathophysiology in the light of new advances. Diabetologia 28:574–578

Unger RH, Cherrington AD (2012) Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. J Clin Invest 122:4–12

Unger RH, Orci L (2010) Paracrinology of islets and the paracrinopathy of diabetes. Proc Natl Acad Sci USA 107:16009–16012

Unger RH, Ohneda A, Aguilar-Parada E, Eisentraut AM (1969) The role of aminogenic glucagon secretion in blood glucose homeostasis. J Clin Invest 48:810–822

Unger RH, Aguilar-Parada E, Müller WA, Eisentraut AM (1970) Studies of pancreatic α-cell function in normal and diabetic subjects. J Clin Invest 49:837–848

Vieira E, Liu YJ, Gylfe E (2004) Involvement of α_1 and β-adrenoceptors in adrenaline stimulation of the glucagon-secreting mouse α-cell. Naunyn Schmiedebergs Arch Pharmacol 369:179–183

Vieira E, Salehi A, Gylfe E (2005) Glucose inhibits glucagon release independently of K_{ATP} channels. Diabetologia 48:A177

Vieira E, Salehi A, Gylfe E (2007) Glucose inhibits glucagon secretion by a direct effect on mouse pancreatic α-cells. Diabetologia 50:370–379

Vignali S, Leiss V, Karl R, Hofmann F, Welling A (2006) Characterization of voltage-dependent sodium and calcium channels in maintain pancreatic α- and β-cells. J Physiol 572:691–706

von Meyenn F, Porstmann T, Gasser E, Selevsek N, Schmidt A, Aebersold R, Stoffel M (2013) Glucagon-induced acetylation of Foxa2 regulates hepatic lipid metabolism. Cell Metab 17:436–447

Vuguin PM, Kedees MH, Cui L, Guz Y, Gelling RW, Nejathaim M, Charron MJ, Teitelman G (2006) Ablation of the glucagon receptor gene increases fetal lethality and produces alterations in islet development and maturation. Endocrinology 147:3995–4006

Wakelam MJO, Murphy GJ, Hruby UJ, Houslay MD (1986) Activation of two signal-transduction systems in hepatocytes by glucagon. Nature 323:68–70

Walker D, De Waard M (1998) Subunit interaction sites in voltage-dependent Ca^{2+} channels: role in channel function. Trends Neurosci 21:148–154

Walker JN, Ramracheya R, Zhang Q, Johnson PR, Braun M, Rorsman P (2011) Regulation of glucagon secretion by glucose: paracrine, intrinsic or both? Diabetes Obes Metab 13(Suppl 1):95–105

Wang JL, McDaniel ML (1990) Secretagogue-induced oscillations of cytoplasmic Ca^{2+} in single β-and α-cells obtained from pancreatic islets by fluorescence-activated cell sorting. Biochem Biophys Res Commun 166:813–818

Wang C, Kerckhofs K, Van de Casteele M, Smolders I, Pipeleers D, Ling Z (2006) Glucose inhibits GABA release by pancreatic β-cells through an increase in GABA shunt activity. Am J Physiol Endocrinol Metab 290:E494–E499

Wang MY, Chen L, Clark GO, Lee Y, Stevens RD, Ilkayeva OR, Wenner BR, Bain JR, Charron MJ, Newgard CB, Unger RH (2010) Leptin therapy in insulin-deficient type I diabetes. Proc Natl Acad Sci USA 107:4813–4819

Wang Y, Li G, Goode J, Paz JC, Ouyang K, Screaton R, Fischer WH, Chen J, Tabas I, Montminy M (2012) Inositol-1,4,5-trisphosphate receptor regulates hepatic gluconeogenesis in fasting and diabetes. Nature 485:128–132

Wang X, Zielinski MC, Misawa R, Wen P, Wang TY, Wang CZ, Witkowski P, Hara M (2013) Quantitative analysis of pancreatic polypeptide cell distribution in the human pancreas. PLoS One 8:e55501

Watanabe C, Seino Y, Miyahira H, Yamamoto M, Fukami A, Ozaki N, Takagishi Y, Sato J, Fukuwatari T, Shibata K, Oiso Y, Murata Y, Hayashi Y (2012) Remodeling of hepatic metabolism and hyperaminoacidemia in mice deficient in proglucagon-derived peptides. Diabetes 61:74–84

Webb GC, Akbar MS, Zhao C, Swift HH, Steiner DF (2002) Glucagon replacement via micro-osmotic pump corrects hypoglycemia and α-cell hyperplasia in prohormone convertase 2 knockout mice. Diabetes 51:398–405

Weckbecker G, Lewis I, Albert R, Schmid HA, Hoyer D, Bruns C (2003) Opportunities in somatostatin research: biological, chemical and therapeutic aspects. Nat Rev Drug Discov 2:999–1017

Weigle DS (1987) Pulsatile secretion of fuel-regulatory hormones. Diabetes 36:764–775

Weigle DS, Goodner CJ (1986) Evidence that the physiological pulse frequency of glucagon secretion optimizes glucose production by perifused rat hepatocytes. Endocrinology 118:1606–1613

Weigle DS, Koerker DJ, Goodner CJ (1984) Pulsatile glucagon delivery enhances glucose production by perifused rat hepatocytes. Am J Physiol 247:E564–E568

Weir GC, Knowlton SD, Atkins RF, McKennan KX, Martin DB (1976) Glucagon secretion from the perfused pancreas of streptozotocin-treated rats. Diabetes 25:275–282

Wendt A, Birnir B, Buschard K, Gromada J, Salehi A, Sewing S, Rorsman P, Braun M (2004) Glucose inhibition of glucagon secretion from rat α-cells is mediated by GABA released from neighboring β-cells. Diabetes 53:1038–1045

Westermark P, Andersson A, Westermark GT (2011) Islet amyloid polypeptide, islet amyloid, and diabetes mellitus. Physiol Rev 91:795–826

Whalley NM, Pritchard LE, Smith DM, White A (2011) Processing of proglucagon to GLP-1 in pancreatic α-cells: is this a paracrine mechanism enabling GLP-1 to act on β-cells? J Endocrinol 211:99–106

Wideman RD, Yu ILY, Webber TD, Verchere CB, Johnson JD, Cheung AT, Kieffer TJ (2006) Improving function and survival of pancreatic islets by endogenous production of glucagon-like peptide 1 (GLP-1). Proc Natl Acad Sci USA 103:13468–13473

Wijesekara N, Dai FF, Hardy AB, Giglou PR, Bhattacharjee A, Koshkin V, Chimienti F, Gaisano HY, Rutter GA, Wheeler MB (2010) β-cell-specific *Znt8* deletion in mice causes marked defects in insulin processing, crystallisation and secretion. Diabetologia 53:1656–1668

Winarto A, Miki T, Seino S, Iwanaga T (2001) Morphological changes in pancreatic islets of K_{ATP} channel-deficient mice: the involvement of K_{ATP} channels in the survival of insulin cells and the maintenance of islet architecture. Arch Histol Cytol 64:59–67

Winnock F, Ling Z, De Proft R, Dejonghe S, Schuit F, Gorus F, Pipeleers D (2002) Correlation between GABA release from rat islet β-cells and their metabolic state. Am J Physiol Endocrinol Metab 282:E937–E942

Wulff H, Castle NA, Pardo LA (2009) Voltage-gated potassium channels as therapeutic targets. Nat Rev Drug Discov 8:982–1001

Wynne K, Park AJ, Small CJ, Meeran K, Ghatei MA, Frost GS, Bloom SR (2006) Oxyntomodulin increases energy expenditure in addition to decreasing energy intake in overweight and obese humans: a randomised controlled trial. Int J Obes (Lond) 30:1729–1736

Xia F, Leung YM, Gaisano G, Gao X, Chen Y, Fox JE, Bhattacharjee A, Wheeler MB, Gaisano HY, Tsushima RG (2007) Targeting of voltage-gated K^+ and Ca^{2+} channels and soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor proteins to cholesterol-rich lipid rafts in pancreatic α-cells: effects on glucagon stimulus-secretion coupling. Endocrinology 148:2157–2167

Xu Y, Xie X (2009) Glucagon receptor mediates calcium signaling by coupling to $G_{\alpha q/11}$ and $G_{\alpha i/o}$ in HEK293 cells. J Recept Signal Transduct Res 29:318–325

Xu E, Kumar M, Zhang Y, Ju W, Obata T, Zhang N, Liu S, Wendt A, Deng S, Ebina Y, Wheeler MB, Braun M, Wang Q (2006) Intra-islet insulin suppresses glucagon release via GABA-$GABA_A$ receptor system. Cell Metab 3:47–58

Yamato E, Ikegami H, Tahara Y, Cha T, Yoneda H, Noma Y, Shima K, Ogihara T (1990) Role of protein kinase C in the regulation of glucagon gene expression by arginine. Biochem Biophys Res Commun 171:898–904

Yan L, Figueroa DJ, Austin CP, Liu Y, Bugianesi RM, Slaughter RS, Kaczorowski GJ, Kohler MG (2004) Expression of voltage-gated potassium channels in human and rhesus pancreatic islets. Diabetes 53:597–607

Yang J, MacDougall ML, McDowell MT, Xi L, Wei R, Zavadoski WJ, Molloy MP, Baker JD, Kuhn M, Cabrera O, Treadway JL (2011) Polyomic profiling reveals significant hepatic metabolic alterations in glucagon-receptor (GCGR) knockout mice: implications on anti-glucagon therapies for diabetes. BMC Genomics 12:281

Yoon JC, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J, Adelmant G, Stafford J, Kahn CR, Granner DK, Newgard CB, Spiegelman BM (2001) Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. Nature 413:131–138

Yoshimoto Y, Fukuyama Y, Horio Y, Inanobe A, Gotoh M, Kurachi Y (1999) Somatostatin induces hyperpolarization in pancreatic islet a cells by activating a G protein-gated K^+ channel. FEBS Lett 444:265–269

Young A (2005) Inhibition of glucagon secretion. Adv Pharmacol 52:151–171

Yu R, Dhall D, Nissen NN, Zhou C, Ren SG (2011) Pancreatic neuroendocrine tumors in glucagon receptor-deficient mice. PLoS One 6:e23397

Yue JT, Burdett E, Coy DH, Giacca A, Efendic S, Vranic M (2012) Somatostatin receptor type 2 antagonism improves glucagon and corticosterone counterregulatory responses to hypoglycemia in streptozotocin-induced diabetic rats. Diabetes 61:197–207

Zhang Y, Zhang N, Gyulkhandanyan AV, Xu E, Gaisano HY, Wheeler MB, Wang Q (2008) Presence of functional hyperpolarisation-activated cyclic nucleotide-gated channels in clonal α cell lines and rat islet α cells. Diabetologia 51:2290–2298

Zhao C, Wilson MC, Schuit F, Halestrap AP, Rutter GA (2001) Expression and distribution of lactate/monocarboxylate transporter isoforms in pancreatic islets and the exocrine pancreas. Diabetes 50:361–366

Zhou H, Zhang T, Harmon JS, Bryan J, Robertson RP (2007) Zinc, not insulin, regulates the rat α-cell response to hypoglycemia *in vivo*. Diabetes 56:1107–1112

Zhou C, Dhall D, Nissen NN, Chen CR, Yu R (2009) Homozygous P86S mutation of the human glucagon receptor is associated with hyperglucagonemia, α-cell hyperplasia, and islet cell tumor. Pancreas 38:941–946

Zhu X, Zhou A, Dey A, Norrbom C, Carroll R, Zhang C, Laurent V, Lindberg I, Ugleholdt R, Holst JJ, Steiner DF (2002) Disruption of PC1/3 expression in mice causes dwarfism and multiple neuroendocrine peptide processing defects. Proc Natl Acad Sci USA 99:10293–10298

Electrophysiology of Islet Cells

10

Gisela Drews, Peter Krippeit-Drews, and Martina Düfer

Contents

Abstract

Stimulus-secretion coupling (SSC) of pancreatic islet cells comprises electrical activity. Changes of the membrane potential (V_m) are regulated by metabolism-dependent alterations in ion channel activity.

This coupling is best explored in β cells. The effect of glucose is directly linked to mitochondrial metabolism as the ATP/ADP ratio determines the open probability of ATP-sensitive K^+ channels (K_{ATP} channels). Nucleotide sensitiv-

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_5,

ity and concentration in the direct vicinity of the channels are controlled by several factors including phospholipids, fatty acids, and kinases, e.g., creatine and adenylate kinase. Closure of K_{ATP} channels leads to depolarization of β cells via a yet unknown depolarizing current. Ca^{2+} influx during action potentials (APs) results in an increase of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) that triggers exocytosis. APs are elicited by opening of voltage-dependent Na^+ and/or Ca^{2+} channels and repolarized by voltage- and/or Ca^{2+}-dependent K^+ channels. At a constant stimulatory glucose concentration, APs are clustered in bursts that are interrupted by hyperpolarized interburst phases. Bursting electrical activity induces parallel fluctuations in $[Ca^{2+}]_c$ and insulin secretion. Bursts are terminated by I_{ksIow} consisting of currents through Ca^{2+}-dependent K^+ channels and K_{ATP} channels. This chapter focuses on structure, characteristics, physiological function, and regulation of ion channels in β cells. Information about pharmacological drugs acting on K_{ATP} channels, K_{ATP} channelopathies, and influence of oxidative stress on K_{ATP} channel function is provided. One focus is the outstanding significance of L-type Ca^{2+} channels for insulin secretion. The role of less well-characterized β cell channels including voltage-dependent Na^+ channels, volume-sensitive anion channels (VSACs), transient receptor potential (TRP)-related channels, and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels is discussed. A model of β cell oscillations provides insight in the interplay of the different channels to induce and maintain electrical activity. Regulation of β cell electrical activity by hormones and the autonomous nervous system is discussed.

α and δ cells are also equipped with K_{ATP} channels and voltage-dependent Na^+, K^+, and Ca^{2+} channels. Yet the SSC of these cells is less clear and is not necessarily dependent on K_{ATP} channel closure. Different ion channels of α and δ cells are introduced and SSC in α cells is described with special respect to paracrine effects of insulin and GABA secreted from β cells.

Keywords

K_{ATP} channels • Voltage-dependent Ca^{2+} channels • Voltage-dependent K^+ channels • Ca^{2+}-activated K^+ channels • K_v channels • K_{Ca} channels • Ca_v channels • SK4 channels • BK channels • Na^+ channels • TRP channels • K_{slow} current • Membrane potential • V_m • Current • Electrical activity • Oscillations • Action potential • Intracellular Ca^{2+} concentration • $[Ca^{2+}]_c$ • Stimulus-secretion coupling • ATP • ADP • Phosphotransfer • SUR1 • Kir6.2 • Sulfonylureas • Diazoxide • Oxidative stress • ROS • Diabetes • Hyperinsulinism • Neonatal diabetes • Mitochondria • Knock-out

Introduction

Diabetes mellitus is the most common endocrine disease. The number of patients with type-2 diabetes is tremendously increasing worldwide. Besides the individual burden the disease causes immense costs for the health care systems. Primarily, diabetes is a β cell disease although the other islet cells, especially glucagon-secreting α cells, are also involved in the manifestation of the disease. Normally, insulin suppresses glucagon secretion. Thus, reduced insulin secretion enhances secretion from α cells and glucagon contributes to a vicious circle that increases blood glucose concentrations. In type-1 diabetes mellitus β cells are destroyed by an autoimmune attack. Type-2 diabetes mellitus manifests when β cells cannot longer compensate for the high insulin demand which accompanies overnutrition and concomitant peripheral insulin resistance.

Hormone secretion of the islets cells is driven by electrical activity. Accordingly, islets cells are equipped with a variety of different ion channels including voltage-dependent and ligand-activated channels. Insulin is the only hormone in the human organism that is able to lower the blood glucose concentration and thus β cells are essential for survival. β-cells are unique as they do not only secrete insulin in response to a stimulus but also adapt the amount of released insulin to the nutrient concentration in the blood. This ensures regulation of the blood glucose concentration in a narrow range. Another unique feature of β cells is the oscillatory activity. The membrane potential oscillates due to fluctuations in the opening and closure of different ion channels. The most important channels involved in this oscillatory pattern are voltage-dependent Ca^{2+} channels and K_{ATP} channels. K_{ATP} channels are regulated by ATP derived from glucose metabolism in β cells and thus provide the link between the stimulus and cell metabolism on one hand and insulin secretion on the other hand. K_{ATP} channels are the target of oral antidiabetic drugs like sulfonylureas and glinides that have been used in the therapy of type-2 diabetes since many years. In recent years with the discovery of new channels in β cells new concepts appeared with the aim to find metabolism-dependent targets to avoid hypoglycemia. The oscillations of the membrane potential are strictly dependent of the surrounding glucose concentration. They comprise bursts with action potentials and silent hyperpolarized phases without electrical activity. The higher the glucose concentration the longer the burst phases. During the burst phases Ca^{2+} enters the cells that trigger insulin secretion. The oscillations of the membrane potential drive fluctuations of the cytosolic Ca^{2+} concentration, and finally oscillations of insulin secretion. Many metabolic factors also fluctuate e.g., ATP and NAD(P)H. Pulsatile insulin secretion is a prerequisite for the normal regulation of the blood glucose concentration. Already in an early phase long before diabetes becomes manifest oscillations are disturbed. Knowledge about ion channels in islet cells and their regulation is essential to understand the molecular basis of diabetes.

The following book chapter focuses on the role of ion channels in the regulation of insulin release but also highlights the role of ion channels in glucagon and somatostatin release.

β Cells

Ion Channels

K_{ATP} Channels

Structure/Assembly – Protein Networks

K_{ATP} channels are hetero-octamers consisting of sulfonylurea receptors (SUR1, SUR2A and B) and K_{IR} channels, either $K_{IR}6.1$ or $K_{IR}6.2$ (Inagaki et al. 1995; Clement et al. 1997; Aguilar-Bryan et al. 1998; Babenko et al. 1998; Bryan et al. 2007). β-cell K_{ATP} channels are composed of four $K_{IR}6.2$ subunits that form the pore and four regulatory SUR1 subunits (Fig. 1; Clement et al. 1997; Inagaki et al. 1997; Shyng and Nichols 1997; Aguilar-Bryan and Bryan 1999).

The $K_{IR}6.2$ Subunit

The $K_{IR}6.2$ subunit is a member of the inward rectifier superfamily. This subunit consists of two membrane-spanning helices, M1 and M2, which are linked by a pore loop containing the pore helix (Fig. 1a and Doyle et al. 1998). A sub-membrane positioned "slide helix" (Fig. 1a) may provide the link between SUR1 and $K_{IR}6.2$ which affects gating of the channel (Babenko 2005). Four $K_{IR}6.2$ subunits are necessary to establish a pore (Doyle et al. 1998; Moreau et al. 2005; Sharma et al. 1999). Usually only truncated $K_{IR}6.2$ proteins ($K_{IR}6.2\Delta C26$) are able to form a functional K_{ATP} channel in the absence of SUR subunits (Tucker et al. 1997) because of the retention of unassembled SUR1 and $K_{IR}6.2$ subunits in the endoplasmic reticulum (Zerangue et al. 1999). However, full length $K_{IR}6.2$ protein can result in functional channels in insect cells using a baculovirus system (Mikhailov et al. 1998). Inhibition by ATP is the most prominent characteristic of K_{ATP} channels. ADP can substitute ATP in the absence of Mg^{2+}, however, with a tenfold lower potency. Biochemical studies have shown that the ATP binding site is located on the large C-terminus of $K_{IR}6.2$ (Tanabe et al. 1999, 2000; Vanoye et al. 2002; Reimann et al. 1999a). Nevertheless, truncation of the $K_{IR}6.2$ N-terminus (Babenko et al. 1999a; Babenko and Bryan 2003) affects the ATP sensitivity.

The SUR1 Subunit

The SUR1 subunit is a typical ABC protein consisting of two bundles of six transmembrane helices (TMD1 and 2) with two cytosolic nucleotide-binding domains (NBD1 and 2, respectively) (Aguilar-Bryan et al. 1995). A bundle of five transmembrane helices (TMD0) together with its linker "L0" completes the subunit at the N-terminus (Fig. 1a).

The TMD0-L0 area interacts with the pore-building $K_{IR}6.2$ subunit to regulate the channel activity (Bryan et al. 2007; Babenko and Bryan 2002, 2003; Chan et al. 2003;

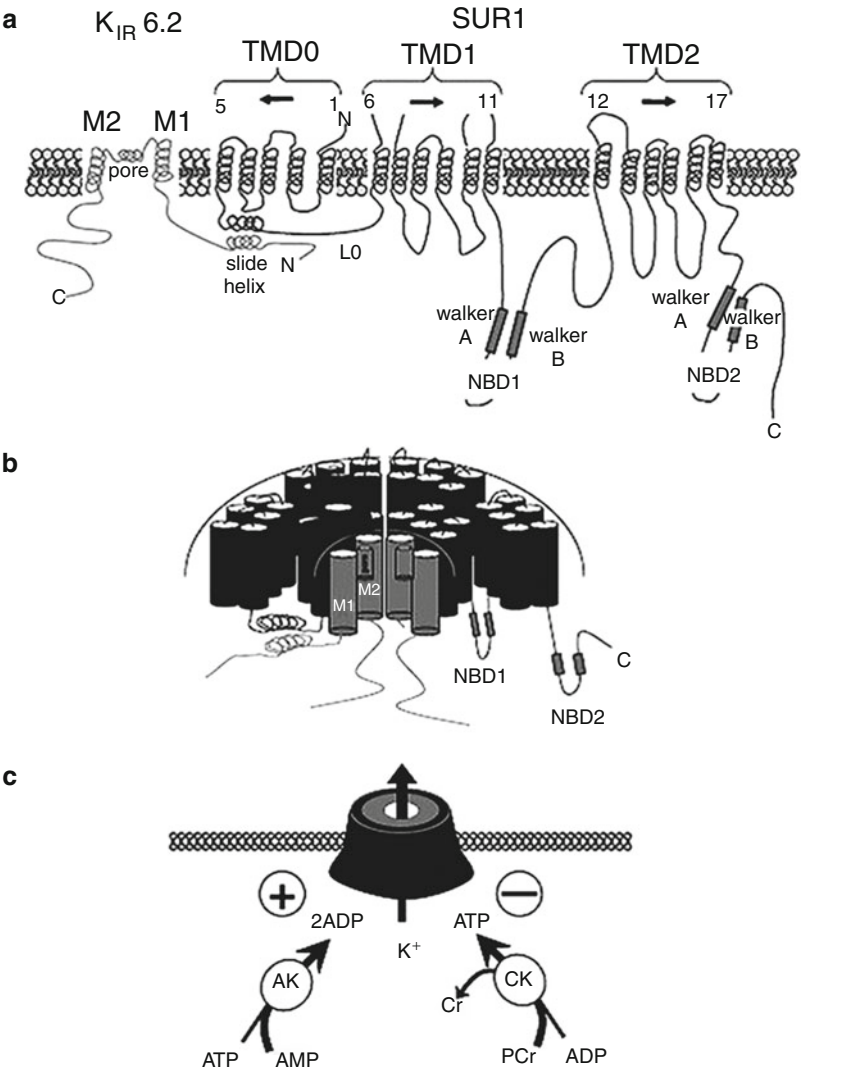

Fig. 1 Topology and homology model of the β cell K_{ATP} channel and regulating enzymes (**a**) The $K_{IR}6.2$ (*gray*) and SUR1 (*black*) topologies are illustrated schematically. The amino (*N*) and carboxyl (*C*) termini are marked. The Walker *A* and *B* consensus motifs are shown in the two nucleotide-binding domains (NBD1 and 2), respectively. The three transmembrane domains (TMD0-2) contain five to six transmembrane helices each (Inagaki et al. 1995; Clement et al. 1997; Aguilar-Bryan et al. 1998; Babenko et al. 1998; Bryan et al. 2007; Inagaki et al. 1997; Shyng and Nichols 1997; Aguilar-Bryan and Bryan 1999; Doyle et al. 1998; Babenko 2005; Moreau et al. 2005; Sharma et al. 1999; Tucker et al. 1997; Zerangue et al. 1999; Mikhailov et al. 1998; Tanabe et al. 1999, 2000). (**b**) The assembly of four subunits (half of a channel) of the hetero-octamer is shown. The complete channel consists of four $K_{IR}6.2$ pore-forming subunits surrounded by four regulating SUR1 subunits. (**c**) A creatine kinase (*CK*) and an adenylate kinase (*AK*) regulate the K_{ATP} channel negatively and positively, respectively. Both enzymes determine the nucleotide concentrations in the vicinity of the channel

Mikhailov et al. 2005; Reimann et al. 1999b). Both NBDs of SUR1 contain Walker A and B motifs (Fig. 1a) which are directly involved in nucleotide binding and modulation of channel activity (Matsuo et al. 2005; Gribble et al. 1997a). The NBDs are the binding sites for MgADP which activates the channel (Gribble et al. 1997a; Ueda et al. 1997), thus having an important role in the metabolic regulation of the channel (Babenko et al. 1999b). The NBDs also bind ATP. While NBD1 is a high-affinity nucleotide-binding site, NBD2 binds MgATP (or MgADP) with low affinity and has intrinsic ATPase activity (Matsuo et al. 2005; de Wet et al. 2007a). A regulatory model proposes that the ATPase activity, and therefore the binding and hydrolysis of MgATP, is required to switch SUR1 into a post-hydrolytic conformation that confers positive regulation to the channel (Proks et al. 2010). By contrast it has been shown (Ortiz et al. 2012) that ATP^{4-} binding to the NBDs, i.e., in the absence of Mg^{2+}, is sufficient to switch SUR1 allosterically into a stimulatory conformation for the Kir6.2 pore. This maneuver also reduces glibenclamide binding suggesting that ATP binding to the NBDs induces the formation of an NBD dimer which reconfigures the TMDs from inward- to outward-facing conformations (Ortiz et al. 2013). Therefore, only ATP-binding-domain and NBD dimerization, without hydrolysis, drives SUR1 from a non-stimulatory, inward-facing conformation with highest affinity for glibenclamide to an outward-facing stimulatory state. ATP-induced inhibition of K_{ATP} channels also occurs via Mg^{2+}-independent binding to the $K_{IR}6.2$ subunit (see above).

The SURs contain the binding sites for the K_{ATP} channel-blocking sulfonylureas such as tolbutamide, glibenclamide, or glimepiride but also for other hypoglycemic agents, e.g., the glinides and K_{ATP} channel openers (for details, see section on "Effects of Drugs").

Usually, the assembly of $K_{IR}6.2$ with SUR1 subunits (Fig. 1b) is required so that only complete hetero-octamers can yield functional K_{ATP} channels (Babenko et al. 1999b). Therefore, the knockout of SUR1 (SUR1KO) (Seghers et al. 2000) and $K_{IR}6.2$ ($K_{IR}6.2$KO) (Miki et al. 1998) abolishes the appearance of K_{ATP} channels in the plasma membrane of β cells (Seghers et al. 2000; Düfer et al. 2004).

The K_{ATP} channel of the β cell plasma membrane cannot be seen as an isolated functioning protein but is part of a protein network that regulates the channel activity.

These interactions comprise exocytotic proteins such as syntaxin-1A which is supposed to directly inhibit K_{ATP} channels (Leung et al. 2007). Another example is the association with the cAMP-sensing protein cAMP-GEFII also known as Epac2 (Eliasson et al. 2003; Leech et al. 2010). It is proposed that the SUR1 subunits of the plasma membrane K_{ATP} channel together with cAMP-GEFII and Rim2, a protein promoting vesicle priming in neurons (Ozaki et al. 2000), interact with SUR1 proteins in the granules. This protein complex controls Cl^- influx into the vesicles and may promote granule priming (Eliasson et al. 2003). This is in accordance with the observation that the cAMP-mediated potentiation of insulin secretion is impaired in SUR1KO mice (Nakazaki et al. 2002).

K_{ATP} channels in pancreatic β cells are not restricted to the plasma membrane but can also be found in membranes of cellular organelles such as secretory vesicles

(Eliasson et al. 1996; Guiot et al. 2007). However, the protein in organelles may differ from the protein in the plasma membrane. The functional significance of vesicular K_{ATP} channels or the vesicular SUR1 subunit has to be established (Guiot et al. 2007).

Electrophysiological Characteristics

The first measurements of single β cell K_{ATP} channel currents were performed in 1984 by Cook and Hales (Cook and Hales 1984) in inside/out patches where they could show the inhibition by cytosolic ATP. In the same year Ashcroft et al. (1984) demonstrated in metabolically intact β cells with cell-attached patches the glucose-induced inhibition of so-called g-channels. Both publications revealed a conductance of about 50 pS in symmetrical 140 mM K^+ concentration and the inward rectification of the I/V curves. The rectification is due to small blocking cations like Na^+ and not an intrinsic voltage dependency of the channel. In addition, different internal blockers of K_{ATP} channels (ATP, Na^+, Ca^{2+}, Mg^{2+}) interfere with different transitions in channel open and closed times and thus modulate the characteristic intrinsic bursts of channel openings (Ashcroft 1988; Woll et al. 1989; Trapp et al. 1998).

Regulation by Metabolism-Derived Nucleotides and Phosphotransfer

Inhibition by ATP

The predominant characteristic of K_{ATP} channels is the inhibition by ATP derived from glucose metabolism. However, the IC_{50} value for K_{ATP} channel inhibition by ATP is in the range of 5–25 μM (Cook and Hales 1984; Schulze et al. 2007; Tarasov et al. 2006), whereas the cytosolic ATP concentration ($[ATP]_c$) amounts to 3–5 mM (Tarasov et al. 2006; Detimary et al. 1998a). This means that K_{ATP} channels would be permanently closed if they really sense $[ATP]_c$. Many attempts have been made to explain this paradox: It was speculated that ATP-consuming pumps like the Na^+, K^+ ATPase build up an ATP gradient between the sub-membrane space and the cytosol (Niki et al. 1989) tremendously lowering [ATP] in the vicinity of the channels. Long-chain acyl CoAs were found to regulate K_{ATP} channels positively (Branstrom et al. 1997a, 1998a, 2004; Larsson et al. 1996a) as well as phosphoinositides, in particular PIP_2 (Baukrowitz et al. 1998; Baukrowitz and Fakler 2000; Schulze et al. 2003; Rapedius et al. 2005; Shyng et al. 2000; Shyng and Nichols 1998). However, these mechanisms are not sufficient to explain the coupling between glucose metabolism and K_{ATP} channel activity.

Phosphotransfer in β Cells

The breakthrough in understanding the coupling of metabolism to V_m came in 1994 when Dukes and coworkers (Dukes et al. 1994) found that solely ATP derived from reduction equivalents produced in glycolysis is used to regulate K_{ATP} channels. It became clear that the β cell senses the actual glucose concentration by registering the rate of the glycolytic flux and transfers this signal to the membrane.

Glycolytic reduction equivalents are shuttled into the mitochondria via the malate-aspartate and the glycerol phosphate shuttle systems. Suppression of these shuttle systems completely inhibits glucose-induced insulin secretion again

demonstrating that only ATP derived from these glycolytic reduction equivalents is able to influence K_{ATP} channel activity (Eto et al. 1999). This concept also explains why pyruvate is no primary secretagogue in β cells (Lenzen 1978; Sener et al. 1978). Obviously, the metabolism of pyruvate and the reduction equivalents generated in the citric acid cycle are used to produce the bulk $[ATP]_c$ which is needed for the energy demands of the cell, e.g., insulin synthesis or Ca^{2+} sequestration. Reports suggesting that anaplerotic feeding of the citric acid cycle markedly influences K_{ATP} channel activity are often based on experiments with lipophilic pyruvate derivatives, e.g., methyl pyruvate (Zawalich and Zawalich 1997), or with α-ketoisocaproate (KIC) (Lenzen et al. 2000). However, since it has been shown that these agents directly inhibit K_{ATP} channels (Düfer et al. 2002; Branstrom et al. 1998b; Lembert and Idahl 1998), the stimulatory effects are rather a consequence of direct interactions than of mitochondrial metabolism.

The discrimination between particular ATP molecules used for either K_{ATP} channel inhibition or energy demands of the cells may be achieved via specialized electron transport chains. Those chains delivering ATP exclusively to the channels may on one side be physically linked to the shuttle systems that transfer glycolytic reduction equivalents to the mitochondrial matrix. On the other side, the F_1/F_0-ATPase of these chains may be tightly coupled to a mitochondrial creatine kinase (CK) (Gerbitz et al. 1996) that directly conveys the energy-rich phosphate from the generated matrix ATP to cytosolic creatine to form phosphocreatine. Phosphocreatine can be channeled through enzyme systems to the plasma membrane where it is reconverted to ATP and creatine by a membrane-associated CK (Fig. 1c; Tarasov et al. 2006; Dzeja and Terzic 2003; Krippeit-Drews et al. 2003). In heart cells the physical association between CK and K_{ATP} channels has been proven (Crawford et al. 2002). Recently the model of a "metabolic barrier" (see section on "Phosphotransfer in β Cells") was evolved whereby ATP-producing and ATP-consuming enzymes like CK and adenylate kinase (AK) determine the ATP concentration in the direct vicinity of the channels and shield the channels against the bulk cellular ATP (Schulze et al. 2007; Stanojevic et al. 2008). This model is strongly supported by the finding that the ATP sensitivity of K_{ATP} channels is reduced in permeabilized cells (open-cell attached configuration) with efficient enzyme activity compared to excised inside/out patches (Schulze et al. 2007; Tarasov et al. 2006). For human β cells the physical association between adenylate kinase and K_{ATP} channels has been shown (Stanojevic et al. 2008). The AK activity may even be intrinsic to the SUR1 subunit of the K_{ATP} channel as suggested by Tarasov and coworkers (Tarasov et al. 2006). The "metabolic barrier" model (Fig. 1c) can explain why K_{ATP} channels in β cells are operative despite the high cellular bulk ATP concentration in the mM range (Detimary et al. 1998a).

β Cell K_{ATP} Channel Regulation by Changes in ATP Sensitivity

It has been shown for heterologous expressed β cell K_{ATP} channels that phosphatidylinositol phosphates (PIPs) antagonize ATP binding to the channel (Baukrowitz et al. 1998; Shyng and Nichols 1998). Therefore, it is thought that activation of

G_q-coupled receptors by cleaving PIP_2 may activate β cell electrical activity and insulin secretion. A proof of this concept for β cell function is missing (Larsson et al. 2000) although the amino acid of Kir6.2 which confers PIP_2-dependent gating is meanwhile identified (Bushman et al. 2013). On the other hand, the K_{ATP} channel activity is modulated by cAMP via its sensor protein Epac (Leech et al. 2010; Kang et al. 2008) which changes the sensitivity of the channel for ATP. Thus, all G_q-protein-coupled receptors may influence K_{ATP} channel activity and thereby β cell function. It has been shown that free fatty acids and conjugated linoleic acids exert their effects on β cells via a G_q-protein-coupled receptor, the GPR40 or FFA_1 receptor (Schmidt et al. 2011; Wagner et al. 2013). Since these compounds stimulate insulin secretion, it may be that at least part of this effect is mediated by an increased sensitivity of the K_{ATP} channel to ATP.

Recently, it has been shown that bile acids are involved in β cell regulation (Düfer et al. 2012a, b). This effect is mediated by the cytosolic farnesoid X receptor (FXR). It has been shown that bile acids increase $[Ca^{2+}]_c$ and insulin secretion and that these effects are brought about by an indirect inhibition of the K_{ATP} current, presumably by increasing the ATP sensitivity of the channels.

Role of K_{ATP} Channels in β Cell Stimulus-Secretion Coupling

Among all ion channels that are operative in pancreatic β cells, K_{ATP} channels play a predominant role for regulation of cell activity as they couple nutrient metabolism to membrane depolarization and finally to adequate insulin secretion. It is known since 1978 that under resting conditions the membrane potential of β cells is mainly dependent on the K^+ permeability of the plasma membrane (Atwater et al. 1978; Meissner et al. 1978) which is mediated by K_{ATP} channels. The decrease in the open probability of K_{ATP} channels is closely correlated with rising glucose concentrations (Ashcroft et al. 1984; Misler et al. 1986). In response to increased ATP synthesis, closure of K_{ATP} channels leads to a gradual decrease of K^+ conductance so that a yet unknown depolarizing current prevails which depolarizes the plasma membrane (Sehlin and Taljedal 1975; Henquin 1978; Rorsman and Trube 1985; Smith et al. 1990a). At approximately −50 mV, the threshold for opening of voltage-dependent Ca^{2+} channels is reached and action potentials appear. The following increase in $[Ca^{2+}]_c$ constitutes the triggering signal for exocytosis (Ashcroft and Rorsman 1989). In response to continuous glucose stimulation, β cells display a characteristic pattern of electrical activity, the so-called slow waves. It is now generally accepted that fluctuations in the K_{ATP} current are a key event for generation of the oscillatory activity. For detailed discussion of this point, see section on "A Model for β Cell Oscillations."

In summary, K_{ATP} channels have several indispensable functions for the β cell, i.e., determination of resting membrane potential, initiation of membrane depolarization in response to nutrient stimulation, and mediation of the close coupling between increasing glucose concentration and electrical activity, and finally K_{ATP} channels are a key regulator of membrane potential oscillations.

Effects of Drugs

K_{ATP} Channel Blockers: K_{ATP} channel inhibitors, i.e., sulfonylureas and glinides, are drugs frequently used to enhance insulin secretion in type-2 diabetics. The ability of tolbutamide to depolarize the membrane potential V_m in islet cells was first shown by Dean and Matthews (1968), and inhibitory action of tolbutamide on single-channel K_{ATP} currents was reported by Trube et al. (1986). As expected from K_{ATP} channel inhibitors, sulfonylureas effectively depolarize β cells even in the absence of glucose. However, in the complete absence of fuels, they cannot imitate the oscillatory pattern characteristic for physiological nutrient stimulation (Henquin 1998).

Sulfonylureas

Sulfonylureas stabilize the closed state and reduce the duration and frequency of the bursts of K_{ATP} channel openings (Gillis et al. 1989). Sulfonylureas have no effect on single-channel conductance (Trube et al. 1986). PIP_2 and acyl-CoA derivatives that induce mechanisms promoting channel opening reduce the maximal sulfonylurea block (Krauter et al. 2001; Koster et al. 1999; Klein et al. 2005), whereas intracellular MgADP enhances the inhibitory potency of sulfonylureas (Zünkler et al. 1988). This contrasts to what is expected from a nucleotide that stimulates channel activity. However, there is evidence that sulfonylureas prevent the activating action of MgADP via SUR1 and thereby the inhibitory effect of the nucleotides (MgADP, ADP, and ATP) at $K_{IR}6.2$ is unmasked (Gribble et al. 1997b; Babenko et al. 1999c). It has been suggested that sulfonylurea binding to one of the four SUR1 subunits is enough to induce channel closure (Dorschner et al. 1999). High-affinity binding sites for sulfonylureas were first identified in the 1980s (Schmid-Antomarchi et al. 1987; Gaines et al. 1988) long before the cloning of SUR1 in 1995 (Aguilar-Bryan et al. 1995). They are located within the C-terminal site between transmembrane segments (TMs) 13–16 of SUR1 (Fig. 1a). For the sulfonylurea glibenclamide which contains a benzamido moiety, TMs 5 and 6 also influence drug binding (Mikhailov et al. 2001). High-affinity binding of tolbutamide to SUR1 is completely abolished when serine 1237, which is positioned in the intracellular loop between TMs 15 and 16 (Fig. 1a), is replaced by tyrosine. Sulfonylureas also bind to pancreatic K_{ATP} channels by a low-affinity binding site which is located on $K_{IR}6.2$ (Gribble et al. 1997b).

Glinides

The so-called glinides summarize drugs of two structurally different classes: the D-phenylalanine derivative nateglinide and the carbamoylbenzoic acid derivative repaglinide. Nateglinide is the first compound lacking a sulfonylurea and benzamido moiety, respectively, that exhibits a mode of action similar to tolbutamide. K_{ATP} channel inhibition is achieved by high-affinity binding to SUR1 which can be prevented by the S1237Y mutation (Chachin et al. 2003; Hansen et al. 2002). In contrast this mutation does not abolish channel inhibition by repaglinide which suggests that repaglinide interacts with different regions located on SUR1 (Hansen et al. 2002; Fuhlendorff et al. 1998) that require

functional coupling to $K_{IR}6.2$ for high-affinity binding (Hansen et al. 2005). Analogous to sulfonylureas the inhibitory potency of both drugs is enhanced by MgADP (Chachin et al. 2003; Dabrowski et al. 2001).

K_{ATP} Channel Openers: The hyperglycemic sulfonamide diazoxide effectively hyperpolarizes the β cell membrane potential and counteracts glucose-stimulated insulin release by opening of K_{ATP} channels (Trube et al. 1986; Henquin and Meissner 1982; Dunne et al. 1987). Diazoxide stimulation requires Mg^{2+} and hydrolyzable ATP and is suggested to act by stabilizing the open state of the channel. In the absence of ATP, ADP is necessary for enhancement of channel activity by diazoxide (Kozlowski et al. 1989; Larsson et al. 1993; Shyng et al. 1997). The regions that are important for ADP-dependent activation contain the second nucleotide-binding fold and the C-terminal site of SUR1 (Matsuoka et al. 2000). It has been suggested that binding sites essential for diazoxide action also include TMs 6–11 (Fig. 1a) and the first nucleotide-binding fold (Babenko et al. 2000). The activating potency of diazoxide is modified by PIP_2 consistent with the idea that the potency of K_{ATP} channel openers is not a fixed parameter but depends on the open-state stability of the channel (Koster et al. 1999).

Diazoxide is the only K_{ATP} channel opener that is successfully used for therapy of hyperinsulinism or inoperable insulinoma (compare section on "Role in Diseases"; Gill et al. 1997).

Efforts have been made to use SUR1-specific K_{ATP} channel openers for protection of β cells from cytokine- or ROS-induced cell damage (Maedler et al. 2004; Kullin et al. 2000). However, up to now it is unclear whether the protective mechanism requires K_{ATP} channel opening to put β cells at rest or results from a direct depolarizing effect on β cell mitochondria (Sandler et al. 2008).

Influence of Oxidative Stress

β-cell damage due to the attack of reactive oxygen or nitrogen species (ROS/RNS) is known to contribute to gluco- and lipotoxicity in the development of diabetes. Furthermore, the procedures to isolate functional islets for islet transplantation are complicated by the negative influence of oxidative stress during the isolation process.

Among a variety of deleterious effects, direct and indirect interactions with K_{ATP} channels are an important pathway by which ROS and RNS, respectively, impair β cell function and inhibit glucose-stimulated insulin secretion (Krippeit-Drews et al. 1999; Akesson and Lundquist 1999). Alterations in electrical activity or K_{ATP} channel current in the presence of hydrogen peroxide (H_2O_2), nitric oxide (NO), or ROS/RNS donors have been described in several studies (Krippeit-Drews et al. 1994, 1995a, 1999; Nakazaki et al. 1995; Tsuura et al. 1994; Drews et al. 2000a). Oxidative stress can affect the physiological function of K_{ATP} channels in a dual way: either by direct interference with channel proteins, e.g., due to oxidation of SH groups (Islam et al. 1993; Krippeit-Drews et al. 1995b), or by indirect mechanisms caused by the inhibitory influence of ROS and RNS on mitochondrial function. In β cells with intact cell metabolism, H_2O_2 has been shown to drastically increase K_{ATP} current (Krippeit-Drews et al. 1994;

Nakazaki et al. 1995). The rise in K_{ATP} current, concomitant membrane hyperpolarization, and inhibition of insulin secretion are the consequences of a dramatic drop in ATP synthesis which is caused by an H_2O_2-induced breakdown of the mitochondrial membrane potential (Krippeit-Drews et al. 1999).

The interactions of RNS with K_{ATP} channel activity are more complex: Membrane hyperpolarization and channel opening due to inhibition of mitochondrial ATP production have been described for NO gas in the μM concentration range and for several NO donors (Krippeit-Drews et al. 1995a; Tsuura et al. 1994). However, prolonged exposure to NO or NO donors exerts a biphasic effect: The first drastic increase in K_{ATP} current is followed by channel inhibition which most likely depends on direct interactions of NO with channel proteins (Drews et al. 2000b). One study reports that sub-μM concentrations of the NO donor NOC-7 suppress K_{ATP} channel activity via a cGMP/PKG-dependent pathway, whereas in agreement with earlier investigations, channel activation was achieved by short-term application of higher concentrations (Sunouchi et al. 2008).

Besides oxidant-induced changes in K_{ATP} channel activity that inevitably lead to impaired insulin secretion, targeting K_{ATP} channels may be an interesting option to interfere with antioxidant defense mechanisms. Inhibition of K_{ATP} channel expression and treatment of β cells with K_{ATP} channel blockers both increase the activity of antioxidant enzymes, thereby protecting the cells from oxidative stress (Gier et al. 2009).

Role in Diseases

Impaired function of K_{ATP} channels can result either in an abnormal increase of insulin secretion or in a pathological reduction of hormone release. The number of mutations on the SUR1 or $K_{IR}6.2$ subunit, respectively, linked to altered K_{ATP} channel activity is steadily increasing. While it is known for decades that decreased K_{ATP} channel activity is a main reason for excessive insulin secretion in patients with congenital hyperinsulinism, there is now increasing knowledge about channel dysfunctions that cause special forms of neonatal diabetes or type-2 diabetes mellitus (see also chapter "► ATP-Sensitive Potassium Channels in Health and Disease").

K_{ATP} Channels and Hyperinsulinism

Congenital hyperinsulinism (CHI) usually presents at birth or within the first year of life and is characterized by excessive insulin secretion in the absence of nutrient stimulation. In approximately 50 % of all CHI patients loss of function mutations located on the SUR1 gene ABCC8 are causing the disease whereas mutations in the $K_{IR}6.2$ gene are much rarer (Dunne et al. 2004; Arnoux et al. 2010). K_{ATP} channel mutations can cause focal and diffuse forms of CHI (Giurgea et al. 2006; Ismail et al. 2011). In principle there are three mechanisms that account for abnormal β cell excitability, i.e., a decreased expression of K_{ATP} channel protein, a decline in intrinsic channel activity, and a reduced potency of physiological regulators to open the channel. Recently, it has been shown that also mutations of the glucokinase may be responsible for CHI (Henquin et al. 2013). Mutations of SUR1 have been

identified that retain "premature" channels in the endoplasmic reticulum, impair K_{ATP} channel trafficking to the plasma membrane, or induce rapid degradation (Reimann et al. 2003; Crane and Aguilar-Bryan 2004; Taschenberger et al. 2002). Other CHI patients carry mutations which lead to a reduced amplitude of K_{ATP} current or to the loss of MgADP sensitivity (Huopio et al. 2000; Straub et al. 2001; Thornton et al. 2003).

K_{ATP} Channels and Diabetes

The first $K_{IR}6.2$ mutations leading to a diabetic phenotype were described by Gloyn et al. in 2004 (Gloyn et al. 2004). Although activating mutations in the KCNJ11 gene are the most common cause of neonatal diabetes (Hattersley and Ashcroft 2005), several mutations in the ABCC8 gene have also been identified (Thomas et al. 1995; Patch et al. 2007; Vaxillaire et al. 2007; Klupa et al. 2008). Gain of function mutations result in an elevated activity of the K_{ATP} channel. The underlying mechanisms include a reduced sensitivity of the channel to ATP, an increased ATPase activity of SUR1, or an abnormal channel activation in response to nucleotide diphosphates or long-chain acyl CoAs (Riedel et al. 2003; Gloyn et al. 2005; Proks et al. 2004; de Wet et al. 2007b; Ellard et al. 2007; Schwanstecher et al. 2002). It is shown that in some $K_{IR}6.2$ mutations, impaired coupling to SUR1 determines the loss of nucleotide inhibition (Tarasov et al. 2007).

The severity of the disease is correlated with the extent of ATP insensitivity and ranges from transient or permanent neonatal diabetes to full DEND syndrome, which is characterized by diabetes and neurological defects (Gloyn et al. 2004; Hattersley and Ashcroft 2005). On the other hand, family studies have shown that the same gene variations can result in different forms of diabetes ranging from neonatal to gestational or late-onset diabetes. This suggests either variation in the penetrance of the channel defect or epigenetic modifications (Yorifuji et al. 2005). Importantly, patients with diabetes due to $K_{IR}6.2$ mutations often benefit from switching insulin therapy to sulfonylureas (Slingerland et al. 2008; Pearson et al. 2006; Flechtner et al. 2006). Short-term investigations suggest that this might also apply to patients with SUR1 mutations (Rafiq et al. 2008). Polymorphisms in the genes encoding $K_{IR}6.2$ or SUR1 not only are associated with neonatal diabetes or DEND syndrome but also have been linked to the development of type-2 diabetes (Gloyn et al. 2003; Laukkanen et al. 2004; Chistiakov et al. 2009; Vaxillaire et al. 2008) or even to secondary failure of sulfonylureas (Sesti et al. 2006). The $K_{IR}6.2$ mutation E23K that increases open channel probability has been extensively studied with respect to its impact on β cell function. However, it is still a matter of debate whether the alterations in K_{ATP} channel activity caused by this mutation can explain a diabetic phenotype (Riedel et al. 2003, 2005; Schwanstecher et al. 2002; Nielsen et al. 2003; Tschritter et al. 2002).

Ca^{2+} Channels

Ca^{2+} influx via voltage-gated Ca^{2+} (Ca_v) channels controls important cellular processes like exocytosis, proliferation, cell viability, gene expression, and cell cycle. In β cells Ca_v channels play a key role in glucose-induced insulin secretion

by mediating Ca^{2+} influx and increasing the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) (Mears 2004; Yang and Berggren 2005, 2006). β-cell Ca_v channel activity and density are decisive for appropriate insulin secretion and up- or downregulation of both parameters can impair β cell function (see also chapter "► Calcium Signaling in the Islets").

Structure/Nomenclature and Occurrence in β Cells

Each Ca_v channel comprises a pore-forming α_1 subunit and auxiliary β, γ, and α_2/δ subunits. The α_1 subunit forms the Ca^{2+}-conducting pore and contains the voltage sensor, the selectivity filter for Ca^{2+}, and the activation and inactivation gates (Yang and Berggren 2006; Catterall 2000). The other subunits modulate channel activation and inactivation and current amplitude and regulate plasma membrane trafficking (Davies et al. 2007; Arikkath and Campbell 2003).

Nomenclature

Multiple nomenclatures for Ca_v channels exist according to their biochemical, biophysical, and pharmacological properties and sequence analysis. According to the primary structure of the α1 subunits, Ca_v channels are divided into three families of closely related members: Ca_v1, Ca_v2, and Ca_v3. $Ca_v1.1$–1.4 channels are L-type Ca^{2+} channels, $Ca_v2.1$ is a P-/Q-type channel, $Ca_v2.2$ belongs to N-type channels, and $Ca_v2.3$ belongs to R-type channels, whereas $Ca_v3.1$–3.3 are T-type channels. Ca_v1 and Ca_v2 channels have a high threshold for voltage-dependent activation and are named HVA (high voltage-activated) Ca^{2+} currents, while T-type Ca^{2+} currents are referred to as LCA (low voltage-activated currents) because they are stimulated by small depolarizations (Catterall et al. 2005).

Ca^{2+} Channels in β Cells

It is still a matter of debate which Ca_v channels are present in β cells and, more important, which are of physiological relevance. The situation is complex because Ca_v channel expression varies between species and often tumor cell lines are used which considerably differ from primary β cells. Long-lasting changes of $[Ca^{2+}]_c$ as required for stimulation of insulin secretion can only be achieved by L-type Ca^{2+} channels which inactivate slowly but not by the rapidly inactivating T-type Ca^{2+} channels. Nevertheless, L-type Ca^{2+} channels are considered to be crucial for β cell function. In mouse β cells a large part of the total voltage-dependent Ca^{2+} current is blocked by L-type Ca^{2+} channel inhibitors (Gilon et al. 1997; Plant 1988a). Moreover, insulin secretion is almost completely blocked by suppression of L-type Ca^{2+} channel activity (Satin et al. 1995; Braun et al. 2008). The existence of $Ca_v1.2$ (characterized by the α_{1C} subunit) and $Ca_v1.3$ (containing the α_{1D} subunit) has been proven in rodent and human islets at the level of genes, mRNA, and proteins (Barg et al. 2001; Horvath et al. 1998; Iwashima et al. 1993; Namkung et al. 2001; Schulla et al. 2003; Seino et al. 1992; Yang et al. 1999), although the relative portion of each channel remains controversial and may depend on the species and the methods used. Noteworthy, polymorphisms in the genes encoding $Ca_v1.2$ and $Ca_v1.3$ are suggested to associate with type-2 diabetes mellitus

(Trombetta et al. 2012; Reinbothe et al. 2013). Studies using mice with genetic ablation of $Ca_v1.2$ and $Ca_v1.3$ channels provide additional insights in the specific functions of these channels (see section on "Role in β Cell Stimulus-Secretion Coupling"). Several non-L-type HVA Ca^{2+} channels have been found in human and rodent β cells and in tumor β cell lines (for details, see reviews by Mears 2004 and Yang and Berggren 2005, 2006).

Electrophysiological Characteristics and Regulation

General Electrophysiological Properties and Influence of Drugs

Ca_v channels are characterized by voltage-dependent activation. Single-channel Ca^{2+} currents and whole-cell Ca^{2+} currents have been measured in various species, whereas L-type currents are best characterized. Since single-channel Ca_v currents are too small to be recorded under physiological conditions, Ba^{2+} has been used as charge carrier because it increases the amplitude of L-type Ca^{2+} channel currents. The single Ca_v channel conductance with extracellular Ba^{2+} is 20–25 pS (Rorsman et al. 1988; Ashcroft et al. 1989). From these data a single-channel conductance of 2 pS has been estimated for physiological Ca^{2+} concentrations. Whole-cell Ca_v currents in mouse β cells are activated at depolarizations to potentials more positive than −50 mV, have a maximum current at −20 mV, and reverse at ∼ +50 mV (Plant 1988a; Rorsman and Trube 1986). In human and rat β cells, additionally T-type Ca^{2+} currents have been detected (Hiriart and Matteson 1988; Misler et al. 1992). β-cell L-type Ca^{2+} currents inactivate in a Ca^{2+}-dependent manner during sustained depolarization (Plant 1988a), and thus inactivation is clearly reduced with Ba^{2+} as charge carrier. A smaller voltage-dependent component of inactivation has also been described (Satin and Cook 1989). To estimate the physiological significance of the different Ca_v channel components for insulin secretion, the correlation between the pharmacological block of channel activity and inhibition of insulin secretion is decisive. Most important in this context is the sensitivity of L-type Ca^{2+} channels to dihydropyridines and D-600 (Plant 1988a; Rorsman and Trube 1986). However, L-type Ca^{2+} channel blockers used for, e.g., treatment of hypertensive patients do not influence glucose metabolism and do not increase the risk of type-2 diabetes mellitus (Taylor et al. 2006). One explanation may be that alternative splicing can modulate the sensitivity of Ca^{2+} channels to Ca^{2+} channel blockers (Zhang et al. 2010).

It is generally accepted that activation of inhibitory G-proteins decreases insulin secretion. It has been shown that the effects of α-adrenergic agonists, somatostatin, and galanin are sensitive to pertussis toxin (Hsu et al. 1991a, b; Nilsson et al. 1989). However, it is highly disputed whether Ca_V channel activity in β cells is regulated by G_i-proteins. Once again the discrepancies may be brought about by the use of tumor cell lines. In insulin-secreting cell lines catecholamines, galanin, and somatostatin seem indeed to inhibit Ca_v channels (Hsu et al. 1991a, b; Aicardi et al. 1991; Homaidan et al. 1991). However, for primary β cells the concept that G_i-stimulating agents inhibit insulin secretion via reduction of Ca_v channel activity has not been confirmed and other mechanisms of action have been proposed (Ullrich and Wollheim 1989; Rorsman et al. 1991; Drews et al. 1990; Debuyser et al. 1991a; Bokvist et al. 1991; Ahren et al. 1986).

Excitosomes

Ca_v channels are not equally distributed in the β cell plasma membrane but are clustered and co-localized with the exocytotic vesicles (Barg et al. 2001; Bokvist et al. 1995; Rutter et al. 2006) (see also chapter "▶ Exocytosis in Islet β-Cells"). The physical neighborhood of Ca_v channels and secretory granules allows a steep local rise of $[Ca^{2+}]_c$ (>15 μM) which is necessary for fast exocytosis with only marginal enhancement of bulk $[Ca^{2+}]_c$ (Barg et al. 2001). Moreover, the formation of excitosomes has been shown for β cells, i.e., the Ca_v channels form a complex with proteins of the exocytotic machinery like syntaxin 1A, SNAP-25, and synaptotagmin (Yang et al. 1999; Wiser et al. 1999). These complexes may fix the channels in the optimal position but also affect channel activity. SNAP-25 possesses distinct inhibitory and activating domains that modulate Ca_v1 channel activity (Ji et al. 2002). These protein networks are suggested to serve as a fine-tuning mechanism of β cell Ca_v1 channel function. Disruption of the integrity of the complexes impairs channel function (Yang et al. 1999; Wiser et al. 1999). Chronic exposure of islets to palmitate, a maneuver that inhibits insulin secretion, disperses the microdomains of localized Ca^{2+} (Hoppa et al. 2009). The steep rise of Ca^{2+} in these microdomains normally triggers exocytosis of closely related granules. Deterioration of the microdomain integrity may contribute to palmitate-induced inhibition of insulin secretion after long-term exposure to the fatty acid. A functional dissociation of Ca^{2+} entry via voltage-gated channels and exocytosis resulting in decreased insulin secretion was also detected in β cells from mice fed with a high-fat diet (Collins et al. 2010). The authors suggest that this observation may depict a novel explanation for the link between obesity and diabetes. Meanwhile it is clear that excitosomes are linked to a larger protein network including other ion channels like K_{ATP} channels and K_v channels and a variety of other proteins like RIM (Rab3A-interacting molecule), MUNC, and GEFII (guanyl nucleotide exchange factor) which interact with each other and the ion channels (Leung et al. 2007). The fine-tuning of β cell activity by this protein networks is achieved, e.g., by coordinated simultaneous effects on exocytotic proteins and ion channel activity and trafficking. Interesting in this context, the eukaryotic translation initiation factor 3 subunit E has been recently identified as a factor that regulates trafficking of $Ca_v1.2$ and thus surface expression of this channel. This trafficking influences Ca^{2+} homeostasis and consequently insulin secretion (Buda et al. 2013). Noteworthy, RIM is under the control of cAMP/PKA and thus this complex network can be modulated by GLP-1 and therapeutically used GLP-1 analogues.

Role in β Cell Stimulus-Secretion Coupling

Ca^{2+} Influx and Electrical Activity

Despite the diversity of Ca_v channels expressed in β cells and species differences, it is unequivocally accepted that L-type Ca^{2+} channels play the paramount role for insulin secretion. In human β cells glucose-induced action potentials and insulin secretion are completely suppressed by blockage of L-type Ca^{2+} channels (Braun et al. 2008). In contrast, depolarization-evoked vesicle exocytosis, measured as changes in cell capacitance, is only marginally influenced by L-type channel

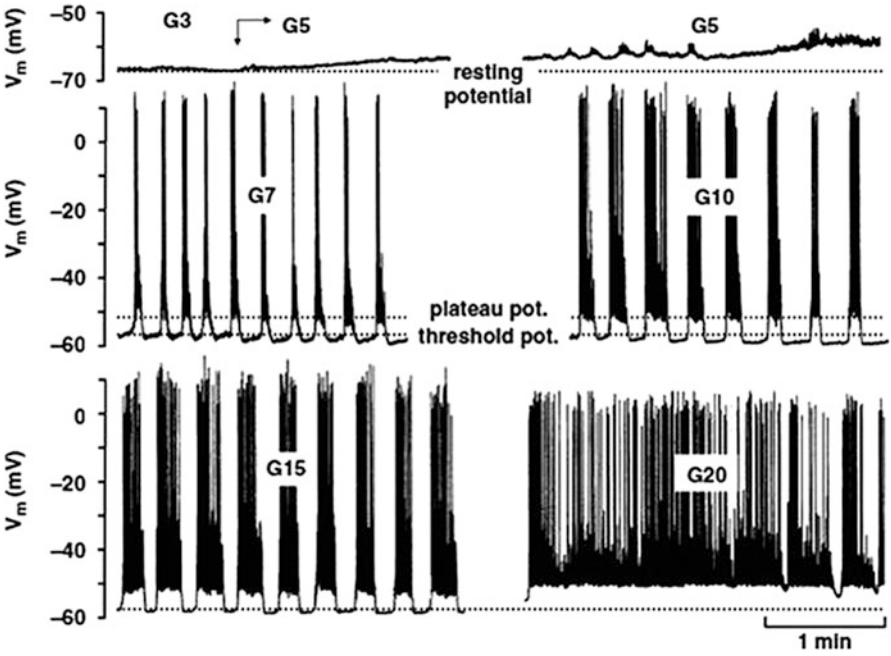

Fig. 2 Microelectrode (*ME*) measurements of membrane potential (V_m) of β cell in intact islets. The glucose concentration is varied from 3 to 20 mM (G3–G20). In the *upper left panel*, V_m started to depolarize when the glucose concentration was switched from 3 to 5 mM (*arrows*). In the *upper right panel*, the cell penetrated by the ME has not yet reached the threshold potential while a neighboring cell is already electrically active. Current through gap junctions from the neighboring cell elicits voltage deflections in the cell impaled by the ME. Note that the burst time increases with increasing glucose concentrations while the interburst time is shortened

blockage. Depolarization-induced exocytosis is markedly suppressed by the P/Q-type blocker omega-agatoxin which does not significantly decrease glucose-induced insulin secretion (Braun et al. 2008). This emphasizes the necessity to properly discriminate between both processes, insulin secretion, and membrane fusion of exocytotic vesicles. A recent paper confirms the outstanding significance of L-type Ca^{2+} channels in INS-1 cells (Nitert et al. 2008). The authors show that glucose-induced insulin secretion and $[Ca^{2+}]_c$ is reduced to basal values by inhibiting L-type Ca^{2+} channels but not markedly influenced by suppressing R-type $Ca_v2.3$ activity. This is just the opposite in delta cells (see section on "Delta Cells"). Notably, a functional variant in the gene encoding $Ca_v2.3$ contributes to the susceptibility of Pima Indians to type 2 diabetes mellitus (Muller et al. 2007). Induction of electrical activity by glucose is a prerequisite for insulin secretion. Glucose evokes electrical activity in β cells that is characterized by bursts of action potentials and hyperpolarized interburst phases (see section on "Regulation of V_m-Independent of K_{ATP} Channels" and Fig. 2). In mouse β cells the action potentials are provoked solely by Ca^{2+} currents; in rat and human β cells, Na^+

channels contribute to electrical activity (Plant 1988a, b; Braun et al. 2008; Gopel et al. 1999; Barnett et al. 1995). The influx of Ca^{2+} from the extracellular space is crucial for glucose-induced insulin secretion; release from the ER can only modulate it (Gilon et al. 1999). Influx ensures high Ca^{2+} concentrations beneath the membrane in the microdomains with complexes containing channels, exocytotic proteins, and vesicles.

Studies with Knockout Mice

Several studies with Ca^{2+} channel knockout mice gave new insights, but until today the problem of the contribution of different channel types to β cell function is not definitely solved.

Schulla et al. (2003) constructed mice with a β cell-specific knockout of the L-type $Ca_v1.2$ channel. About 45 % of the Ca^{2+} channel current was removed by this maneuver, but the remaining current was insensitive to the L-type channel blocker isradipine suggesting that $Ca_v1.2$ carries the L-type current in β cells. $Ca_v1.2$KO mice were glucose intolerant compared to WT mice. The first phase of insulin secretion and the rapid component of exocytosis were significantly reduced. The authors take their results as disruption of the $Ca_v1.2$ channel/granule complexes. However, no information is given about effects of the $Ca_v1.2$ knockout on the second phase of insulin secretion (>15 min). Moreover, it is remarkable that complete loss of $Ca_v1.2$ channel activity influences electrical activity and $[Ca^{2+}]_c$ only marginally. The knockout of another important L-type Ca^{2+} channel present in β cells was also investigated. Barg and coworkers (Barg et al. 2001) did not detect a significant effect of the $Ca_v1.3$ knockout on β cell Ca^{2+} currents in accordance with the observation of Platzer et al. (2000) that fasting insulin and glucose serum concentrations are equal in $Ca_v1.3$KO mice and WT animals. Moreover, no change in these parameters was obtained after a glucose challenge. In contrast, Namkung and coworkers (2001) found a severe impairment of glucose tolerance and reduced serum insulin concentrations in $Ca_v1.3$ channel knockout mice compared to their littermates. The situation is even more complex because the $Ca_v1.3$ knockout seems to be counteracted by upregulation of the $Ca_v1.2$ gene (Namkung et al. 2001).

Two papers describe reduced insulin tolerance in mice lacking R-type $Ca_v2.3$ channels (Pereverzev et al. 2002; Matsuda et al. 2001). One of these papers shows that the effect is accompanied by reduced glucose-induced insulin secretion (Pereverzev et al. 2002). However, further studies are needed to clarify the role of $Ca_v2.3$ channels for insulin secretion because the effect of the knockout on glucose tolerance was marked in male but not in female animals and was lost in aged animals (Pereverzev et al. 2002). Jing et al. (2005) suggest a role of $Ca_v2.3$ channels in vesicle recruitment because in their study the $Ca_v2.3$ knockout primarily affects second phase of insulin secretion.

K_v and K_{Ca} Channels

Pancreatic β cells express a variety of K^+ channels regulated by voltage (K_v channels) and/or by the intracellular Ca^{2+} concentration (K_{Ca} channels).

While the primary function of K_v channels, i.e., the repolarization of action potentials, is well accepted for years, the importance of K_{Ca} channels is less clear. Recent studies show that K_{Ca} channels play a role for the regulation of the characteristic membrane potential oscillations but are also involved in determining the glucose responsiveness of pancreatic β cells.

Characteristics of K_v and K_{Ca} Channels in β Cells

K_v Channels

K_v channels belong to the family of K^+ channels with six transmembrane regions of which 11 subfamilies have been described up to now. In primary β cells K_v channels of five subfamilies (K_v1, 2, 3, 6, and 9) have been detected (Philipson et al. 1991; Yan et al. 2004; Roe et al. 1996; Göpel et al. 2000; MacDonald and Wheeler 2003; Jacobson and Philipson 2007a). The members of K_v1, K_v2, and K_v3 form functional channels as homo- or hetero-tetramers, whereas K_v6 and 9 are silent subunits that have been shown to modulate K_v2 and K_v3 channel currents by co-assembly in heterologous expression systems (Kerschensteiner and Stocker 1999; Sano et al. 2002). In clonal and primary β cells, K_v currents consist of at least 2 components: one 4-aminopyridine-insensitive current without inactivation (delayed-rectifier current, K_{DR}) that can be blocked by TEA^+ in the low mM concentration range and one inactivating current (A-current) that is inhibited by 4-aminopyridine (Düfer et al. 2004; MacDonald et al. 2001, 2002a; Smith et al. 1989; Su et al. 2001). The inactivating component is maximal at +30 mV, and its contribution to whole-cell K_v current gets progressively smaller with increasing depolarization (Smith et al. 1990b). K_v channels underlying the A-currents require membrane depolarization more positive than −40 mV for activation, whereas delayed-rectifier currents are active at V_m above −20 mV (Herrington et al. 2005). Consequently, in β cells K_v channels are not operative at resting membrane potential (Henquin 1990). A-type currents in clonal or primary β cells can be mediated by K_v1.4, K_v3.3, and K_v3.4 or K_v4.x (MacDonald and Wheeler 2003). Among K_{DR} channels K_v2.1 seems to play a predominant role for β cells where it is clustered with Ca_v1.2 and SNARE proteins in cholesterol-rich lipid rafts (Xia et al. 2004; Herrington 2007).

K_v currents can be modulated by hormones and neurotransmitters. GIP has been shown to diminish the A-currents in pancreatic β cells in a PKA-dependent manner (Kim et al. 2005). GLP-1 receptor activation reduces K_v currents (MacDonald et al. 2002b), thereby antagonizing membrane repolarization – a mechanism that may contribute to the stimulatory effect of GLP-1 on insulin secretion. Recently, it has been shown that incretin-mediated acetylation and/or phosphorylation of K_v2.1 channels is involved in the antiapoptotic action of GLP-1 (Kim et al. 2012). Other modulators of K_v channels are non-esterified fatty acids. Phospholipase A_2-β-mediated hydrolysis of membrane phospholipids has been shown to reduce peak K_v current (Jacobson et al. 2007a) and islet-PLA_2-β-overexpressing β cells display reduced K_v2.1 currents with alterations in electrical activity and increased insulin secretion (Bao et al. 2008).

K_{Ca} Channels

K_{Ca} channels can be divided into three groups with respect to their single-channel conductance: large-conductance BK channels ($K_{Ca}1.1$; maxi-K), intermediate-conductance SK4 channels ($K_{Ca}3.1$; IK1), and small-conductance K_{Ca} channels (SK1, SK2, and SK3).

The existence of BK channels in pancreatic β cells and insulin-secreting cell lines has been verified by several groups (Braun et al. 2008; MacDonald et al. 2002a; Ribalet et al. 1988; Satin et al. 1989; Kukuljan et al. 1991; Düfer et al. 2011). BK channels are hetero-octamers of 4 α subunits forming the channel pore and 4 β subunits with regulatory functions. BK channels have a single-channel conductance of ~150−300 pS and are sensitive to low concentrations of TEA^+, charybdotoxin, and iberiotoxin. They are active at nM Ca^{2+} concentrations and Ca^{2+} sensitivity is increased with membrane depolarization. With respect to the contribution of BK channels to action potentials, reports are contradictory: BK channel knockout or inhibition with iberiotoxin has been shown to increase the duration of action potentials in murine β cells (Düfer et al. 2011), whereas others report no effect of iberiotoxin on the shape of action potentials (Kukuljan et al. 1991). Two studies show an elevated amplitude of action potentials in a subset of human β cells and in murine β cells, respectively, in response to BK channel inhibition (Braun et al. 2008; Houamed et al. 2010). Interestingly, genetic ablation of functional BK channels impairs glucose tolerance and increases islet cell apoptosis (Düfer et al. 2011).

The intermediate-conductance K_{Ca} channel has been cloned from human pancreas in 1997 (Ishii et al. 1997) and SK4 mRNA and protein, respectively, is expressed in murine islets (Düfer et al. 2009; Tamarina et al. 2003). SK4 channel opening is largely independent of V_m (Vogalis et al. 1998; Jensen et al. 1998) but strictly regulated by $[Ca^{2+}]_c$ (Vogalis et al. 1998; Ledoux et al. 2006). Single-channel currents with SK4 channel characteristics have been observed in clonal and primary β cells (Düfer et al. 2009; Kozak et al. 1998). Genetic ablation of SK4 (SK4KO) channels increases the duration and frequency of Ca^{2+} action potentials, and pharmacological channel inhibition alters the oscillatory pattern of $[Ca^{2+}]_c$ in WT β cells (Düfer et al. 2009). In addition, glucose responsiveness of V_m and of $[Ca^{2+}]_c$ are shifted to lower glucose concentrations in SK4KO β cells. Compared to their littermates SK4KO animals exhibit an improved glucose tolerance but no change in insulin sensitivity.

In mouse islets mRNA of the small-conductance K_{Ca} channel SK1 has been observed, and for SK2 and SK3 protein co-localization with insulin has been verified in dissociated islet cells (Tamarina et al. 2003). SK3 protein has also been detected in human islets (Jacobson et al. 2010). Up to now there is no investigation characterizing single-channel currents of SK1–SK3 in β cells, but it has been shown that SK1–SK3 channel inhibitors influence membrane potential and Ca^{2+} oscillations (Tamarina et al. 2003; Zhang et al. 2005).

Contribution of K_{Ca} Channels to K_{slow} Currents

In 1999 a K^+ current activating with increasing Ca^{2+} influx during burst phases of glucose-stimulated β cells was detected (Göpel et al. 1999). The current, termed K_{slow} due to its delayed and slow onset, strongly depends on $[Ca^{2+}]_c$. It can be modulated by Ca^{2+} influx via L-type Ca^{2+} channels and by Ca^{2+} release of the endoplasmic reticulum (Haspel et al. 2005; Goforth et al. 2002). Further analysis suggested that approximately 50 % of K_{slow} could be ascribed to K_{ATP} current (Kanno et al. 2002a). Another significant component is SK channels. For murine β cells it has been shown that knockout or pharmacological inhibition of SK4 channels significantly reduced K_{slow} (Düfer et al. 2009). Although K_{slow} currents are not sensitive to apamin, a blocker of the small-conductance SK channel (Göpel et al. 1999; Goforth et al. 2002), there is one study suggesting involvement of SK3 channels in generation of K_{slow} (Zhang et al. 2005).

Significance of K_v and K_{Ca} Channels for β Cell Electrical Activity

Role of K_v and K_{Ca} Channels for Action Potentials

The primary function of K_v channels in β cells is action potential repolarization (Rorsman and Trube 1986; Smith et al. 1990b; Henquin 1990). Increasing K^+ outward current repolarizes V_m and terminates Ca^{2+} action potentials prior to Ca^{2+}-dependent inactivation of L-type Ca^{2+} channels. Inhibition of K_v channels with TEA^+ or several spider toxins extends action potential duration. Consequently, blockade of K_v channels is a potent tool to augment insulin release (Su et al. 2001; Herrington 2007; Atwater et al. 1979). As activation of K_v channels requires membrane depolarization, targeting K_v channels affects insulin secretion only in the presence of elevated glucose concentrations or other depolarizing stimuli (MacDonald and Wheeler 2003; Henquin 1990).

At least in rodent β cells, the most important K_v channel underlying the K_{DR} current is $K_v2.1$ (Roe et al. 1996). Inhibition or knockout of this channel reduces K_{DR} currents by >80 %, broadens single action potentials, and increases insulin secretion (MacDonald et al. 2002a; Herrington et al. 2006; Jacobson et al. 2007a). In human β cells ~50 % of K_{DR} currents are sensitive to the $K_v2.1$ blockers stromatoxin and hanatoxin, respectively (Braun et al. 2008; Herrington et al. 2005). Experiments with K_v1 channel antagonists show that $K_v1.1$, $K_v1.2$, and $K_v1.3$ channels do not markedly contribute to the regulation of insulin secretion in primary β cells whereas an adenoviral approach with dominant-negative $K_v1.4$ suggests involvement of this channel in the generation of KDR currents (MacDonald et al. 2001; Herrington et al. 2005).

Action potentials and insulin secretion can also be modulated by K_{Ca} channels: Inhibition of SK4 channels with TRAM-34 or genetic channel ablation leads to action potential broadening, increases the frequency of glucose-induced Ca^{2+} action potentials, and elevates Ca^{2+} influx (Düfer et al. 2009). Interestingly, inhibition of SK4 channels not only does affect glucose-stimulated β cell activity but also shifts the threshold for glucose responsiveness of V_m, $[Ca^{2+}]_c$ and insulin secretion to lower glucose concentrations (Düfer et al. 2009).

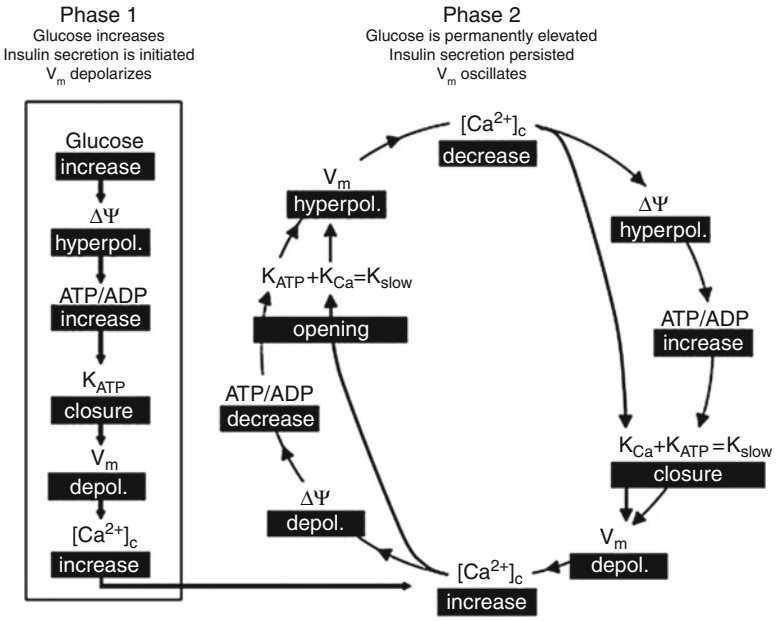

Fig. 3 Model for V_m oscillation in WT β cells. *Phase 1* describes the consensus model of β cell activation by glucose. *Phase 2* indicates that Ca^{2+} influx increases the K_{slow} current (for details, see text) which counterbalances the depolarization. During the hyperpolarized phase, $[Ca^{2+}]_c$ is lowered and the cell depolarizes again. Thus, V_m oscillates at a constant stimulatory glucose concentration

Blockade of small-conductance SK channels has also been shown to increase the frequency of action potentials and to increase glucose-stimulated insulin release (Jacobson et al. 2010; Zhang et al. 2005). In INS1 cells knockdown of SK1 channels alters the resting membrane potential (Andres et al. 2009). It is suggested that Ca^{2+}-activated K^+ channels of the BK type play a significant role for action potential repolarization in human and murine β cells (Braun et al. 2008; Düfer et al. 2011).

Role of K_{Ca} Channels in Oscillations of V_m

For decades it was discussed whether K_{Ca} channels participate in the regulation of the characteristic membrane potential oscillations of β cells (Henquin 1990; Kukuljan et al. 1991; Ribalet and Beigelman 1980; Ämmälä et al. 1991; Atwater et al. 1980). At present, it is generally accepted that periodic activation of K_{ATP} channels is a key event that determines oscillations in V_m (Rolland et al. 2002a; Krippeit-Drews et al. 2000; compare section on "Characteristics of K_v and K_{Ca} Channels in β Cells" and see Fig. 3). Early studies investigating the effect of elevated Ca^{2+} influx on membrane potential already suggested that activation of a K_{Ca} current could modulate the length of the hyperpolarized interburst intervals (Rosario et al. 1993). As blockage of BK channels does not influence membrane potential oscillations (Henquin 1990; Kukuljan et al. 1991; Houamed et al. 2010;

Atwater et al. 1979), these channels are not considered to play a role for regulation of the burst pattern. However, with the detection of a Ca^{2+}-dependent, sulfonylurea-insensitive component of K_{slow}, it became obvious that activation of K_{Ca} channels plays an important role for induction of the electrically silent interburst phases (Göpel et al. 1999; Goforth et al. 2002; Kanno et al. 2002a). Although the precise nature of the underlying ion channels remains to be identified (compare "Characteristics of K_v and K_{Ca} Channels in β Cells"), the sensitivity of K_{slow} to SK channel blockers and the ability of these drugs to alter oscillations in V_m and $[Ca^{2+}]_c$, respectively, clearly point to an involvement of small- and intermediate-conductance K_{Ca} channels in the regulation of membrane potential oscillations (Düfer et al. 2009; Zhang et al. 2005).

Other Ion Channels

Na^+ Channels

Plant (1988b) was the first to report the existence of voltage-dependent Na^+ channels in the pancreatic β cell of the mouse. Strangely, in mouse β cells, Na^+ channels are fully inactivated at the resting potential (Plant 1988b) and seem to have no physiological function. This is different in the β cells of dogs (Pressel and Misler 1991) and humans (Pressel and Misler 1990). In these species glucose-induced electrical activity consists largely of Na^+ action potentials (Na^+ APs) inhibitable by tetrodotoxin (TTX). Na^+ influx depolarizes the cell membrane to voltages where L-type Ca^{2+} channels open. In human β cells, Na^+ APs play a major role at a V_m negative to −45 mV and disappear due to Na^+ channel inactivation at a V_m positive to −40 mV, i.e. at glucose concentrations higher than 10 mM (Barnett et al. 1995). More recent work confirms the role of Na^+ APs in human β cells (Braun et al. 2008). Half-maximal inactivation of the Na^+ channel was found at ∼ −45 mV, and TTX is more potent to inhibit glucose-induced insulin secretion at low than at high glucose concentrations. Quantitative RT-PCR identified $Na_v1.6$ and $Na_v1.7$ channels to be expressed in equal amounts in human β cells (Braun et al. 2008).

Volume-Sensitive Anion Channels (VSACs)

In 1994 Britsch and coworkers (1994) published that osmotic cell swelling markedly increased glucose-induced electrical activity. They ascribed the underlying depolarization to activation of a volume-sensitive anion current (VSAC). This current was later confirmed and electrophysiologically characterized (Kinard and Satin 1995; Best et al. 1996a; Drews et al. 1998) (see also chapter "▶ Anionic Transporters and Channels"). The existence of this current is well established; however, its role for the physiological function of β cells – besides cell volume regulation – is not fully understood, although it has been extensively studied by Best and coworkers (Best 1999, 2002; Best and Benington 1998; Best et al. 1996b, 1997, 1999, 2000, 2001, 2004a, b; Miley et al. 1999). Since E_{Cl} is about −30 mV (Kinard and Satin 1995; Drews et al. 1998), the VSAC will provide a depolarizing current at most physiological potentials. Inhibition of K_{ATP} channels by cell metabolism or antidiabetic drugs leads to depolarization of β cells, but the underlying current for the depolarization is unknown. Whether VSAC is this "unknown

current" or contributes to it is still conflicting (Best et al. 1996a; Best and Benington 1998). More recently, it has been shown that glucose activates the VSAC by incorporating the channel protein in the plasma membrane of INS-1E cells (Jakab et al. 2006). However, this effect was elicited by 20 mM glucose and could be mimicked by the non-metabolizable 3-O-methylglucose and may therefore be caused by cell swelling.

Transient Receptor Potential (TRP)-Related Channels

On the search for the unknown depolarizing current, TRP channels were also regarded as potential candidates, but at the resting β cell, no activation mechanism for these channels has been described so far (Jacobson and Philipson 2007b). Members of all three subfamilies of TRP channels (C-form for canonical, M-form for melastatin, V-form for vanilloid) have been found in either primary β cells or insulin-secreting cell lines (Jacobson and Philipson 2007b).

According to Islam (2011), many TRP channels are indeed involved in β cell function. He proposes that these channels and their depolarizing currents switch β cells from a "ready" to an "on" mode in response to various stimuli. TRPC1 and TRPC4 channels found in islets and β cell lines (Sakura and Ashcroft 1997; Roe et al. 1998; Qian et al. 2002) are nonselective cation channels which are activated by either $G_{q/11}$ protein or IP_3 or by Ca^{2+} release from intracellular stores (Jacobson and Philipson 2007b; Roe et al. 1998; Qian et al. 2002; Gustafsson et al. 2005) and may therefore be counted among the store-operated Ca^{2+} channels (Dyachok and Gylfe 2001). Worley and coworkers (1994) presented evidence that β cells also possess store-operated nonselective monovalent cation channels. These channels may be TRP channels (Qian et al. 2002) and were suggested to be TRPM4 (Cheng et al. 2007) or TRPM5 channels (Prawitt et al. 2003), but do obviously not represent the acetylcholine-induced Na^+ current which is independent of Ca^{2+} stores (Rolland et al. 2002b). Another signaling pathway in which TRP channels are involved is the action of incretins such as GLP1 (Leech and Habener 1997; Miura and Matsui 2003) though the exact nature of the channel(s) involved remains undefined. Nevertheless, these TRP channels are candidates to account for the GLP1-induced depolarization which is independent from K_{ATP} channel inhibition (Britsch et al. 1995). Steroidal compounds often have rapid effects on membrane surface receptors. Wagner and coworkers (Wagner et al. 2008) have recently shown that pregnenolone sulfate activates TRPM3 channels, thereby increasing $[Ca^{2+}]_c$ and insulin secretion. Thus, a cross talk between steroidal and insulin-signaling endocrine systems is enabled (for review, see Thiel et al. 2013), although TRPM3 seems to have nothing to do with glucose-induced stimulus-secretion coupling (Klose et al. 2011).

TRP channels may also be involved in β cell destruction during the development of diabetes as TRPM2 channels were identified to be activated by H_2O_2 (Qian et al. 2002; Togashi et al. 2006). Since TRPM2 channels are unspecific cation channels (Jacobson and Philipson 2007), these channels can account for the excessive unspecific Ca^{2+} influx in response to H_2O_2 in β cells (Krippeit-Drews et al. 1999). Moreover, the H_2O_2-induced ATP depletion may release Ca^{2+} from intracellular stores (Krippeit-Drews et al. 1999) and in turn open release-activated

Ca^{2+} channels or another group of unspecific cation channels belonging to TRPC4 (Jacobson and Philipson 2007b). Thus, TRP channels may be involved in Ca^{2+} overload of β cells in response to oxidative stress which is causative for subsequent cell death.

A channel of the vanilloid subfamily, TRPV1, was found to be expressed in primary β cells and in pancreatic neurons (Akiba et al. 2004) which may link regulation of food intake and pancreatic endocrine function.

Hyperpolarization-Activated Cyclic Nucleotide-Gated (HCN) Channels

HCN channels are pacemaker channels of oscillations in a variety of cells (Noma et al. 1977; Yanagihara and Irisawa 1980; Ludwig et al. 1998; Santoro et al. 1998; Seifert et al. 1999; Robinson and Siegelbaum 2003). Since β cells are oscillating, it is tempting to speculate that HCN channels are involved in the pattern of electrical activity. In addition, it has been shown that cAMP has a depolarizing effect on β cells (Debuyser et al. 1991b) which may contribute to the depolarizing effect of GLP-1 (see section "Regulation by Hormones and Neurotransmitters"). To our knowledge there are to date only two reports dealing with HCN channels in β cells (El-Kholy et al. 2007; Zhang et al. 2009). Expression of a dominant-negative HCN2 channel abolished endogenous HCN currents in rat pancreatic β cells and HCN2 also seems to be the predominant channel in MIN6 cells and mouse islets. Both papers fail to establish a physiological role of these channels for glucose-induced electrical activity or insulin secretion (El-Kholy et al. 2007; Zhang et al. 2009), but it was suggested that under pathological conditions such as hypokalemia, the channel may play a protective role.

Cell Membrane Potential (V_m)

V_m of β cells is unique due to its regulation by glucose. It links signals derived from glucose metabolism to insulin secretion by determining $[Ca^{2+}]_c$.

Regulation by Glucose

Glucose enters β cells mainly via the high-K_m Glut-2 transporter (Johnson et al. 1990). As this transporter is not rate-limiting for glucose uptake, β cell cytosolic glucose concentration is rapidly adapted upon changes in blood glucose concentration. Glucose induces insulin secretion by activating a triggering pathway (closure of K_{ATP} channels, depolarization of V_m, and increase in $[Ca^{2+}]_c$) and an amplifying pathway (sensitization of the exocytotic machinery for $[Ca^{2+}]_c$) that is independent of changes in K_{ATP} channel activity and V_m (Henquin 2000; Aizawa et al. 2002). The triggering Ca^{2+} signal is essential. All physiological or pharmacological maneuvers lowering or enhancing $[Ca^{2+}]_c$ impair or improve insulin secretion. The triggering pathway is superior to the amplifying pathway. As long as the triggering signal $[Ca^{2+}]_c$ is slight, amplifying signals are without effect. Thus, low glucose can stimulate amplifying signals but they are silent without an adequate increase of the triggering Ca^{2+} signal. In this case an augmentation of $[Ca^{2+}]_c$,

regardless by which means (metabolism-derived or metabolism-independent signal), unmasks the amplifying pathway. The amplifying mechanism strongly depends on metabolism; however, the signal(s) responsible for this phenomenon is not yet identified.

Regulation of V_m by K_{ATP} Channels

In the presence of functional K_{ATP} channels, the actual plasma glucose concentration determines the activity of K_{ATP} channels. At a subthreshold glucose concentration, V_m is silent (~ -70 mV) and is mainly determined by the K_{ATP} current (Ashcroft and Rorsman 1990). With increasing glucose concentration, glucose metabolism and thus ATP formation rise and more and more K_{ATP} channels close until the K_{ATP} current is reduced to a level at which the unknown depolarizing current exceeds the hyperpolarizing current through K_{ATP} channels. V_m depolarizes to the threshold for the opening of voltage-dependent ion channels (Ca_v and Na_v channels, depending on the species), and action potentials start from a plateau potential (see Fig. 2). At a suprathreshold glucose concentration, V_m starts to oscillate. The knowledge about the nature of these oscillations mainly derived from mouse β cells. The depolarized burst phases with action potentials and the silent hyperpolarized interburst phases are glucose dependent. With increasing glucose concentration burst phases are prolonged and interburst phases are shortened until continuous activity is reached at glucose concentrations above ~25 mM (Fig. 2). Each action potential is terminated by deactivation of Ca^{2+} channels which is achieved by opening of K_v and K_{Ca} channels (see section on "Significance of K_v and K_{Ca} Channels for β Cell Electrical Activity" and Smith et al. 1990b), a maneuver that repolarizes V_m to the plateau potential from which the next action potential starts. However, the question remains which mechanisms drive the unique glucose-induced oscillations of V_m with bursts of action potentials and silent interburst phases.

A Model for β Cell Oscillations

$[Ca^{2+}]_c$ plays a pivotal role in insulin secretion. It has been suggested that the glucose-induced increase in $[Ca^{2+}]_c$ augments the mitochondrial Ca^{2+} concentration ($[Ca^{2+}]_m$) with subsequent activation of Ca^{2+}-dependent dehydrogenases and ATP production (Kennedy and Wollheim 1998). However, this positive feedback mechanism is not compatible with oscillations that require a negative feedback process. The following model suggests that the positive feedback mechanism that is induced upon a glucose rise converts into a negative feedback mechanism during sustained glucose elevation (see Fig. 3 and Kennedy et al. 2002). During phase 1, glucose increases and stimulates the β cell. The metabolism of the sugar leads to the production of reduction equivalents which enter the respiratory chains. This hyperpolarizes the mitochondrial membrane potential $\Delta\Psi$. The resulting H^+ gradient is used by the F_1/F_0 ATPase and leads to ATP production (and phosphocreatine production, see "Regulation by Metabolism-Derived Nucleotides and Phosphotransfer"), closure of K_{ATP} channels, depolarization of V_m, increase of $[Ca^{2+}]_c$, and finally insulin secretion. During phase 2 (see Fig. 3), glucose is steadily

increased which keeps up insulin secretion; however, the β cell now undergoes oscillatory activity. The increase in $[Ca^{2+}]_c$ depolarizes ΔΨ which diminishes ATP production and leads to reopening of some K_{ATP} channels. In addition, elevated $[Ca^{2+}]_c$ activates K_{Ca} channels. As a consequence of both processes, V_m hyperpolarizes which lowers $[Ca^{2+}]_c$. Subsequently, K_{Ca} channel activity decreases whereas ΔΨ hyperpolarizes. The enhanced ATP formation leads to closure of K_{ATP} channels and finally $[Ca^{2+}]_c$ increases. With this rise in $[Ca^{2+}]_c$, the next cycle starts. This model assumes that during sustained glucose elevation, an increase in $[Ca^{2+}]_c$ does not enhance but diminishes ATP production. This hypothesis is meanwhile supported by many observations: (1) Stimulation of Ca^{2+} influx reduces the ATP/ADP ratio (Detimary et al. 1998b). (2) Ca^{2+} influx depolarizes ΔΨ (Krippeit-Drews et al. 2000). (3) K_{ATP} channel activity oscillates and these oscillations are driven by $[Ca^{2+}]_c$ oscillations (Rolland et al. 2002a; Larsson et al. 1996b). (4) ΔΨ oscillates in dependence of the Ca^{2+} fluctuations (Krippeit-Drews et al. 2000; Kindmark et al. 2001). (5) $[Ca^{2+}]_c$ drives NADH oscillations (Luciani et al. 2006). This model implicates that burst phases of V_m are terminated by activation of K_{slow} composed of K_{ATP} and Ca^{2+}-dependent K^+ currents. K_{slow} counterbalances the depolarizing current and finally hyperpolarizes the plasma membrane below the threshold for L-type Ca^{2+} channel opening (Göpel et al. 1999; Düfer et al. 2009; Kanno et al. 2002a; Rolland et al. 2002a; Krippeit-Drews et al. 2000; Larsson et al. 1996b). This model is excellently supported by mathematical simulations of β cell bursting (Chay and Keizer 1983; Magnus and Keizer 1998; Bertram and Sherman 2004).

β-cell oscillatory activity is considered to be a prerequisite for pulsatile insulin secretion. Interestingly, oscillations of the membrane potential persist in β cells without functional K_{ATP} channels (SUR1KO) (Düfer et al. 2004). This demonstrates that mechanisms exist that can substitute for K_{ATP} channels to hyperpolarize V_m and sustain oscillations (see "Regulation of V_m-Independent of K_{ATP} Channels" for further details).

Regulation of V_m-Independent of K_{ATP} Channels

It is meanwhile well accepted that glucose can mediate insulin secretion by a K_{ATP} channel-independent pathway (Sato et al. 1999; Komatsu et al. 2001). Interestingly, V_m of β cells lacking functional K_{ATP} channels is also regulated by glucose. As expected, SUR1KO β cells display action potentials even at very low glucose concentration but surprisingly still exhibit an oscillatory pattern of electrical activity with burst and interburst phases (Düfer et al. 2004). Action potential frequency, percentage of time with action potentials, and interburst length change in response to an alteration of the glucose concentration. Compatibly, glucose depolarizes V_m of β cells from $K_{IR}6.2$ knockout mice (Ravier et al. 2009). Since oscillations require a hyperpolarizing current, these results suggest that other hyperpolarizing mechanisms besides K_{ATP} channels are regulated directly either by glucose or by signals deriving from the glucose metabolism. Additional hyperpolarizing mechanisms may be upregulated as a result of K_{ATP} channel loss. As mentioned above, K_{slow} currents are good candidates that may contribute to β cell hyperpolarization (Göpel et al. 1999). The K_{Ca} component of the K_{slow} current may gain importance in β

cells lacking K_{ATP} channels. Another possibility proposed recently is the activation of the Na^+, K^+-ATPase by glucose metabolism and insulin. The stimulation of the pump induces a hyperpolarizing current sufficient to maintain oscillatory electrical activity when the membrane resistance is high due to the lack of K_{ATP} channel conductance (Düfer et al. 2009).

Regulation by Hormones and Neurotransmitters

Glucose-stimulated insulin secretion is modulated by a variety of hormones and neurotransmitters which affects V_m of β cells besides other steps of the stimulus-secretion coupling.

GLP-1

GLP-1 that is produced in the neuroendocrine L-cells of the intestine is the most important representative of the incretin hormones, a group of intestinal hormones that increase insulin secretion in the presence of glucose. For several years the genetically engineered GLP-1 analogue exenatide and other GLP-1 analogues are used in the treatment of type-2 diabetes mellitus. GLP-1 exerts direct effect on β cells by binding to G-protein-coupled receptors that stimulate the adenylate cyclase and increase the cAMP concentration. cAMP can either activate protein kinase A or Epac (exchange protein activated by cAMP). Both pathways have been identified in β cells (Seino 2012). However, the downstream pathways eliciting insulin secretion have not been completely identified yet. It has been suggested that GLP-1 depolarizes V_m by closing K_{ATP} channels (Holz et al. 1993; Gromada et al. 1998; Light et al. 2002). However, this mode of action of GLP-1 is inconsistent with other findings. Some studies propose that the insulinotropic effect of GLP-1 is mediated by its effects on unspecific cation currents (Leech and Habener 1998; Kato et al. 1996); others attribute it to L-type Ca^{2+} currents (Britsch et al. 1995; Gromada et al. 1998; Suga et al. 1997) or Ca^{2+} mobilization from intracellular stores (Gromada et al. 1995).

Noradrenaline and Galanin

The autonomic nervous system has important modulating effects on insulin secretion by adapting hormone release to food intake or increased physical or psychic stress. The sympathetic neurotransmitter (nor)adrenaline and the co-transmitter galanin suppress insulin secretion (Drews et al. 1990), while the parasympathetic neurotransmitter acetylcholine enhances hormone secretion (Gilon and Henquin 2001). Noradrenaline and galanin act on several steps in β cell stimulus-secretion coupling including the membrane potential. After binding to α_2 and specific galanin receptors, respectively, noradrenaline and galanin hyperpolarize V_m via G_i-protein-coupled processes (Nilsson et al. 1989; Drews et al. 1990; Ullrich and Wollheim 1988); however, the underlying mechanisms are still unclear. For insulin-secreting tumor cell lines, it has been proposed that the sympathetic neurotransmitters activate K_{ATP} channels and that this mechanism hyperpolarizes the β cells (Dunne et al. 1989; Zhao et al. 2008). However, this mode of action was never confirmed with primary β cells. In 1991 Rorsman and coworkers described the

activation of a sulfonylurea-insensitive low-conductance K^+ current by clonidine (Rorsman et al. 1991). It was concluded that adrenaline shares this target because it acts via the same receptors. This assumption is supported by the findings that noradrenaline and galanin are able to hyperpolarize mouse β cells in the absence of K_{ATP} channels (Düfer et al. 2004; Sieg et al. 2004). Inhibition of L-type Ca^{2+} channel current by galanin or catecholamines was solely described for insulin-secreting tumor cell lines (Hsu et al. 1991a; Homaidan et al. 1991) but not approved in primary β cells (Bokvist et al. 1991).

Somatostatin

Somatostatin is released from delta cells of the islets of Langerhans and inhibits insulin secretion by a paracrine effect. Like noradrenaline and galanin, it hyperpolarizes V_m (Nilsson et al. 1989). The mode of action is not identified, but for primary β cells, a similar mechanism is suggested as for adrenaline and galanin (Rorsman et al. 1991). The hyperpolarization is not mediated by opening of K_{ATP} channels (Düfer et al. 2004). Somatostatin and its analogues octreotide or lanreotide are used in the treatment of congenital hyperinsulinism of infancy (CHI). In most cases the disease is due to mutations in one of the subunits of K_{ATP} channels, i.e., Kir6.2 or SUR1, or to mutations in the glucokinase gene. Thus, it is plausible that somatostatin or analogues can exert beneficial effects in patients affected with CHI.

Acetylcholine

The parasympathetic neurotransmitter acetylcholine has complex effects on β cells that result under physiological conditions in an augmentation of insulin secretion. The effect of the transmitter on β cells is mediated by M_3 receptors. Membrane depolarization is one mechanism contributing to the insulinotropic effect of acetylcholine. The depolarization is caused by activation of a Na^+ current and the subsequent stimulation of Ca^{2+} influx. The Na^+ current is not voltage-dependent and not regulated by store depletion. Surprisingly, the activation of the Na^+ current occurs independent of G-proteins. It is suggested that distinct Na^+ channels are directly coupled to muscarinic receptors in β cells via an unknown transduction mechanism (Rolland et al. 2002b; Gilon and Henquin 2001; Miura et al. 1996). It has been shown that Ca^{2+} store depletion triggers Ca^{2+} or unspecific cation influx in β cells (Roe et al. 1998; Miura et al. 1997). Therefore, another possibility for an acetylcholine-induced depolarization is emptying of Ca^{2+} stores by IP_3 with subsequent induction of store-dependent Ca^{2+} influx. However, to our knowledge it has only been shown for insulin-secreting cell lines but not for primary β cells that acetylcholine stimulates this pathway (Mears and Zimliki 2004).

Insulin

It is attractive to assume that insulin influences its own secretion by a feedback mechanism. However, the concept that insulin has an autocrine effect is controversial. Numerous papers on this topic demonstrate negative feedback, positive feedback, or no effect of insulin on β cell function (for review, see Leibiger and

Berggren 2008). The K_{ATP} channel has been identified as a target for insulin. Khan and coworkers (2001) show that insulin activates K_{ATP} channels leading to hyperpolarization of V_m which would suppress insulin secretion. It is suggested that this effect of insulin on K_{ATP} channels is mediated by PI_3 kinase/PI(3,4,5)P_3 signaling that alters the ATP sensitivity of K_{ATP} channels (Khan et al. 2001; Persaud et al. 2002). Insulin hyperpolarizes V_m in SUR1KO mouse β-cells showing that the negative feedback of insulin on V_m is present in the absence of K_{ATP} channels. Düfer and coworkers (2009) provide evidence that this negative feedback is due to the activation of the Na^+, K^+-ATPase by insulin. This mechanism may gain importance in cells with a high membrane resistance where small current changes can induce large effects on V_m.

α-Cells

Ion Channels

Most studies addressing the expression and function of ion channels in pancreatic α cells have been performed with rodent islet preparations. In α-cells there have been identified at least four different types of K^+ channels, four types of voltage-gated Ca^{2+} channels, a Na^+ channel, and the $GABA_A$ receptor Cl^- channel (Yan et al. 2004; Gromada et al. 1997; Rorsman et al. 1989). Recent studies also prove evidence for a regulatory function of HCN channels (Zhang et al. 2008) and ionotropic glutamate receptors (Cabrera et al. 2008) (see also chapter "▶ Physiological and Pathophysiological Control of Glucagon Secretion by Pancreatic α-Cells").

K_{ATP} Channels

K_{ATP} currents have been observed in clonal glucagon-secreting αTC6 cells (Rajan et al. 1993; Ronner et al. 1993) as well as in rodent α cells (Barg et al. 2000; Bokvist et al. 1999; Leung et al. 2005), and co-localization of $K_{IR}6.2$ or SUR1 mRNA, respectively, with glucagon has been shown in intact islets (Bokvist et al. 1999). Up to now a direct proof for K_{ATP} channel activity in human α cells is still missing. In accordance with the characteristics of K_{ATP} channel regulation in β cells, the sensitivity of K_{ATP} channels toward ATP inhibition is much higher in excised patches ($K_i \sim 17$ μM) compared to intact α cells ($K_i \sim 940$ μM) (Bokvist et al. 1999; Gromada et al. 2007). With regard to nucleotide sensitivity, there seem to exist species differences: A reduction of the ATP sensitivity by PIP_2 was reported for rat (Bokvist et al. 1999) but not for murine α cells (Leung et al. 2005), and the K_i value for ATP in intact murine α cells is about sixfold higher (Leung et al. 2005) compared to rats. ATP sensitivity of α cell K_{ATP} channels has been shown to be reduced by insulin (Ravier and Rutter 2005; Leung et al. 2006), and it has been suggested that the mediator inducing channel opening is not insulin but Zn^{2+} (Zhou et al. 2007).

Other K^+ Channels

Besides ATP-regulated K^+ channels, α cells are also equipped with voltage-activated K^+ channels. In human α cells $K_v3.1$ and $K_v6.1$ have been identified on mRNA level (Yan et al. 2004), and $K_v4.3$ was detected in mouse α cells (Göpel et al. 2000a). BK channels are suggested to be present due to the sensitivity of glucagon secretion to iberiotoxin (Spigelman et al. 2010).

Two groups of currents, a TEA^+-resistant but 4-aminopyridine-sensitive transient K^+ current (A-current) (Göpel et al. 2000a, b; Leung et al. 2005) and a TEA^+-sensitive delayed-rectifier K^+ current (K_{DR}), have been detected in mouse α cells (Leung et al. 2005; Göpel et al. 2000b). The A-current might, at least in part, be attributable to $K_v4.3$ channels (Göpel et al. 2000a). In addition a G-protein-coupled K^+ current composed of $K_{IR}3.2c$ and $K_{IR}3.4$ that is activated by GTP via the somatostatin receptor has been described by Yoshimoto et al. (1999).

Ca^{2+} Channels

Ca^{2+}-dependent action potentials in α cells have been described first by Rorsman and Hellman (Rorsman and Hellman 1988) in FACS-purified cells of guinea pigs.

Currents through L-type Ca^{2+} channels were reported in α cells of several species. Channel opening starts at membrane depolarization above −50 mV, and the current through these channels mediates about 50–60 % of the Ca^{2+} influx induced by membrane depolarization in rat and mouse α cells (Barg et al. 2000; Vignali et al. 2006). L-type Ca^{2+} currents are suggested to account for most of the Ca^{2+} increase required for glucagon secretion in response to adrenaline or forskolin stimulation (Gromada et al. 1997). Comparative experiments with knockout animals suggest that L-type Ca^{2+} current in α cells is mediated by $Ca_V1.2$ and 1.3 (Vignali et al. 2006).

N-type Ca^{2+} channels seem to play a role for regulation of exocytosis under resting conditions in rat α cells (Gromada et al. 1997) and for glucose-induced glucagon secretion (see section "Regulation of Electrical Activity" and Olsen et al. 2005). In mouse α cells about 25 % of the depolarization-evoked Ca^{2+} current could be ascribed to omega-conotoxin-GVIA-sensitive N-type Ca^{2+} channels (Barg et al. 2000). However, expression of N-type Ca^{2+} channel mRNA ($Ca_V2.2$) was not found in murine α cells (Vignali et al. 2006).

R-type Ca^{2+} channels that are blockable by the $Ca_V2.3$ channel inhibitor SNX 482 account for ∼30 % of Ca^{2+} influx in murine α cells (Vignali et al. 2006) but seem not to play any role for glucose-regulated glucagon secretion in rat α cells (Olsen et al. 2005). Low-voltage-activated T-type Ca^{2+} currents have been measured in mouse and guinea pig α cells (Göpel et al. 2000a; Leung et al. 2005; Rorsman and Hellman 1988), whereas one study failed to detect these channels in murine α cells (Vignali et al. 2006). As these channels activate at relatively negative membrane potential of ∼ −60 mV, it is suggested that they are involved in the initiation of Ca^{2+} action potentials (Gromada et al. 2007).

Na^+ Channels

The Na^+ channels expressed in α cells are inhibited by tetrodotoxin and activate at potentials more positive than −30 mV. Maximum peak current is achieved between −10 and 0 mV. Inactivation of Na^+ channels occurs with $V_{1/2}$ of ∼ −50 mV (Göpel et al. 2000a). This clearly contrasts to mouse β cells where $V_{1/2}$ is ∼ −100 mV and no Na^+ current could be evoked by depolarizations starting from the resting membrane potential (compare section "Other Ion Channels" and "Na^+ Channels"). The importance of Na^+ channels in α cells is underlined by the fact that tetrodotoxin strongly inhibits glucagon secretion (Göpel et al. 2000a).

$GABA_A$ Cl^- Channels

The existence of Cl^- currents activated by GABA in α cells was primarily described by Rorsman et al. (1989) for cells isolated from guinea pigs. $GABA_A$ receptor mRNA and protein expression have been identified in clonal and primary α cells (Bailey et al. 2007; Wendt et al. 2004; Xu et al. 2006). In patch-clamped α cells, application of GABA terminates action potentials. The GABA-activated current as well as GABA-induced inhibition of glucagon release is sensitive to the $GABA_A$ receptor antagonist bicuculline (Rorsman et al. 1989; Gaskins et al. 1995). Translocation of $GABA_A$ receptors and Cl^- currents has been shown to be potentiated by insulin (Xu et al. 2006).

HCN Channels

There is one report (Zhang et al. 2008) showing mRNA and protein expression of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in αTC6 cells and rat α cells. Blockade of HCN channels resulted in elevation of $[Ca^{2+}]_c$ and increased glucagon secretion in clonal and primary α cells.

Ionotropic Glutamate Receptors

Cabrera et al. (2008) demonstrate that human α cells express glutamate receptors of the AMPA/kainate type which are Na^+-permeable nonselective cation channels. Stimulation of these receptors results in activation of an NBQX-sensitive inward current and in glucagon secretion. The authors suggest that glutamate release from α cells provides an autocrine positive feedback mechanism where activation of AMPA and kainate receptors triggers membrane depolarization and promotes opening of voltage-gated Ca^{2+} channels.

Regulation of Electrical Activity

As expected from an electrically excitable cell, the degree of membrane depolarization and the extent of glucagon release are closely coupled in α cells. In the absence of glucose, α cells are electrically active and display Na^+- and Ca^{2+}-dependent action potentials (Rorsman and Hellman 1988; Wesslen et al. 1987). In contrast to β cells where Ca^{2+} action potentials are induced when V_m is depolarized above −50 mV, action potentials in α cells start at a more

hyperpolarized membrane potential of ~ −70 to −60 mV (Barg et al. 2000; Göpel et al. 2000b; Gromada et al. 2004). It is suggested that in mouse α cells, electrical activity is initiated by opening of T-type Ca^{2+} channels. Further depolarization leads to opening of Na^+ and L-type Ca^{2+} channels, and activation of K_{DR} channels and A-currents induces action potential repolarization. In rat α cells there is no proof for the existence of T-type Ca^{2+} channels, but it is suggested that due to the low K^+ conductance, V_m is sufficiently depolarized for Na^+ and Ca^{2+} channel activation (Gromada et al. 2007). In low glucose K_v channels positively regulate glucagon release as their opening prevents depolarization-induced inactivation of Na^+ or Ca^{2+} channels (Spigelman et al. 2010).

Regarding the influence of nutrients, hormones, or drugs acting on ion channels, one must clearly discriminate between studies made with single cells and those with α cells within intact islets. Studies performed with intact islets more precisely reflect the situation *in vivo*. However, such investigations have the drawback that direct effects of nutrients or drugs on ion channels cannot be discriminated from indirect mechanisms mediated by paracrine regulators.

α cells of intact islets are spontaneously active and increasing glucose results in membrane hyperpolarization (Hjortoe et al. 2004; Manning Fox et al. 2006). Reports about glucose-dependent regulation of electrical activity in single isolated α cells are inconsistent. Varying glucose between 5 and 20 mM has no effect on action potential frequency in guinea pig α cells (Rorsman and Hellman 1988). In FACS-purified α cells of rats and in single mouse α cells, increasing glucose above 10 mM results in increased membrane depolarization with reduced action potential amplitude (Gromada et al. 2004; Franklin et al. 2005). In contrast, the same groups also report for both species membrane hyperpolarization below the threshold for action potentials in response to high glucose (Barg et al. 2000; Bokvist et al. 1999).

Recently, it has been shown that in isolated α cells, glucose-mediated K_{ATP} channel closure induces a sequence of events similar to the stimulus-secretion cascade of β cells: Elevating glucose decreases K_{ATP} current which triggers Ca^{2+} influx and exocytosis (Olsen et al. 2005; Franklin et al. 2005). As the α cells' ATP/ADP ratio is higher than in β cells, K_{ATP} current is much lower which allows spontaneous electrical activity even in the absence of glucose (Olsen et al. 2005). Interestingly, in contrast to β cells, the potency of glucose to inhibit K_{ATP} current seems to be very low. One study described that inhibition of K^+ conductance by 20 mM glucose amounts to only 1/3 of tolbutamide inhibition (Olsen et al. 2005), whereas another investigation completely failed to detect any inhibitory effect of 15 mM glucose on K_{ATP} current (Quoix et al. 2009).

Regardless of what happens on the single-cell level, there is much evidence that the primary mechanisms governing glucagon secretion are mediated by paracrine signaling pathways. Insulin and GABA which are secreted from neighboring β cells as well as somatostatin from delta cells hyperpolarize the α cell via activation of K_{ATP} channels, $GABA_A$ Cl^- channels, and G-protein-coupled K^+ channels, respectively (compare section "Ion Channels"). However, glucose-induced inhibition of glucagon release can also occur independently of K_{ATP} channels and somatostatin signaling (Cheng-Xue et al. 2013). The importance of a glucose-mediated direct

inhibition of glucagon secretion is still in debate. This pathway suggests that with high glucose concentrations, membrane depolarization via closure of K_{ATP} channels might exceed the stimulatory range and lead to reduction of exocytosis via inactivation of Na^+ and N-type Ca^{2+} channels (MacDonald et al. 2007).

Delta Cells

Less than 10 % of the islet cells are delta cells producing somatostatin (Kanno et al. 2002b). Somatostatin is known to act as a paracrine regulator that inhibits insulin and glucagon secretion (Nilsson et al. 1989; Ullrich et al. 1990; Wollheim et al. 1990; Schuit et al. 1989).

Delta cells (Guiot et al. 2007; Gopel et al. 2000b; Berts et al. 1996; Suzuki et al. 1997, 1999) and derived tumor cells (Branstrom et al. 1997b) are equipped with K_{ATP} channels and respond to an increase in glucose concentration with depolarization (Efendic et al. 1979). Delta cells were supposed to have a similar glucose-induced stimulus-secretion coupling than β-cells (Göpel et al. 2000b) although they are already stimulated at lower glucose concentrations (~3 mM) (Nadal et al. 1999) possibly because of a lower density of K_{ATP} channels (Quesada et al. 1999). In contrast, Zhang and coworkers (Zhang et al. 2007) have shown that the β cell-specific stimulus-secretion coupling is not necessarily valid for delta cells. They approved that at low glucose concentrations, V_m and $[Ca^{2+}]_c$ are at least partly dependent on K_{ATP} channel activity and Ca^{2+} influx through L-type Ca^{2+} channels but that neither exocytosis nor somatostatin secretion is influenced by L-type Ca^{2+} channel blockers. They show that, especially in high glucose concentrations, somatostatin secretion is completely independent on K_{ATP} channel activity but influenced by inhibitors of R-type Ca^{2+} channel ($Ca_v2.3$) blockers. In addition they illustrated that exocytosis and secretion crucially depend on Ca^{2+}-induced Ca^{2+} release (CICR) through ryanodine receptors (RyR3 type). It is suggested that K_{ATP} channel closure initially depolarizes delta cells in response to rising glucose concentrations but that R-type rather than L-type Ca^{2+} channels and CICR are responsible for somatostatin secretion. Accordingly, somatostatin release at high glucose concentrations is tolbutamide insensitive and even exists in SUR1KO mice (Zhang et al. 2007). Due to the limited number of studies, the exact nature of stimulus-secretion coupling in delta cells remains elusive.

Cross-References

- ▶ Anionic Transporters and Channels
- ▶ ATP-Sensitive Potassium Channels in Health and Disease
- ▶ Calcium Signaling in the Islets
- ▶ Exocytosis in Islet β-Cells
- ▶ Physiological and Pathophysiological Control of Glucagon Secretion by Pancreatic α-Cells

References

Aguilar-Bryan L, Bryan J (1999) Molecular biology of adenosine triphosphate-sensitive potassium channels. Endocr Rev 20:101–135

Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP, Boyd AE 3rd, Gonzalez G, Herrera-Sosa H, Nguy K, Bryan J, Nelson DA (1995) Cloning of the β cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science 268:423–426

Aguilar-Bryan L, Clement JP, Gonzalez G, Kunjilwar K, Babenko A, Bryan J (1998) Toward understanding the assembly and structure of K_{ATP} channels. Physiol Rev 78:227–245

Ahren B, Arkhammar P, Berggren PO, Nilsson T (1986) Galanin inhibits glucose-stimulated insulin release by a mechanism involving hyperpolarization and lowering of cytoplasmic free Ca^{2+} concentration. Biochem Biophys Res Commun 140:1059–1063

Aicardi G, Pollo A, Sher E, Carbone E (1991) Noradrenergic inhibition and voltage-dependent facilitation of omega-conotoxin-sensitive Ca channels in insulin-secreting RINm5F cells. FEBS Lett 281:201–204

Aizawa T, Sato Y, Komatsu M (2002) Importance of nonionic signals for glucose-induced biphasic insulin secretion. Diabetes 51(Suppl 1):S96–S98

Akesson B, Lundquist I (1999) Nitric oxide and hydroperoxide affect islet hormone release and Ca^{2+} efflux. Endocrine 11:99–107

Akiba Y, Kato S, Katsube K, Nakamura M, Takeuchi K, Ishii H, Hibi T (2004) Transient receptor potential vanilloid subfamily 1 expressed in pancreatic islet β cells modulates insulin secretion in rats. Biochem Biophys Res Commun 321:219–225

Ämmälä C, Larsson O, Berggren PO, Bokvist K, Juntti-Berggren L, Kindmark H, Rorsman P (1991) Inositol trisphosphate-dependent periodic activation of a Ca^{2+}activated K^+ conductance in glucose-stimulated pancreatic β-cells. Nature 353:849–852

Andres MA, Baptista NC, Efird JT, Ogata KK, Bellinger FP, Zeyda T (2009) Depletion of SK1 channel subunits leads to constitutive insulin secretion. FEBS Lett 583:369–376

Arikkath J, Campbell KP (2003) Auxiliary subunits: essential components of the voltage-gated calcium channel complex. Curr Opin Neurobiol 13:298–307

Arnoux JB, de Lonlay P, Ribeiro MJ, Hussain K, Blankenstein O, Mohnike K, Valayannopoulos V, Robert JJ, Rahier J, Sempoux C, Bellanne C, Verkarre V, Aigrain Y, Jaubert F, Brunelle F, Nihoul-Fekete C (2010) Congenital hyperinsulinism. Early Hum Dev 86:287–294

Ashcroft FM (1988) Adenosine 5′-triphosphate-sensitive potassium channels. Annu Rev Neurosci 11:97–118

Ashcroft FM, Rorsman P (1989) Electrophysiology of the pancreatic β-cell. Prog Biophys Mol Biol 54:87–143

Ashcroft FM, Rorsman P (1990) ATP-sensitive K^+ channels: a link between β-cell metabolism and insulin secretion. Biochem Soc Trans 18:109–111

Ashcroft FM, Harrison DE, Ashcroft SJ (1984) Glucose induces closure of single potassium channels in isolated rat pancreatic β-cells. Nature 312:446–448

Ashcroft FM, Rorsman P, Trube G (1989) Single calcium channel activity in mouse pancreatic β-cells. Ann N Y Acad Sci 560:410–412

Atwater I, Ribalet B, Rojas E (1978) Cyclic changes in potential and resistance of the β-cell membrane induced by glucose in islets of Langerhans from mouse. J Physiol 278:117–139

Atwater I, Ribalet B, Rojas E (1979) Mouse pancreatic β-cells: tetraethylammonium blockage of the potassium permeability increase induced by depolarization. J Physiol 288:561–574

Atwater I, Dawson CM, Scott A, Eddlestone G, Rojas E (1980) The nature of the oscillatory behaviour in electrical activity from pancreatic β-cell. Horm Metab Res Suppl Suppl 10:100–107

Babenko AP (2005) K_{ATP} channels "vingt ans apres": ATG to PDB to mechanism. J Mol Cell Cardiol 39:79–98

Babenko AP, Bryan J (2002) SUR-dependent modulation of K_{ATP} channels by an N-terminal KIR6.2 peptide. Defining intersubunit gating interactions. J Biol Chem 277:43997–44004

Babenko AP, Bryan J (2003) Sur domains that associate with and gate K_{ATP} pores define a novel gatekeeper. J Biol Chem 278:41577–41580

Babenko AP, Aguilar-Bryan L, Bryan J (1998) A view of sur/KIR6.X, K_{ATP} channels. Annu Rev Physiol 60:667–687

Babenko AP, Gonzalez G, Bryan J (1999a) The N-terminus of KIR6.2 limits spontaneous bursting and modulates the ATP-inhibition of K_{ATP} channels. Biochem Biophys Res Commun 255:231–238

Babenko AP, Gonzalez G, Aguilar-Bryan L, Bryan J (1999b) Sulfonylurea receptors set the maximal open probability, ATP sensitivity and plasma membrane density of K_{ATP} channels. FEBS Lett 445:131–136

Babenko AP, Gonzalez G, Bryan J (1999c) The tolbutamide site of SUR1 and a mechanism for its functional coupling to K_{ATP} channel closure. FEBS Lett 459:367–376

Babenko AP, Gonzalez G, Bryan J (2000) Pharmaco-topology of sulfonylurea receptors. Separate domains of the regulatory subunits of K_{ATP} channel isoforms are required for selective interaction with K^+ channel openers. J Biol Chem 275:717–720

Bailey SJ, Ravier MA, Rutter GA (2007) Glucose-dependent regulation of gamma-aminobutyric acid (GABA A) receptor expression in mouse pancreatic islet α-cells. Diabetes 56:320–327

Bao S, Jacobson DA, Wohltmann M, Bohrer A, Jin W, Philipson LH, Turk J (2008) Glucose homeostasis, insulin secretion, and islet phospholipids in mice that overexpress iPLA2β in pancreatic β-cells and in iPLA2β-null mice. Am J Physiol Endocrinol Metab 294:E217–E229

Barg S, Galvanovskis J, Gopel SO, Rorsman P, Eliasson L (2000) Tight coupling between electrical activity and exocytosis in mouse glucagon-secreting α-cells. Diabetes 49:1500–1510

Barg S, Ma X, Eliasson L, Galvanovskis J, Gopel SO, Obermuller S, Platzer J, Renstrom E, Trus M, Atlas D, Striessnig J, Rorsman P (2001) Fast exocytosis with few Ca^{2+} channels in insulin-secreting mouse pancreatic B cells. Biophys J 81:3308–3323

Barnett DW, Pressel DM, Misler S (1995) Voltage-dependent Na^+ and Ca^{2+} currents in human pancreatic islet β-cells: evidence for roles in the generation of action potentials and insulin secretion. Pflugers Arch 431:272–282

Baukrowitz T, Fakler B (2000) K_{ATP} channels gated by intracellular nucleotides and phospholipids. Eur J Biochem 267:5842–5848

Baukrowitz T, Schulte U, Oliver D, Herlitze S, Krauter T, Tucker SJ, Ruppersberg JP, Fakler B (1998) PIP2 and PIP as determinants for ATP inhibition of K_{ATP} channels. Science 282:1141–1144

Bertram R, Sherman A (2004) A calcium-based phantom bursting model for pancreatic islets. Bull Math Biol 66:1313–1344

Berts A, Ball A, Dryselius G, Gylfe E, Hellman B (1996) Glucose stimulation of somatostatin-producing islet cells involves oscillatory Ca^{2+} signaling. Endocrinology 137:693–697

Best L (1999) Cell-attached recordings of the volume-sensitive anion channel in rat pancreatic β-cells. Biochim Biophys Acta 1419:248–256

Best L (2002) Study of a glucose-activated anion-selective channel in rat pancreatic β-cells. Pflugers Arch 445:97–104

Best L, Benington S (1998) Effects of sulphonylureas on the volume-sensitive anion channel in rat pancreatic β-cells. Br J Pharmacol 125:874–878

Best L, Miley HE, Yates AP (1996a) Activation of an anion conductance and β-cell depolarization during hypotonically induced insulin release. Exp Physiol 81:927–933

Best L, Sheader EA, Brown PD (1996b) A volume-activated anion conductance in insulin-secreting cells. Pflugers Arch 431:363–370

Best L, Brown PD, Tomlinson S (1997) Anion fluxes, volume regulation and electrical activity in the mammalian pancreatic β-cell. Exp Physiol 82:957–966

Best L, Miley HE, Brown PD, Cook LJ (1999) Methylglyoxal causes swelling and activation of a volume-sensitive anion conductance in rat pancreatic β-cells. J Membr Biol 167:65–71

Best L, Brown PD, Sheader EA, Yates AP (2000) Selective inhibition of glucose-stimulated β-cell activity by an anion channel inhibitor. J Membr Biol 177:169–175

Best L, Speake T, Brown P (2001) Functional characterisation of the volume-sensitive anion channel in rat pancreatic β-cells. Exp Physiol 86:145–150

Best L, Davies S, Brown PD (2004a) Tolbutamide potentiates the volume-regulated anion channel current in rat pancreatic β cells. Diabetologia 47:1990–1997

Best L, Yates AP, Decher N, Steinmeyer K, Nilius B (2004b) Inhibition of glucose-induced electrical activity in rat pancreatic β-cells by DCPIB, a selective inhibitor of volume-sensitive anion currents. Eur J Pharmacol 489:13–19

Bokvist K, Ammala C, Berggren PO, Rorsman P, Wahlander K (1991) α 2-adrenoreceptor stimulation does not inhibit L-type calcium channels in mouse pancreatic β-cells. Biosci Rep 11:147–157

Bokvist K, Eliasson L, Ammala C, Renstrom E, Rorsman P (1995) Co-localization of L-type Ca^{2+} channels and insulin-containing secretory granules and its significance for the initiation of exocytosis in mouse pancreatic β-cells. EMBO J 14:50–57

Bokvist K, Olsen HL, Hoy M, Gotfredsen CF, Holmes WF, Buschard K, Rorsman P, Gromada J (1999) Characterisation of sulphonylurea and ATP-regulated K^+ channels in rat pancreatic α-cells. Pflugers Arch 438:428–436

Branstrom R, Corkey BE, Berggren PO, Larsson O (1997a) Evidence for a unique long chain acyl-CoA ester binding site on the ATP-regulated potassium channel in mouse pancreatic β cells. J Biol Chem 272:17390–17394

Branstrom R, Hoog A, Wahl MA, Berggren PO, Larsson O (1997b) RIN14B: a pancreatic delta-cell line that maintains functional ATP-dependent K^+ channels and capability to secrete insulin under conditions where it no longer secretes somatostatin. FEBS Lett 411:301–307

Branstrom R, Leibiger IB, Leibiger B, Corkey BE, Berggren PO, Larsson O (1998a) Long chain coenzyme A esters activate the pore-forming subunit (Kir6.2) of the ATP-regulated potassium channel. J Biol Chem 273:31395–31400

Branstrom R, Efendic S, Berggren PO, Larsson O (1998b) Direct inhibition of the pancreatic β-cell ATP-regulated potassium channel by α-ketoisocaproate. J Biol Chem 273:14113–14118

Branstrom R, Aspinwall CA, Valimaki S, Ostensson CG, Tibell A, Eckhard M, Brandhorst H, Corkey BE, Berggren PO, Larsson O (2004) Long-chain CoA esters activate human pancreatic β-cell K_{ATP} channels: potential role in type 2 diabetes. Diabetologia 47:277–283

Braun M, Ramracheya R, Bengtsson M, Zhang Q, Karanauskaite J, Partridge C, Johnson PR, Rorsman P (2008) Voltage-gated ion channels in human pancreatic β-cells: electrophysiological characterization and role in insulin secretion. Diabetes 57:1618–1628

Britsch S, Krippeit-Drews P, Gregor M, Lang F, Drews G (1994) Effects of osmotic changes in extracellular solution on electrical activity of mouse pancreatic β-cells. Biochem Biophys Res Commun 204:641–645

Britsch S, Krippeit-Drews P, Lang F, Gregor M, Drews G (1995) Glucagon-like peptide-1 modulates Ca^{2+} current but not K^+ ATP current in intact mouse pancreatic β-cells. Biochem Biophys Res Commun 207:33–39

Bryan J, Munoz A, Zhang X, Düfer M, Drews G, Krippeit-Drews P, Aguilar-Bryan L (2007) ABCC8 and ABCC9: ABC transporters that regulate K^+ channels. Pflugers Arch 453:703–718

Buda P, Reinbothe T, Nagaraj V, Mahdi T, Luan C, Tang Y, Axelsson AS, Li D, Rosengren AH, Renstrom E, Zhang E (2013) Eukaryotic translation initiation factor 3 subunit e controls intracellular calcium homeostasis by regulation of $Ca_v1.2$ surface expression. PLoS One 8: e64462

Bushman JD, Zhou Q, Shyng SL (2013) A Kir6.2 pore mutation causes inactivation of ATP-sensitive potassium channels by disrupting PIP2-dependent gating. PLoS One 8: e63733

Cabrera O, Jacques-Silva MC, Speier S, Yang SN, Kohler M, Fachado A, Vieira E, Zierath JR, Kibbey R, Berman DM, Kenyon NS, Ricordi C, Caicedo A, Berggren PO (2008) Glutamate is a positive autocrine signal for glucagon release. Cell Metab 7:545–554

Catterall WA (2000) Structure and regulation of voltage-gated Ca^{2+} channels. Annu Rev Cell Dev Biol 16:521–555

Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J (2005) International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. Pharmacol Rev 57:411–425

Chachin M, Yamada M, Fujita A, Matsuoka T, Matsushita K, Kurachi Y (2003) Nateglinide, a D-phenylalanine derivative lacking either a sulfonylurea or benzamido moiety, specifically inhibits pancreatic β-cell-type K_{ATP} channels. J Pharmacol Exp Ther 304:1025–1032

Chan KW, Zhang H, Logothetis DE (2003) N-terminal transmembrane domain of the SUR controls trafficking and gating of Kir6 channel subunits. EMBO J 22:3833–3843

Chay TR, Keizer J (1983) Minimal model for membrane oscillations in the pancreatic β-cell. Biophys J 42:181–190

Cheng H, Beck A, Launay P, Gross SA, Stokes AJ, Kinet JP, Fleig A, Penner R (2007) TRPM4 controls insulin secretion in pancreatic β-cells. Cell Calcium 41:51–61

Cheng-Xue R, Gomez-Ruiz A, Antoine N, Noel LA, Chae HY, Ravier MA, Chimienti F, Schuit FC, Gilon P (2013) Tolbutamide controls glucagon release from mouse islets differently than glucose: involvement of K_{ATP} channels from both α-cells and δ-cells. Diabetes 62:1612–1622

Chistiakov DA, Potapov VA, Khodirev DC, Shamkhalova MS, Shestakova MV, Nosikov VV (2009) Genetic variations in the pancreatic ATP-sensitive potassium channel, β-cell dysfunction, and susceptibility to type 2 diabetes. Acta Diabetol 46:43–49

Clement JP, Kunjilwar K, Gonzalez G, Schwanstecher M, Panten U, Aguilar-Bryan L, Bryan J (1997) Association and stoichiometry of K_{ATP} channel subunits. Neuron 18:827–838

Collins SC, Hoppa MB, Walker JN, Amisten S, Abdulkader F, Bengtsson M, Fearnside J, Ramracheya R, Toye AA, Zhang Q, Clark A, Gauguier D, Rorsman P (2010) Progression of diet-induced diabetes in C57BL6J mice involves functional dissociation of Ca^{2+} channels from secretory vesicles. Diabetes 59:1192–1201

Cook DL, Hales CN (1984) Intracellular ATP directly blocks K^+ channels in pancreatic β-cells. Nature 311:271–273

Crane A, Aguilar-Bryan L (2004) Assembly, maturation, and turnover of K_{ATP} channel subunits. J Biol Chem 279:9080–9090

Crawford RM, Ranki HJ, Botting CH, Budas GR, Jovanovic A (2002) Creatine kinase is physically associated with the cardiac ATP-sensitive K^+ channel in vivo. FASEB J 16:102–104

Dabrowski M, Wahl P, Holmes WE, Ashcroft FM (2001) Effect of repaglinide on cloned β cell, cardiac and smooth muscle types of ATP-sensitive potassium channels. Diabetologia 44:747–756

Davies A, Hendrich J, Van Minh AT, Wratten J, Douglas L, Dolphin AC (2007) Functional biology of the α(2)delta subunits of voltage-gated calcium channels. Trends Pharmacol Sci 28:220–228

de Wet H, Mikhailov MV, Fotinou C, Dreger M, Craig TJ, Venien-Bryan C, Ashcroft FM (2007a) Studies of the ATPase activity of the ABC protein SUR1. FEBS J 274:3532–3544

de Wet H, Rees MG, Shimomura K, Aittoniemi J, Patch AM, Flanagan SE, Ellard S, Hattersley AT, Sansom MS, Ashcroft FM (2007b) Increased ATPase activity produced by mutations at arginine-1380 in nucleotide-binding domain 2 of ABCC8 causes neonatal diabetes. Proc Natl Acad Sci USA 104:18988–18992

Dean PM, Matthews EK (1968) Electrical activity in pancreatic islet cells. Nature 219:389–390

Debuyser A, Drews G, Henquin JC (1991a) Adrenaline inhibition of insulin release: role of the repolarization of the B cell membrane. Pflugers Arch 419:131–137

Debuyser A, Drews G, Henquin JC (1991b) Adrenaline inhibition of insulin release: role of cyclic AMP. Mol Cell Endocrinol 78:179–186

Detimary P, Dejonghe S, Ling Z, Pipeleers D, Schuit F, Henquin JC (1998a) The changes in adenine nucleotides measured in glucose-stimulated rodent islets occur in β cells but not in α cells and are also observed in human islets. J Biol Chem 273:33905–33908

Detimary P, Gilon P, Henquin JC (1998b) Interplay between cytoplasmic Ca^{2+} and the ATP/ADP ratio: a feedback control mechanism in mouse pancreatic islets. Biochem J 333(Pt 2):269–274

Dorschner H, Brekardin E, Uhde I, Schwanstecher C, Schwanstecher M (1999) Stoichiometry of sulfonylurea-induced ATP-sensitive potassium channel closure. Mol Pharmacol 55:1060–1066

Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science 280:69–77

Drews G, Debuyser A, Nenquin M, Henquin JC (1990) Galanin and epinephrine act on distinct receptors to inhibit insulin release by the same mechanisms including an increase in K^+ permeability of the β-cell membrane. Endocrinology 126:1646–1653

Drews G, Zempel G, Krippeit-Drews P, Britsch S, Busch GL, Kaba NK, Lang F (1998) Ion channels involved in insulin release are activated by osmotic swelling of pancreatic β-cells. Biochim Biophys Acta 1370:8–16

Drews G, Krämer C, Düfer M, Krippeit-Drews P (2000a) Contrasting effects of alloxan on islets and single mouse pancreatic β-cells. Biochem J 352(Pt 2):389–397

Drews G, Krämer C, Krippeit-Drews P (2000b) Dual effect of NO on K^+_{ATP} current of mouse pancreatic β-cells: stimulation by deenergizing mitochondria and inhibition by direct interaction with the channel. Biochim Biophys Acta 1464:62–68

Düfer M, Krippeit-Drews P, Buntinas L, Siemen D, Drews G (2002) Methyl pyruvate stimulates pancreatic β-cells by a direct effect on K_{ATP} channels, and not as a mitochondrial substrate. Biochem J 368:817–825

Düfer M, Haspel D, Krippeit-Drews P, Aguilar-Bryan L, Bryan J, Drews G (2004) Oscillations of membrane potential and cytosolic Ca^{2+} concentration in $SUR1^{-/-}$ β cells. Diabetologia 47:488–498

Düfer M, Neye Y, Krippeit-Drews P, Drews G (2004) Direct interference of HIV protease inhibitors with pancreatic β-cell function. Naunyn Schmiedebergs Arch Pharmacol 369:583–590

Düfer M, Gier B, Wolpers D, Ruth P, Krippeit Drews P, Drews G (2009) SK4 channels are involved in the regulation of glucose homeostasis and pancreatic β-cell function. Diabetes 58:1835–1843

Düfer M, Haspel D, Krippeit-Drews P, Aguilar-Bryan L, Bryan J, Drews G (2009) Activation of the Na^+/K^+-ATPase by insulin and glucose as a putative negative feedback mechanism in pancreatic β-cells. Pflugers Arch 457:1351–1360

Düfer M, Neye Y, Hörth K, Krippeit-Drews P, Hennige A, Widmer H, McClafferty H, Shipston MJ, Haring HU, Ruth P, Drews G (2011) BK channels affect glucose homeostasis and cell viability of murine pancreatic β cells. Diabetologia 54:423–432

Düfer M, Hörth K, Wagner R, Schittenhelm B, Prowald S, Wagner TF, Oberwinkler J, Lukowski R, Gonzalez FJ, Krippeit-Drews P, Drews G (2012a) Bile acids acutely stimulate insulin secretion of mouse β-cells via farnesoid X receptor activation and K_{ATP} channel inhibition. Diabetes 61:1479–1489

Düfer M, Hörth K, Krippeit-Drews P, Drews G (2012b) The significance of the nuclear farnesoid X receptor (FXR) in β cell function. Islets 4:333–338

Dukes ID, McIntyre MS, Mertz RJ, Philipson LH, Roe MW, Spencer B, Worley JF 3rd (1994) Dependence on NADH produced during glycolysis for β-cell glucose signaling. J Biol Chem 269:10979–10982

Dunne MJ, Illot MC, Peterson OH (1987) Interaction of diazoxide, tolbutamide and ATP4- on nucleotide-dependent K^+ channels in an insulin-secreting cell line. J Membr Biol 99:215–224

Dunne MJ, Bullett MJ, Li GD, Wollheim CB, Petersen OH (1989) Galanin activates nucleotide-dependent K^+ channels in insulin-secreting cells via a pertussis toxin-sensitive G-protein. EMBO J 8:413–420

Dunne MJ, Cosgrove KE, Shepherd RM, Aynsley-Green A, Lindley KJ (2004) Hyperinsulinism in infancy: from basic science to clinical disease. Physiol Rev 84:239–275

Dyachok O, Gylfe E (2001) Store-operated influx of Ca^{2+} in pancreatic β-cells exhibits graded dependence on the filling of the endoplasmic reticulum. J Cell Sci 114:2179–2186

Dzeja PP, Terzic A (2003) Phosphotransfer networks and cellular energetics. J Exp Biol 206:2039–2047

Efendic S, Enzmann F, Nylen A, Uvnas-Wallensten K, Luft R (1979) Effect of glucose/sulfonylurea interaction on release of insulin, glucagon, and somatostatin from isolated perfused rat pancreas. Proc Natl Acad Sci USA 76:5901–5904

Eliasson L, Renstrom E, Ammala C, Berggren PO, Bertorello AM, Bokvist K, Chibalin A, Deeney JT, Flatt PR, Gabel J, Gromada J, Larsson O, Lindstrom P, Rhodes CJ, Rorsman P (1996) PKC-dependent stimulation of exocytosis by sulfonylureas in pancreatic β cells. Science 271:813–815

Eliasson L, Ma X, Renstrom E, Barg S, Berggren PO, Galvanovskis J, Gromada J, Jing X, Lundquist I, Salehi A, Sewing S, Rorsman P (2003) SUR1 regulates PKA-independent cAMP-induced granule priming in mouse pancreatic β-cells. J Gen Physiol 121:181–197

El-Kholy W, MacDonald PE, Fox JM, Bhattacharjee A, Xue T, Gao X, Zhang Y, Stieber J, Li RA, Tsushima RG, Wheeler MB (2007) Hyperpolarization-activated cyclic nucleotide-gated channels in pancreatic β-cells. Mol Endocrinol 21:753–764

Ellard S, Flanagan SE, Girard CA, Patch AM, Harries LW, Parrish A, Edghill EL, Mackay DJ, Proks P, Shimomura K, Haberland H, Carson DJ, Shield JP, Hattersley AT, Ashcroft FM (2007) Permanent neonatal diabetes caused by dominant, recessive, or compound heterozygous SUR1 mutations with opposite functional effects. Am J Hum Genet 81:375–382

Eto K, Tsubamoto Y, Terauchi Y, Sugiyama T, Kishimoto T, Takahashi N, Yamauchi N, Kubota N, Murayama S, Aizawa T, Akanuma Y, Aizawa S, Kasai H, Yazaki Y, Kadowaki T (1999) Role of NADH shuttle system in glucose-induced activation of mitochondrial metabolism and insulin secretion. Science 283:981–985

Flechtner I, de Lonlay P, Polak M (2006) Diabetes and hypoglycaemia in young children and mutations in the Kir6.2 subunit of the potassium channel: therapeutic consequences. Diabetes Metab 32:569–580

Franklin I, Gromada J, Gjinovci A, Theander S, Wollheim CB (2005) β-cell secretory products activate α-cell ATP-dependent potassium channels to inhibit glucagon release. Diabetes 54:1808–1815

Fuhlendorff J, Rorsman P, Kofod H, Brand CL, Rolin B, MacKay P, Shymko R, Carr RD (1998) Stimulation of insulin release by repaglinide and glibenclamide involves both common and distinct processes. Diabetes 47:345–351

Gaines KL, Hamilton S, Boyd AE 3rd (1988) Characterization of the sulfonylurea receptor on β cell membranes. J Biol Chem 263:2589–2592

Gaskins HR, Baldeon ME, Selassie L, Beverly JL (1995) Glucose modulates gamma-aminobutyric acid release from the pancreatic β TC6 cell line. J Biol Chem 270:30286–30289

Gerbitz KD, Gempel K, Brdiczka D (1996) Mitochondria and diabetes. Genetic, biochemical, and clinical implications of the cellular energy circuit. Diabetes 45:113–126

Gier B, Krippeit-Drews P, Sheiko T, Aguilar-Bryan L, Bryan J, Düfer M, Drews G (2009) Suppression of K_{ATP} channel activity protects murine pancreatic β cells against oxidative stress. J Clin Invest 119:3246–3256

Gill GV, Rauf O, MacFarlane IA (1997) Diazoxide treatment for insulinoma: a national UK survey. Postgrad Med J 73:640–641

Gillis KD, Gee WM, Hammoud A, McDaniel ML, Falke LC, Misler S (1989) Effects of sulfonamides on a metabolite-regulated ATPi-sensitive K^+ channel in rat pancreatic β-cells. Am J Physiol 257:C1119–C1127

Gilon P, Henquin JC (2001) Mechanisms and physiological significance of the cholinergic control of pancreatic β-cell function. Endocr Rev 22:565–604

Gilon P, Yakel J, Gromada J, Zhu Y, Henquin JC, Rorsman P (1997) G protein-dependent inhibition of L-type Ca^{2+} currents by acetylcholine in mouse pancreatic β-cells. J Physiol 499(Pt 1):65–76

Gilon P, Arredouani A, Gailly P, Gromada J, Henquin JC (1999) Uptake and release of Ca^{2+} by the endoplasmic reticulum contribute to the oscillations of the cytosolic Ca^{2+} concentration triggered by Ca^{2+} influx in the electrically excitable pancreatic β-cell. J Biol Chem 274:20197–20205

Giurgea I, Sanlaville D, Fournet JC, Sempoux C, Bellanne-Chantelot C, Touati G, Hubert L, Groos MS, Brunelle F, Rahier J, Henquin JC, Dunne MJ, Jaubert F, Robert JJ, Nihoul-Fekete C, Vekemans M, Junien C, de Lonlay P (2006) Congenital hyperinsulinism and mosaic abnormalities of the ploidy. J Med Genet 43:248–254

Gloyn AL, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G, Walker M, Levy JC, Sampson M, Halford S, McCarthy MI, Hattersley AT, Frayling TM (2003) Large-scale association studies of variants in genes encoding the pancreatic β-cell K_{ATP} channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. Diabetes 52:568–572

Gloyn AL, Pearson ER, Antcliff JF, Proks P, Bruining GJ, Slingerland AS, Howard N, Srinivasan S, Silva JM, Molnes J, Edghill EL, Frayling TM, Temple IK, Mackay D, Shield JP, Sumnik Z, van Rhijn A, Wales JK, Clark P, Gorman S, Aisenberg J, Ellard S, Njolstad PR, Ashcroft FM, Hattersley AT (2004) Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med 350:1838–1849

Gloyn AL, Reimann F, Girard C, Edghill EL, Proks P, Pearson ER, Temple IK, Mackay DJ, Shield JP, Freedenberg D, Noyes K, Ellard S, Ashcroft FM, Gribble FM, Hattersley AT (2005) Relapsing diabetes can result from moderately activating mutations in KCNJ11. Hum Mol Genet 14:925–934

Goforth PB, Bertram R, Khan FA, Zhang M, Sherman A, Satin LS (2002) Calcium-activated K^+ channels of mouse β-cells are controlled by both store and cytoplasmic Ca^{2+}: experimental and theoretical studies. J Gen Physiol 120:307–322

Göpel SO, Kanno T, Barg S, Eliasson L, Galvanovskis J, Renstrom E, Rorsman P (1999) Activation of Ca^{2+}-dependent K^+ channels contributes to rhythmic firing of action potentials in mouse pancreatic β cells. J Gen Physiol 114:759–770

Göpel SO, Kanno T, Barg S, Rorsman P (2000) Patch-clamp characterisation of somatostatin-secreting -cells in intact mouse pancreatic islets. J Physiol 528:497–507

Göpel SO, Kanno T, Barg S, Weng XG, Gromada J, Rorsman P (2000a) Regulation of glucagon release in mouse -cells by K_{ATP} channels and inactivation of TTX-sensitive Na^+ channels. J Physiol 528:509–520

Göpel SO, Kanno T, Barg S, Rorsman P (2000b) Patch-clamp characterisation of somatostatin-secreting -cells in intact mouse pancreatic islets. J Physiol 528:497–507

Gribble FM, Tucker SJ, Ashcroft FM (1997a) The essential role of the Walker A motifs of SUR1 in K-ATP channel activation by Mg-ADP and diazoxide. EMBO J 16:1145–1152

Gribble FM, Tucker SJ, Ashcroft FM (1997b) The interaction of nucleotides with the tolbutamide block of cloned ATP-sensitive K^+ channel currents expressed in Xenopus oocytes: a reinterpretation. J Physiol 504(Pt 1):35–45

Gribble FM, Tucker SJ, Haug T, Ashcroft FM (1998) MgATP activates the β cell K_{ATP} channel by interaction with its SUR1 subunit. Proc Natl Acad Sci USA 95:7185–7190

Gromada J, Dissing S, Bokvist K, Renstrom E, Frokjaer-Jensen J, Wulff BS, Rorsman P (1995) Glucagon-like peptide I increases cytoplasmic calcium in insulin-secreting β TC3-cells by enhancement of intracellular calcium mobilization. Diabetes 44:767–774

Gromada J, Bokvist K, Ding WG, Barg S, Buschard K, Renstrom E, Rorsman P (1997) Adrenaline stimulates glucagon secretion in pancreatic α-cells by increasing the Ca^{2+} current and the number of granules close to the L-type Ca^{2+} channels. J Gen Physiol 110:217–228

Gromada J, Bokvist K, Ding WG, Holst JJ, Nielsen JH, Rorsman P (1998) Glucagon-like peptide 1 (7-36) amide stimulates exocytosis in human pancreatic β-cells by both proximal and distal regulatory steps in stimulus-secretion coupling. Diabetes 47:57–65

Gromada J, Ma X, Hoy M, Bokvist K, Salehi A, Berggren PO, Rorsman P (2004) ATP-sensitive K^+ channel-dependent regulation of glucagon release and electrical activity by glucose in wild-type and $SUR1^{-/-}$ mouse α-cells. Diabetes 53(Suppl 3):S181–S189

Gromada J, Franklin I, Wollheim CB (2007) α-cells of the endocrine pancreas: 35 years of research but the enigma remains. Endocr Rev 28:84–116

Guiot Y, Stevens M, Marhfour I, Stiernet P, Mikhailov M, Ashcroft SJ, Rahier J, Henquin JC, Sempoux C (2007) Morphological localisation of sulfonylurea receptor 1 in endocrine cells of human, mouse and rat pancreas. Diabetologia 50:1889–1899

Gustafsson AJ, Ingelman-Sundberg H, Dzabic M, Awasum J, Nguyen KH, Ostenson CG, Pierro C, Tedeschi P, Woolcott O, Chiounan S, Lund PE, Larsson O, Islam MS (2005) Ryanodine receptor-operated activation of TRP-like channels can trigger critical Ca^{2+} signaling events in pancreatic β-cells. FASEB J 19:301–303

Hansen AM, Christensen IT, Hansen JB, Carr RD, Ashcroft FM, Wahl P (2002) Differential interactions of nateglinide and repaglinide on the human β-cell sulphonylurea receptor 1. Diabetes 51:2789–2795

Hansen AM, Hansen JB, Carr RD, Ashcroft FM, Wahl P (2005) Kir6.2-dependent high-affinity repaglinide binding to β-cell K_{ATP} channels. Br J Pharmacol 144:551–557

Haspel D, Krippeit-Drews P, Aguilar-Bryan L, Bryan J, Drews G, Düfer M (2005) Crosstalk between membrane potential and cytosolic Ca^{2+} concentration in β cells from $Sur1^{-/-}$ mice. Diabetologia 48:913–921

Hattersley AT, Ashcroft FM (2005) Activating mutations in Kir6.2 and neonatal diabetes: new clinical syndromes, new scientific insights, and new therapy. Diabetes 54:2503–2513

Henquin JC (1978) D-glucose inhibits potassium efflux from pancreatic islet cells. Nature 271:271–273

Henquin JC (1990) Role of voltage- and Ca^{2+}-dependent K^+ channels in the control of glucose-induced electrical activity in pancreatic β-cells. Pflugers Arch 416:568–572

Henquin JC (1998) A minimum of fuel is necessary for tolbutamide to mimic the effects of glucose on electrical activity in pancreatic β-cells. Endocrinology 139:993–998

Henquin JC (2000) Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 49:1751–1760

Henquin JC, Meissner HP (1982) Opposite effects of tolbutamide and diazoxide on $^{86}Rb^+$ fluxes and membrane potential in pancreatic B cells. Biochem Pharmacol 31:1407–1415

Henquin JC, Sempoux C, Marchandise J, Godecharles S, Guiot Y, Nenquin M, Rahier J (2013) Congenital hyperinsulinism caused by hexokinase I expression or glucokinase-activating mutation in a subset of β-cells. Diabetes 62:1689–1696

Herrington J (2007) Gating modifier peptides as probes of pancreatic β-cell physiology. Toxicon 49:231–238

Herrington J, Sanchez M, Wunderler D, Yan L, Bugianesi RM, Dick IE, Clark SA, Brochu RM, Priest BT, Kohler MG, McManus OB (2005) Biophysical and pharmacological properties of the voltage-gated potassium current of human pancreatic β-cells. J Physiol 567:159–175

Herrington J, Zhou YP, Bugianesi RM, Dulski PM, Feng Y, Warren VA, Smith MM, Kohler MG, Garsky VM, Sanchez M, Wagner M, Raphaelli K, Banerjee P, Ahaghotu C, Wunderler D, Priest BT, Mehl JT, Garcia ML, McManus OB, Kaczorowski GJ, Slaughter RS (2006) Blockers of the delayed-rectifier potassium current in pancreatic β-cells enhance glucose-dependent insulin secretion. Diabetes 55:1034–1042

Hiriart M, Matteson DR (1988) Na channels and two types of Ca channels in rat pancreatic B cells identified with the reverse hemolytic plaque assay. J Gen Physiol 91:617–639

Hjortoe GM, Hagel GM, Terry BR, Thastrup O, Arkhammar PO (2004) Functional identification and monitoring of individual α and β cells in cultured mouse islets of Langerhans. Acta Diabetol 41:185–193

Holz GG, Kuhtreiber WM, Habener JF (1993) Pancreatic β-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). Nature 361:362–365

Homaidan FR, Sharp GW, Nowak LM (1991) Galanin inhibits a dihydropyridine-sensitive Ca^{2+} current in the RINm5f cell line. Proc Natl Acad Sci USA 88:8744–8748

Hoppa MB, Collins S, Ramracheya R, Hodson L, Amisten S, Zhang Q, Johnson P, Ashcroft FM, Rorsman P (2009) Chronic palmitate exposure inhibits insulin secretion by dissociation of Ca^{2+} channels from secretory granules. Cell Metab 10:455–465

Horvath A, Szabadkai G, Varnai P, Aranyi T, Wollheim CB, Spat A, Enyedi P (1998) Voltage dependent calcium channels in adrenal glomerulosa cells and in insulin producing cells. Cell Calcium 23:33–42

Houamed KM, Sweet IR, Satin LS (2010) BK channels mediate a novel ionic mechanism that regulates glucose-dependent electrical activity and insulin secretion in mouse pancreatic β-cells. J Physiol 588:3511–3523

Hsu WH, Xiang HD, Rajan AS, Boyd AE 3rd (1991a) Activation of α 2-adrenergic receptors decreases Ca^{2+} influx to inhibit insulin secretion in a hamster β-cell line: an action mediated by a guanosine triphosphate-binding protein. Endocrinology 128:958–964

Hsu WH, Xiang HD, Rajan AS, Kunze DL, Boyd AE 3rd (1991b) Somatostatin inhibits insulin secretion by a G-protein-mediated decrease in Ca^{2+} entry through voltage-dependent Ca^{2+} channels in the β cell. J Biol Chem 266:837–843

Huopio H, Reimann F, Ashfield R, Komulainen J, Lenko HL, Rahier J, Vauhkonen I, Kere J, Laakso M, Ashcroft F, Otonkoski T (2000) Dominantly inherited hyperinsulinism caused by a mutation in the sulfonylurea receptor type 1. J Clin Invest 106:897–906

Inagaki N, Gonoi T, Clement JP, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J (1995) Reconstitution of IK_{ATP}: an inward rectifier subunit plus the sulfonylurea receptor. Science 270:1166–1170

Inagaki N, Gonoi T, Seino S (1997) Subunit stoichiometry of the pancreatic β-cell ATP-sensitive K^+ channel. FEBS Lett 409:232–236

Ishii TM, Silvia C, Hirschberg B, Bond CT, Adelman JP, Maylie J (1997) A human intermediate conductance calcium-activated potassium channel. Proc Natl Acad Sci USA 94:11651–11656

Islam MS (2011) TRP channels of islets. Adv Exp Med Biol 704:811–830

Islam MS, Berggren PO, Larsson O (1993) Sulfhydryl oxidation induces rapid and reversible closure of the ATP-regulated K^+ channel in the pancreatic β-cell. FEBS Lett 319:128–132

Ismail D, Smith VV, de Lonlay P, Ribeiro MJ, Rahier J, Blankenstein O, Flanagan SE, Bellanne-Chantelot C, Verkarre V, Aigrain Y, Pierro A, Ellard S, Hussain K (2011) Familial focal congenital hyperinsulinism. J Clin Endocrinol Metab 96:24–28

Iwashima Y, Pugh W, Depaoli AM, Takeda J, Seino S, Bell GI, Polonsky KS (1993) Expression of calcium channel mRNAs in rat pancreatic islets and downregulation after glucose infusion. Diabetes 42:948–955

Jacobson DA, Philipson LH (2007a) Action potentials and insulin secretion: new insights into the role of K_v channels. Diabetes Obes Metab 9(Suppl 2):89–98

Jacobson DA, Philipson LH (2007b) TRP channels of the pancreatic β cell. Handb Exp Pharmacol 409–424

Jacobson DA, Weber CR, Bao S, Turk J, Philipson LH (2007a) Modulation of the pancreatic islet β-cell-delayed rectifier potassium channel $K_v2.1$ by the polyunsaturated fatty acid arachidonate. J Biol Chem 282:7442–7449

Jacobson DA, Kuznetsov A, Lopez JP, Kash S, Ammala CE, Philipson LH (2007b) $K_v2.1$ ablation alters glucose-induced islet electrical activity, enhancing insulin secretion. Cell Metab 6:229–235

Jacobson DA, Mendez F, Thompson M, Torres J, Cochet O, Philipson LH (2010) Calcium-activated and voltage-gated potassium channels of the pancreatic islet impart distinct and complementary roles during secretagogue induced electrical responses. J Physiol 588:3525–3537

Jakab M, Grundbichler M, Benicky J, Ravasio A, Chwatal S, Schmidt S, Strbak V, Furst J, Paulmichl M, Ritter M (2006) Glucose induces anion conductance and cytosol-to-membrane transposition of ICln in INS-1E rat insulinoma cells. Cell Physiol Biochem 18:21–34

Jensen BS, Strobaek D, Christophersen P, Jorgensen TD, Hansen C, Silahtaroglu A, Olesen SP, Ahring PK (1998) Characterization of the cloned human intermediate-conductance Ca^{2+}-activated K^+ channel. Am J Physiol 275:C848–C856

Ji J, Yang SN, Huang X, Li X, Sheu L, Diamant N, Berggren PO, Gaisano HY (2002) Modulation of L-type Ca^{2+} channels by distinct domains within SNAP-25. Diabetes 51:1425–1436

Jing X, Li DQ, Olofsson CS, Salehi A, Surve VV, Caballero J, Ivarsson R, Lundquist I, Pereverzev A, Schneider T, Rorsman P, Renstrom E (2005) $Ca_v2.3$ calcium channels control second-phase insulin release. J Clin Invest 115:146–154

Johnson JH, Newgard CB, Milburn JL, Lodish HF, Thorens B (1990) The high Km glucose transporter of islets of Langerhans is functionally similar to the low affinity transporter of liver and has an identical primary sequence. J Biol Chem 265:6548–6551

Kang G, Leech CA, Chepurny OG, Coetzee WA, Holz GG (2008) Role of the cAMP sensor Epac as a determinant of K_{ATP} channel ATP sensitivity in human pancreatic β-cells and rat INS-1 cells. J Physiol 586:1307–1319

Kanno T, Rorsman P, Gopel SO (2002a) Glucose-dependent regulation of rhythmic action potential firing in pancreatic β-cells by K_{ATP}-channel modulation. J Physiol 545:501–507

Kanno T, Gopel SO, Rorsman P, Wakui M (2002b) Cellular function in multicellular system for hormone-secretion: electrophysiological aspect of studies on α-, β-and δ-cells of the pancreatic islet. Neurosci Res 42:79–90

Kato M, Ma HT, Tatemoto K (1996) GLP-1 depolarizes the rat pancreatic β cell in a Na^+-dependent manner. Regul Pept 62:23–27

Kennedy ED, Wollheim CB (1998) Role of mitochondrial calcium in metabolism-secretion coupling in nutrient-stimulated insulin release. Diabetes Metab 24:15–24

Kennedy RT, Kauri LM, Dahlgren GM, Jung SK (2002) Metabolic oscillations in β-cells. Diabetes 51(Suppl 1):S152–S161

Kerschensteiner D, Stocker M (1999) Heteromeric assembly of $K_v2.1$ with $K_v9.3$: effect on the state dependence of inactivation. Biophys J 77:248–257

Khan FA, Goforth PB, Zhang M, Satin LS (2001) Insulin activates ATP-sensitive K^+ channels in pancreatic β-cells through a phosphatidylinositol 3-kinase-dependent pathway. Diabetes 50:2192–2198

Kim SJ, Choi WS, Han JS, Warnock G, Fedida D, McIntosh CH (2005) A novel mechanism for the suppression of a voltage-gated potassium channel by glucose-dependent insulinotropic polypeptide: protein kinase A-dependent endocytosis. J Biol Chem 280:28692–28700

Kim SJ, Widenmaier SB, Choi WS, Nian C, Ao Z, Warnock G, McIntosh CH (2012) Pancreatic β-cell prosurvival effects of the incretin hormones involve post-translational modification of $K_v2.1$ delayed rectifier channels. Cell Death Differ 19:333–344

Kinard TA, Satin LS (1995) An ATP-sensitive Cl^- channel current that is activated by cell swelling, cAMP, and glyburide in insulin-secreting cells. Diabetes 44:1461–1466

Kindmark H, Kohler M, Brown G, Branstrom R, Larsson O, Berggren PO (2001) Glucose-induced oscillations in cytoplasmic free Ca^{2+} concentration precede oscillations in mitochondrial membrane potential in the pancreatic β-cell. J Biol Chem 276:34530–34536

Klein A, Lichtenberg J, Stephan D, Quast U (2005) Lipids modulate ligand binding to sulphonylurea receptors. Br J Pharmacol 145:907–915

Klose C, Straub I, Riehle M, Ranta F, Krautwurst D, Ullrich S, Meyerhof W, Harteneck C (2011) Fenamates as TRP channel blockers: mefenamic acid selectively blocks TRPM3. Br J Pharmacol 162:1757–1769

Klupa T, Kowalska I, Wyka K, Skupien J, Patch AM, Flanagan SE, Noczynska A, Arciszewska M, Ellard S, Hattersley AT, Sieradzki J, Mlynarski W, Malecki MT (2008) Mutations in the ABCC8 gene are associated with a variable clinical phenotype. Clin Endocrinol 71:358–362

Komatsu M, Sato Y, Aizawa T, Hashizume K (2001) K_{ATP} channel-independent glucose action: an elusive pathway in stimulus-secretion coupling of pancreatic β-cell. Endocr J 48:275–288

Koster JC, Sha Q, Nichols CG (1999) Sulfonylurea and K^+-channel opener sensitivity of K_{ATP} channels. Functional coupling of Kir6.2 and SUR1 subunits. J Gen Physiol 114:203–213

Kozak JA, Misler S, Logothetis DE (1998) Characterization of a Ca^{2+}-activated K^+ current in insulin-secreting murine βTC-3 cells. J Physiol 509(Pt 2):355–370

Kozlowski RZ, Hales CN, Ashford ML (1989) Dual effects of diazoxide on ATP-K^+ currents recorded from an insulin-secreting cell line. Br J Pharmacol 97:1039–1050

Krauter T, Ruppersberg JP, Baukrowitz T (2001) Phospholipids as modulators of K_{ATP} channels: distinct mechanisms for control of sensitivity to sulphonylureas, K^+ channel openers, and ATP. Mol Pharmacol 59:1086–1093

Krippeit-Drews P, Lang F, Haussinger D, Drews G (1994) H_2O_2 induced hyperpolarization of pancreatic β-cells. Pflugers Arch 426:552–554

Krippeit-Drews P, Kröncke KD, Welker S, Zempel G, Roenfeldt M, Ammon HP, Lang F, Drews G (1995a) The effects of nitric oxide on the membrane potential and ionic currents of mouse pancreatic B cells. Endocrinology 136:5363–5369

Krippeit-Drews P, Zempel G, Ammon HP, Lang F, Drews G (1995b) Effects of membrane-permeant and -impermeant thiol reagents on Ca^{2+} and K^+ channel currents of mouse pancreatic B cells. Endocrinology 136:464–467

Krippeit-Drews P, Krämer C, Welker S, Lang F, Ammon HP, Drews G (1999) Interference of H_2O_2 with stimulus-secretion coupling in mouse pancreatic β-cells. J Physiol 514(Pt 2):471–481

Krippeit-Drews P, Düfer M, Drews G (2000) Parallel oscillations of intracellular calcium activity and mitochondrial membrane potential in mouse pancreatic β-cells. Biochem Biophys Res Commun 267:179–183

Krippeit-Drews P, Bäcker M, Düfer M, Drews G (2003) Phosphocreatine as a determinant of K_{ATP} channel activity in pancreatic β-cells. Pflugers Arch 445:556–562

Kukuljan M, Goncalves AA, Atwater I (1991) Charybdotoxin-sensitive K_{Ca} channel is not involved in glucose-induced electrical activity in pancreatic β-cells. J Membr Biol 119:187–195

Kullin M, Li Z, Hansen JB, Bjork E, Sandler S, Karlsson FA (2000) K_{ATP} channel openers protect rat islets against the toxic effect of streptozotocin. Diabetes 49:1131–1136

Larsson O, Ammala C, Bokvist K, Fredholm B, Rorsman P (1993) Stimulation of the K_{ATP} channel by ADP and diazoxide requires nucleotide hydrolysis in mouse pancreatic β-cells. J Physiol 463:349–365

Larsson O, Deeney JT, Branstrom R, Berggren PO, Corkey BE (1996a) Activation of the ATP-sensitive K^+ channel by long chain acyl-CoA. A role in modulation of pancreatic β-cell glucose sensitivity. J Biol Chem 271:10623–10626

Larsson O, Kindmark H, Brandstrom R, Fredholm B, Berggren PO (1996b) Oscillations in K_{ATP} channel activity promote oscillations in cytoplasmic free Ca^{2+} concentration in the pancreatic β cell. Proc Natl Acad Sci USA 93:5161–5165

Larsson O, Barker CJ, Berggren PO (2000) Phosphatidylinositol 4,5-bisphosphate and ATP-sensitive potassium channel regulation: a word of caution. Diabetes 49:1409–1412

Laukkanen O, Pihlajamaki J, Lindstrom J, Eriksson J, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Tuomilehto J, Uusitupa M, Laakso M (2004) Polymorphisms of the SUR1 (ABCC8) and Kir6.2 (KCNJ11) genes predict the conversion from impaired glucose tolerance to type 2 diabetes. J Clin Endocrinol Metab 89:6286–6290, The Finnish Diabetes Prevention Study

Ledoux J, Werner ME, Brayden JE, Nelson MT (2006) Calcium-activated potassium channels and the regulation of vascular tone. Physiology (Bethesda) 21:69–78

Leech CA, Habener JF (1997) Insulinotropic glucagon-like peptide-1-mediated activation of non-selective cation currents in insulinoma cells is mimicked by maitotoxin. J Biol Chem 272:17987–17993

Leech CA, Habener JF (1998) A role for Ca^{2+}-sensitive nonselective cation channels in regulating the membrane potential of pancreatic β-cells. Diabetes 47:1066–1073

Leech CA, Dzhura I, Chepurny OG, Schwede F, Genieser HG, Holz GG (2010) Facilitation of β-cell K_{ATP} channel sulfonylurea sensitivity by a cAMP analog selective for the cAMP-regulated guanine nucleotide exchange factor Epac. Islets 2:72–81

Leibiger IB, Berggren PO (2008) Insulin signaling in the pancreatic β-cell. Annu Rev Nutr 28:233–251

Lembert N, Idahl LA (1998) α-ketoisocaproate is not a true substrate for ATP production by pancreatic β-cell mitochondria. Diabetes 47:339–344

Lenzen S (1978) Effects of α-ketocarboxylic acids and 4-pentenoic acid on insulin secretion from the perfused rat pancreas. Biochem Pharmacol 27:1321–1324

Lenzen S, Lerch M, Peckmann T, Tiedge M (2000) Differential regulation of $[Ca^{2+}]_i$ oscillations in mouse pancreatic islets by glucose, α-ketoisocaproic acid, glyceraldehyde and glycolytic intermediates. Biochim Biophys Acta 1523:65–72

Leung YM, Ahmed I, Sheu L, Tsushima RG, Diamant NE, Hara M, Gaisano HY (2005) Electrophysiological characterization of pancreatic islet cells in the mouse insulin promoter-green fluorescent protein mouse. Endocrinology 146:4766–4775

Leung YM, Ahmed I, Sheu L, Gao X, Hara M, Tsushima RG, Diamant NE, Gaisano HY (2006) Insulin regulates islet α-cell function by reducing K_{ATP} channel sensitivity to adenosine 5′-triphosphate inhibition. Endocrinology 147:2155–2162

Leung YM, Kwan EP, Ng B, Kang Y, Gaisano HY (2007) SNAREing voltage-gated K^+ and ATP-sensitive K^+ channels: tuning β-cell excitability with syntaxin-1A and other exocytotic proteins. Endocr Rev 28:653–663

Light PE, Manning Fox JE, Riedel MJ, Wheeler MB (2002) Glucagon-like peptide-1 inhibits pancreatic ATP-sensitive potassium channels via a protein kinase A- and ADP-dependent mechanism. Mol Endocrinol 16:2135–2144

Luciani DS, Misler S, Polonsky KS (2006) Ca^{2+} controls slow NAD(P)H oscillations in glucose-stimulated mouse pancreatic islets. J Physiol 572:379–392

Ludwig A, Zong X, Jeglitsch M, Hofmann F, Biel M (1998) A family of hyperpolarization-activated mammalian cation channels. Nature 393:587–591

MacDonald PE, Wheeler MB (2003) Voltage-dependent K^+ channels in pancreatic β cells: role, regulation and potential as therapeutic targets. Diabetologia 46:1046–1062

MacDonald PE, Ha XF, Wang J, Smukler SR, Sun AM, Gaisano HY, Salapatek AM, Backx PH, Wheeler MB (2001) Members of the K_v1 and K_v2 voltage-dependent K^+ channel families regulate insulin secretion. Mol Endocrinol 15:1423–1435

MacDonald PE, Sewing S, Wang J, Joseph JW, Smukler SR, Sakellaropoulos G, Saleh MC, Chan CB, Tsushima RG, Salapatek AM, Wheeler MB (2002a) Inhibition of $K_v2.1$ voltage-dependent K^+ channels in pancreatic β-cells enhances glucose-dependent insulin secretion. J Biol Chem 277:44938–44945

MacDonald PE, Salapatek AM, Wheeler MB (2002b) Glucagon-like peptide-1 receptor activation antagonizes voltage-dependent repolarizing K^+ currents in β-cells: a possible glucose-dependent insulinotropic mechanism. Diabetes 51(Suppl 3):S443–S447

MacDonald PE, De Marinis YZ, Ramracheya R, Salehi A, Ma X, Johnson PR, Cox R, Eliasson L, Rorsman P (2007) A K ATP channel-dependent pathway within α cells regulates glucagon release from both rodent and human islets of Langerhans. PLoS Biol 5:e143

Maedler K, Storling J, Sturis J, Zuellig RA, Spinas GA, Arkhammar PO, Mandrup-Poulsen T, Donath MY (2004) Glucose- and interleukin-1β-induced β-cell apoptosis requires Ca^{2+} influx and extracellular signal-regulated kinase (ERK) 1/2 activation and is prevented by a sulfonylurea receptor 1/inwardly rectifying K^+ channel 6.2 (SUR/Kir6.2) selective potassium channel opener in human islets. Diabetes 53:1706–1713

Magnus G, Keizer J (1998) Model of β-cell mitochondrial calcium handling and electrical activity. I cytoplasmic variables. Am J Physiol 274:C1158–C1173

Manning Fox JE, Gyulkhandanyan AV, Satin LS, Wheeler MB (2006) Oscillatory membrane potential response to glucose in islet β-cells: a comparison of islet-cell electrical activity in mouse and rat. Endocrinology 147:4655–4663

Matsuda Y, Saegusa H, Zong S, Noda T, Tanabe T (2001) Mice lacking $Ca_v2.3$ (α1E) calcium channel exhibit hyperglycemia. Biochem Biophys Res Commun 289:791–795

Matsuo M, Kimura Y, Ueda K (2005) K_{ATP} channel interaction with adenine nucleotides. J Mol Cell Cardiol 38:907–916

Matsuoka T, Matsushita K, Katayama Y, Fujita A, Inageda K, Tanemoto M, Inanobe A, Yamashita S, Matsuzawa Y, Kurachi Y (2000) C-terminal tails of sulfonylurea receptors control ADP-induced activation and diazoxide modulation of ATP-sensitive K^+ channels. Circ Res 87:873–880

Mears D (2004) Regulation of insulin secretion in islets of Langerhans by Ca^{2+} channels. J Membr Biol 200:57–66

Mears D, Zimliki CL (2004) Muscarinic agonists activate Ca^{2+} store-operated and -independent ionic currents in insulin-secreting HIT-T15 cells and mouse pancreatic β-cells. J Membr Biol 197:59–70

Meissner HP, Henquin JC, Preissler M (1978) Potassium dependence of the membrane potential of pancreatic β-cells. FEBS Lett 94:87–89

Mikhailov MV, Proks P, Ashcroft FM, Ashcroft SJ (1998) Expression of functionally active ATP-sensitive K-channels in insect cells using baculovirus. FEBS Lett 429:390–394

Mikhailov MV, Mikhailova EA, Ashcroft SJ (2001) Molecular structure of the glibenclamide binding site of the β-cell K_{ATP} channel. FEBS Lett 499:154–160

Mikhailov MV, Campbell JD, de Wet H, Shimomura K, Zadek B, Collins RF, Sansom MS, Ford RC, Ashcroft FM (2005) 3-D structural and functional characterization of the purified K_{ATP} channel complex Kir6.2-SUR1. EMBO J 24:4166–4175

Miki T, Nagashima K, Tashiro F, Kotake K, Yoshitomi H, Tamamoto A, Gonoi T, Iwanaga T, Miyazaki J, Seino S (1998) Defective insulin secretion and enhanced insulin action in K_{ATP} channel-deficient mice. Proc Natl Acad Sci USA 95:10402–10406

Miley HE, Brown PD, Best L (1999) Regulation of a volume-sensitive anion channel in rat pancreatic β-cells by intracellular adenine nucleotides. J Physiol 515(Pt 2):413–417

Misler S, Falke LC, Gillis K, McDaniel ML (1986) A metabolite-regulated potassium channel in rat pancreatic B cells. Proc Natl Acad Sci USA 83:7119–7123

Misler S, Barnett DW, Gillis KD, Pressel DM (1992) Electrophysiology of stimulus-secretion coupling in human β-cells. Diabetes 41:1221–1228

Miura Y, Matsui H (2003) Glucagon-like peptide-1 induces a cAMP-dependent increase of $[Na^+]_i$ associated with insulin secretion in pancreatic β-cells. Am J Physiol Endocrinol Metab 285:E1001–E1009

Miura Y, Gilon P, Henquin JC (1996) Muscarinic stimulation increases Na^+ entry in pancreatic β-cells by a mechanism other than the emptying of intracellular Ca^{2+} pools. Biochem Biophys Res Commun 224:67–73

Miura Y, Henquin JC, Gilon P (1997) Emptying of intracellular Ca^{2+} stores stimulates Ca^{2+} entry in mouse pancreatic β-cells by both direct and indirect mechanisms. J Physiol 503(Pt 2):387–398

Moreau C, Prost AL, Derand R, Vivaudou M (2005) SUR, ABC proteins targeted by K_{ATP} channel openers. J Mol Cell Cardiol 38:951–963

Muller YL, Hanson RL, Zimmerman C, Harper I, Sutherland J, Kobes S, International Type 2 Diabetes 1q Consortium, Knowler WC, Bogardus C, Baier LJ (2007) Variants in the Ca V 2.3 (α 1E) subunit of voltage-activated Ca^{2+} channels are associated with insulin resistance and type 2 diabetes in Pima Indians. Diabetes 56:3089–3094

Nadal A, Quesada I, Soria B (1999) Homologous and heterologous asynchronicity between identified α-, β- and δ-cells within intact islets of Langerhans in the mouse. J Physiol 517 (Pt 1):85–93

Nakazaki M, Kakei M, Koriyama N, Tanaka H (1995) Involvement of ATP-sensitive K^+ channels in free radical-mediated inhibition of insulin secretion in rat pancreatic β-cells. Diabetes 44:878–883

Nakazaki M, Crane A, Hu M, Seghers V, Ullrich S, Aguilar-Bryan L, Bryan J (2002) cAMP-activated protein kinase-independent potentiation of insulin secretion by cAMP is impaired in SUR1 null islets. Diabetes 51:3440–3449

Namkung Y, Skrypnyk N, Jeong MJ, Lee T, Lee MS, Kim HL, Chin H, Suh PG, Kim SS, Shin HS (2001) Requirement for the L-type Ca^{2+} channel α(1D) subunit in postnatal pancreatic β cell generation. J Clin Invest 108:1015–1022

Nielsen EM, Hansen L, Carstensen B, Echwald SM, Drivsholm T, Glumer C, Thorsteinsson B, Borch-Johnsen K, Hansen T, Pedersen O (2003) The E23K variant of Kir6.2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. Diabetes 52:573–577

Niki I, Ashcroft FM, Ashcroft SJ (1989) The dependence on intracellular ATP concentration of ATP-sensitive K-channels and of Na, K-ATPase in intact HIT-T15 β-cells. FEBS Lett 257:361–364

Nilsson T, Arkhammar P, Rorsman P, Berggren PO (1989) Suppression of insulin release by galanin and somatostatin is mediated by a G-protein. An effect involving repolarization and reduction in cytoplasmic free Ca^{2+} concentration. J Biol Chem 264:973–980

Nitert MD, Nagorny CL, Wendt A, Eliasson L, Mulder H (2008) $Ca_v1.2$ rather than $Ca_v1.3$ is coupled to glucose-stimulated insulin secretion in INS-1 832/13 cells. J Mol Endocrinol 41:1–11

Noma A, Yanagihara K, Irisawa H (1977) Inward current of the rabbit sinoatrial node cell. Pflugers Arch 372:43–51

Olsen HL, Theander S, Bokvist K, Buschard K, Wollheim CB, Gromada J (2005) Glucose stimulates glucagon release in single rat α-cells by mechanisms that mirror the stimulus-secretion coupling in β-cells. Endocrinology 146:4861–4870

Ortiz D, Voyvodic P, Gossack L, Quast U, Bryan J (2012) Two neonatal diabetes mutations on transmembrane helix 15 of SUR1 increase affinity for ATP and ADP at nucleotide binding domain 2. J Biol Chem 287:17985–17995

Ortiz D, Gossack L, Quast U, Bryan J (2013) Reinterpreting the action of ATP analogs on K_{ATP} channels. J Biol Chem 288:18894–18902

Ozaki N, Shibasaki T, Kashima Y, Miki T, Takahashi K, Ueno H, Sunaga Y, Yano H, Matsuura Y, Iwanaga T, Takai Y, Seino S (2000) cAMP-GEFII is a direct target of cAMP in regulated exocytosis. Nat Cell Biol 2:805–811

Patch AM, Flanagan SE, Boustred C, Hattersley AT, Ellard S (2007) Mutations in the ABCC8 gene encoding the SUR1 subunit of the K_{ATP} channel cause transient neonatal diabetes, permanent neonatal diabetes or permanent diabetes diagnosed outside the neonatal period. Diabetes Obes Metab 9(Suppl 2):28–39

Pearson ER, Flechtner I, Njolstad PR, Malecki MT, Flanagan SE, Larkin B, Ashcroft FM, Klimes I, Codner E, Iotova V, Slingerland AS, Shield J, Robert JJ, Holst JJ, Clark PM, Ellard S, Sovik O, Polak M, Hattersley AT (2006) Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. N Engl J Med 355:467–477

Pereverzev A, Mikhna M, Vajna R, Gissel C, Henry M, Weiergraber M, Hescheler J, Smyth N, Schneider T (2002) Disturbances in glucose-tolerance, insulin release, and stress-induced hyperglycemia upon disruption of the $Ca_v2.3$ (α 1E) subunit of voltage-gated Ca^{2+} channels. Mol Endocrinol 16:884–895

Persaud SJ, Asare-Anane H, Jones PM (2002) Insulin receptor activation inhibits insulin secretion from human islets of Langerhans. FEBS Lett 510:225–228

Philipson LH, Hice RE, Schaefer K, LaMendola J, Bell GI, Nelson DJ, Steiner DF (1991) Sequence and functional expression in *Xenopus* oocytes of a human insulinoma and islet potassium channel. Proc Natl Acad Sci USA 88:53–57

Plant TD (1988a) Properties and calcium-dependent inactivation of calcium currents in cultured mouse pancreatic β-cells. J Physiol 404:731–747

Plant TD (1988b) Na^+ currents in cultured mouse pancreatic β-cells. Pflugers Arch 411:429–435

Platzer J, Engel J, Schrott-Fischer A, Stephan K, Bova S, Chen H, Zheng H, Striessnig J (2000) Congenital deafness and sinoatrial node dysfunction in mice lacking class D L-type Ca^{2+} channels. Cell 102:89–97

Prawitt D, Monteilh-Zoller MK, Brixel L, Spangenberg C, Zabel B, Fleig A, Penner R (2003) TRPM5 is a transient Ca^{2+}-activated cation channel responding to rapid changes in $[Ca^{2+}]_i$. Proc Natl Acad Sci USA 100:15166–15171

Pressel DM, Misler S (1990) Sodium channels contribute to action potential generation in canine and human pancreatic islet B cells. J Membr Biol 116:273–280

Pressel DM, Misler S (1991) Role of voltage-dependent ionic currents in coupling glucose stimulation to insulin secretion in canine pancreatic islet β-cells. J Membr Biol 124:239–253

Proks P, Antcliff JF, Lippiat J, Gloyn AL, Hattersley AT, Ashcroft FM (2004) Molecular basis of Kir6.2 mutations associated with neonatal diabetes or neonatal diabetes plus neurological features. Proc Natl Acad Sci USA 101:17539–17544

Proks P, de Wet H, Ashcroft FM (2010) Activation of the K_{ATP} channel by Mg-nucleotide interaction with SUR1. J Gen Physiol 136:389–405

Qian F, Huang P, Ma L, Kuznetsov A, Tamarina N, Philipson LH (2002) TRP genes: candidates for nonselective cation channels and store-operated channels in insulin-secreting cells. Diabetes 51(Suppl 1):S183–S189

Quesada I, Nadal A, Soria B (1999) Different effects of tolbutamide and diazoxide in α, β-, and δ-cells within intact islets of Langerhans. Diabetes 48:2390–2397

Quoix N, Cheng-Xue R, Mattart L, Zeinoun Z, Guiot Y, Beauvois MC, Henquin JC, Gilon P (2009) Glucose and pharmacological modulators of ATP-sensitive K^+ channels control $[Ca^{2+}]_c$ by different mechanisms in isolated mouse α-cells. Diabetes 58:412–421

Rafiq M, Flanagan SE, Patch AM, Shields BM, Ellard S, Hattersley AT (2008) Effective treatment with oral sulfonylureas in patients with diabetes due to sulfonylurea receptor 1 (SUR1) mutations. Diabetes Care 31:204–209

Rajan AS, Aguilar-Bryan L, Nelson DA, Nichols CG, Wechsler SW, Lechago J, Bryan J (1993) Sulfonylurea receptors and ATP-sensitive K^+ channels in clonal pancreatic α cells. Evidence for two high affinity sulfonylurea receptors. J Biol Chem 268:15221–15228

Rapedius M, Soom M, Shumilina E, Schulze D, Schonherr R, Kirsch C, Lang F, Tucker SJ, Baukrowitz T (2005) Long chain CoA esters as competitive antagonists of phosphatidylinositol 4,5-bisphosphate activation in Kir channels. J Biol Chem 280:30760–30767

Ravier MA, Rutter GA (2005) Glucose or insulin, but not zinc ions, inhibit glucagon secretion from mouse pancreatic α-cells. Diabetes 54:1789–1797

Ravier MA, Nenquin M, Miki T, Seino S, Henquin JC (2009) Glucose controls cytosolic Ca^{2+} and insulin secretion in mouse islets lacking adenosine triphosphate-sensitive K^+ channels owing to a knockout of the pore-forming subunit Kir6.2. Endocrinology 150:33–45

Reimann F, Ryder TJ, Tucker SJ, Ashcroft FM (1999a) The role of lysine 185 in the kir6.2 subunit of the ATP-sensitive channel in channel inhibition by ATP. J Physiol 520 (Pt 3):661–669

Reimann F, Tucker SJ, Proks P, Ashcroft FM (1999b) Involvement of the n-terminus of Kir6.2 in coupling to the sulphonylurea receptor. J Physiol 518(Pt 2):325–336

Reimann F, Huopio H, Dabrowski M, Proks P, Gribble FM, Laakso M, Otonkoski T, Ashcroft FM (2003) Characterisation of new K_{ATP}-channel mutations associated with congenital hyperinsulinism in the Finnish population. Diabetologia 46:241–249

Reinbothe TM, Alkayyali S, Ahlqvist E, Tuomi T, Isomaa B, Lyssenko V, Renstrom E (2013) The human L-type calcium channel $Ca_v1.3$ regulates insulin release and polymorphisms in CACNA1D associate with type 2 diabetes. Diabetologia 56:340–349

Ribalet B, Beigelman PM (1980) Calcium action potentials and potassium permeability activation in pancreatic β-cells. Am J Physiol 239:C124–C133

Ribalet B, Eddlestone GT, Ciani S (1988) Metabolic regulation of the K_{ATP} and a maxi-K(V) channel in the insulin-secreting RINm5F cell. J Gen Physiol 92:219–237

Riedel MJ, Boora P, Steckley D, de Vries G, Light PE (2003) Kir6.2 polymorphisms sensitize β-cell ATP-sensitive potassium channels to activation by acyl CoAs: a possible cellular mechanism for increased susceptibility to type 2 diabetes? Diabetes 52:2630–2635

Riedel MJ, Steckley DC, Light PE (2005) Current status of the E23K Kir6.2 polymorphism: implications for type-2 diabetes. Hum Genet 116:133–145

Robinson RB, Siegelbaum SA (2003) Hyperpolarization-activated cation currents: from molecules to physiological function. Annu Rev Physiol 65:453–480

Roe MW, Worley JF 3rd, Mittal AA, Kuznetsov A, DasGupta S, Mertz RJ, Witherspoon SM 3rd, Blair N, Lancaster ME, Mclntyre MS, Shehee WR, Dukes ID, Philipson LH (1996) Expression and function of pancreatic β-cell delayed rectifier K^+ channels. Role in stimulus-secretion coupling. J Biol Chem 271:32241–32246

Roe MW, Worley JF 3rd, Qian F, Tamarina N, Mittal AA, Dralyuk F, Blair NT, Mertz RJ, Philipson LH, Dukes ID (1998) Characterization of a Ca^{2+} release-activated nonselective cation current regulating membrane potential and $[Ca^{2+}]_i$ oscillations in transgenically derived β-cells. J Biol Chem 273:10402–10410

Rolland JF, Henquin JC, Gilon P (2002a) Feedback control of the ATP-sensitive K^+ current by cytosolic Ca^{2+} contributes to oscillations of the membrane potential in pancreatic β-cells. Diabetes 51:376–384

Rolland JF, Henquin JC, Gilon P (2002b) G protein-independent activation of an inward Na^+ current by muscarinic receptors in mouse pancreatic β-cells. J Biol Chem 277:38373–38380

Ronner P, Matschinsky FM, Hang TL, Epstein AJ, Buettger C (1993) Sulfonylurea-binding sites and ATP-sensitive K^+ channels in α-TC glucagonoma and β-TC insulinoma cells. Diabetes 42:1760–1772

Rorsman P, Hellman B (1988) Voltage-activated currents in guinea pig pancreatic α 2 cells. Evidence for Ca^{2+}-dependent action potentials. J Gen Physiol 91:223–242

Rorsman P, Trube G (1985) Glucose dependent K^+-channels in pancreatic β-cells are regulated by intracellular ATP. Pflugers Arch 405:305–309

Rorsman P, Trube G (1986) Calcium and delayed potassium currents in mouse pancreatic β-cells under voltage-clamp conditions. J Physiol 374:531–550

Rorsman P, Ashcroft FM, Trube G (1988) Single Ca channel currents in mouse pancreatic β-cells. Pflugers Arch 412:597–603

Rorsman P, Berggren PO, Bokvist K, Ericson H, Mohler H, Ostenson CG, Smith PA (1989) Glucose-inhibition of glucagon secretion involves activation of GABAA-receptor chloride channels. Nature 341:233–236

Rorsman P, Bokvist K, Ammala C, Arkhammar P, Berggren PO, Larsson O, Wahlander K (1991) Activation by adrenaline of a low-conductance G protein-dependent K^+ channel in mouse pancreatic B cells. Nature 349:77–79

Rosario LM, Barbosa RM, Antunes CM, Silva AM, Abrunhosa AJ, Santos RM (1993) Bursting electrical activity in pancreatic β-cells: evidence that the channel underlying the burst is sensitive to Ca^{2+} influx through L-type Ca^{2+} channels. Pflugers Arch 424:439–447

Rutter GA, Tsuboi T, Ravier MA (2006) Ca^{2+} microdomains and the control of insulin secretion. Cell Calcium 40:539–551

Sakura H, Ashcroft FM (1997) Identification of four *trp1* gene variants murine pancreatic β-cells. Diabetologia 40:528–532

Sandler S, Andersson AK, Larsson J, Makeeva N, Olsen T, Arkhammar PO, Hansen JB, Karlsson FA, Welsh N (2008) Possible role of an ischemic preconditioning-like response mechanism in K_{ATP} channel opener-mediated protection against streptozotocin-induced suppression of rat pancreatic islet function. Biochem Pharmacol 76:1748–1756

Sano Y, Mochizuki S, Miyake A, Kitada C, Inamura K, Yokoi H, Nozawa K, Matsushime H, Furuichi K (2002) Molecular cloning and characterization of $K_v6.3$, a novel modulatory subunit for voltage-gated K^+ channel $K_v2.1$. FEBS Lett 512:230–234

Santoro B, Liu DT, Yao H, Bartsch D, Kandel ER, Siegelbaum SA, Tibbs GR (1998) Identification of a gene encoding a hyperpolarization-activated pacemaker channel of brain. Cell 93:717–729

Satin LS, Cook DL (1989) Calcium current inactivation in insulin-secreting cells is mediated by calcium influx and membrane depolarization. Pflugers Arch 414:1–10

Satin LS, Hopkins WF, Fatherazi S, Cook DL (1989) Expression of a rapid, low-voltage threshold K current in insulin-secreting cells is dependent on intracellular calcium buffering. J Membr Biol 112:213–222

Satin LS, Tavalin SJ, Kinard TA, Teague J (1995) Contribution of L- and non-L-type calcium channels to voltage-gated calcium current and glucose-dependent insulin secretion in HIT-T15 cells. Endocrinology 136:4589–4601

Sato Y, Anello M, Henquin JC (1999) Glucose regulation of insulin secretion independent of the opening or closure of adenosine triphosphate-sensitive K^+ channels in β cells. Endocrinology 140:2252–2257

Schmid-Antomarchi H, de Weille J, Fosset M, Lazdunski M (1987) The antidiabetic sulfonylurea glibenclamide is a potent blocker of the ATP-modulated K^+ channel in insulin secreting cells. Biochem Biophys Res Commun 146:21–25

Schmidt J, Liebscher K, Merten N, Grundmann M, Mielenz M, Sauerwein H, Christiansen E, Due-Hansen ME, Ulven T, Ullrich S, Gomeza J, Drewke C, Kostenis E (2011) Conjugated linoleic acids mediate insulin release through islet G protein-coupled receptor FFA1/GPR40. J Biol Chem 286:11890–11894

Schuit FC, Derde MP, Pipeleers DG (1989) Sensitivity of rat pancreatic A and B cells to somatostatin. Diabetologia 32:207–212

Schulla V, Renstrom E, Feil R, Feil S, Franklin I, Gjinovci A, Jing XJ, Laux D, Lundquist I, Magnuson MA, Obermuller S, Olofsson CS, Salehi A, Wendt A, Klugbauer N, Wollheim CB, Rorsman P, Hofmann F (2003) Impaired insulin secretion and glucose tolerance in β cell-selective $Ca_v1.2$ Ca^{2+} channel null mice. EMBO J 22:3844–3854

Schulze D, Rapedius M, Krauter T, Baukrowitz T (2003) Long-chain acyl-CoA esters and phosphatidylinositol phosphates modulate ATP inhibition of K_{ATP} channels by the same mechanism. J Physiol 552:357–367

Schulze DU, Düfer M, Wieringa B, Krippeit-Drews P, Drews G (2007) An adenylate kinase is involved in K_{ATP} channel regulation of mouse pancreatic β cells. Diabetologia 50:2126–2134

Schwanstecher C, Meyer U, Schwanstecher M (2002) K(IR)6.2 polymorphism predisposes to type 2 diabetes by inducing overactivity of pancreatic β-cell ATP- sensitive K^+ channels. Diabetes 51:875–879

Seghers V, Nakazaki M, DeMayo F, Aguilar-Bryan L, Bryan J (2000) Sur1 knockout mice. A model for K_{ATP} channel-independent regulation of insulin secretion. J Biol Chem 275:9270–9277

Sehlin J, Taljedal IB (1975) Glucose-induced decrease in Rb^+ permeability in pancreatic β cells. Nature 253:635–636

Seifert R, Scholten A, Gauss R, Mincheva A, Lichter P, Kaupp UB (1999) Molecular characterization of a slowly gating human hyperpolarization-activated channel predominantly expressed in thalamus, heart, and testis. Proc Natl Acad Sci USA 96:9391–9396

Seino S (2012) Cell signalling in insulin secretion: the molecular targets of ATP, cAMP and sulfonylurea. Diabetologia 55:2096–2108

Seino S, Chen L, Seino M, Blondel O, Takeda J, Johnson JH, Bell GI (1992) Cloning of the α 1 subunit of a voltage-dependent calcium channel expressed in pancreatic β cells. Proc Natl Acad Sci USA 89:584–588

Sener A, Kawazu S, Hutton JC, Boschero AC, Devis G, Somers G, Herchuelz A, Malaisse WJ (1978) The stimulus-secretion coupling of glucose-induced insulin release. Effect of exogenous pyruvate on islet function. Biochem J 176:217–232

Sesti G, Laratta E, Cardellini M, Andreozzi F, Del Guerra S, Irace C, Gnasso A, Grupillo M, Lauro R, Hribal ML, Perticone F, Marchetti P (2006) The E23K variant of KCNJ11 encoding the pancreatic β-cell adenosine 5′-triphosphate-sensitive potassium channel subunit Kir6.2 is associated with an increased risk of secondary failure to sulfonylurea in patients with type 2 diabetes. J Clin Endocrinol Metab 91:2334–2339

Sharma N, Crane A, Clement JP, Gonzalez G, Babenko AP, Bryan J, Aguilar-Bryan L (1999) The C terminus of SUR1 is required for trafficking of K_{ATP} channels. J Biol Chem 274:20628–20632

Shyng S, Nichols CG (1997) Octameric stoichiometry of the K_{ATP} channel complex. J Gen Physiol 110:655–664

Shyng SL, Nichols CG (1998) Membrane phospholipid control of nucleotide sensitivity of K_{ATP} channels. Science 282:1138–1141

Shyng S, Ferrigni T, Nichols CG (1997) Regulation of K_{ATP} channel activity by diazoxide and MgADP. Distinct functions of the two nucleotide binding folds of the sulfonylurea receptor. J Gen Physiol 110:643–654

Shyng SL, Cukras CA, Harwood J, Nichols CG (2000) Structural determinants of PIP(2) regulation of inward rectifier K_{ATP} channels. J Gen Physiol 116:599–608

Sieg A, Su J, Munoz A, Buchenau M, Nakazaki M, Aguilar-Bryan L, Bryan J, Ullrich S (2004) Epinephrine-induced hyperpolarization of islet cells without K_{ATP} channels. Am J Physiol Endocrinol Metab 286:E463–E471

Slingerland AS, Hurkx W, Noordam K, Flanagan SE, Jukema JW, Meiners LC, Bruining GJ, Hattersley AT, Hadders-Algra M (2008) Sulphonylurea therapy improves cognition in a patient with the V59M KCNJ11 mutation. Diabet Med 25:277–281

Smith PA, Bokvist K, Rorsman P (1989) Demonstration of A-currents in pancreatic islet cells. Pflugers Arch 413:441–443

Smith PA, Ashcroft FM, Rorsman P (1990a) Simultaneous recordings of glucose dependent electrical activity and ATP-regulated K^+-currents in isolated mouse pancreatic β-cells. FEBS Lett 261:187–190

Smith PA, Bokvist K, Arkhammar P, Berggren PO, Rorsman P (1990b) Delayed rectifying and calcium-activated K^+ channels and their significance for action potential repolarization in mouse pancreatic β-cells. J Gen Physiol 95:1041–1059

Spigelman AF, Dai X, MacDonald PE (2010) Voltage-dependent K^+ channels are positive regulators of α cell action potential generation and glucagon secretion in mice and humans. Diabetologia 53:1917–1926

Stanojevic V, Habener JF, Holz GG, Leech CA (2008) Cytosolic adenylate kinases regulate K-ATP channel activity in human β-cells. Biochem Biophys Res Commun 368:614–619

Straub SG, Cosgrove KE, Ammala C, Shepherd RM, O'Brien RE, Barnes PD, Kuchinski N, Chapman JC, Schaeppi M, Glaser B, Lindley KJ, Sharp GW, Aynsley-Green A, Dunne MJ (2001) Hyperinsulinism of infancy: the regulated release of insulin by K_{ATP} channel-independent pathways. Diabetes 50:329–339

Su J, Yu H, Lenka N, Hescheler J, Ullrich S (2001) The expression and regulation of depolarization-activated K^+ channels in the insulin-secreting cell line INS-1. Pflugers Arch 442:49–56

Suga S, Kanno T, Nakano K, Takeo T, Dobashi Y, Wakui M (1997) GLP-I(7-36) amide augments Ba^{2+} current through L-type Ca^{2+} channel of rat pancreatic β-cell in a cAMP-dependent manner. Diabetes 46:1755–1760

Sunouchi T, Suzuki K, Nakayama K, Ishikawa T (2008) Dual effect of nitric oxide on ATP-sensitive K^+ channels in rat pancreatic β cells. Pflugers Arch 456:573–579

Suzuki M, Fujikura K, Inagaki N, Seino S, Takata K (1997) Localization of the ATP-sensitive K^+ channel subunit Kir6.2 in mouse pancreas. Diabetes 46:1440–1444

Suzuki M, Fujikura K, Kotake K, Inagaki N, Seino S, Takata K (1999) Immuno-localization of sulphonylurea receptor 1 in rat pancreas. Diabetologia 42:1204–1211

Tamarina NA, Wang Y, Mariotto L, Kuznetsov A, Bond C, Adelman J, Philipson LH (2003) Small-conductance calcium-activated K^+ channels are expressed in pancreatic islets and regulate glucose responses. Diabetes 52:2000–2006

Tanabe K, Tucker SJ, Matsuo M, Proks P, Ashcroft FM, Seino S, Amachi T, Ueda K (1999) Direct photoaffinity labeling of the Kir6.2 subunit of the ATP-sensitive K^+ channel by 8-azido-ATP. J Biol Chem 274:3931–3933

Tanabe K, Tucker SJ, Ashcroft FM, Proks P, Kioka N, Amachi T, Ueda K (2000) Direct photoaffinity labeling of Kir6.2 by [gamma-(32)P]ATP-[gamma]4-azidoanilide. Biochem Biophys Res Commun 272:316–319

Tarasov AI, Girard CA, Ashcroft FM (2006) ATP sensitivity of the ATP-sensitive K^+ channel in intact and permeabilized pancreatic β-cells. Diabetes 55:2446–2454

Tarasov AI, Girard CA, Larkin B, Tammaro P, Flanagan SE, Ellard S, Ashcroft FM (2007) Functional analysis of two Kir6.2 (KCNJ11) mutations, K170T and E322K, causing neonatal diabetes. Diabetes Obes Metab 9(Suppl 2):46–55

Taschenberger G, Mougey A, Shen S, Lester LB, LaFranchi S, Shyng SL (2002) Identification of a familial hyperinsulinism-causing mutation in the sulfonylurea receptor 1 that prevents normal trafficking and function of K_{ATP} channels. J Biol Chem 277:17139–17146

Taylor EN, Hu FB, Curhan GC (2006) Antihypertensive medications and the risk of incident type 2 diabetes. Diabetes Care 29:1065–1070

Thiel G, Muller I, Rossler OG (2013) Signal transduction via TRPM3 channels in pancreatic β-cells. J Mol Endocrinol 50:R75–R83

Thomas PM, Cote GJ, Wohllk N, Haddad B, Mathew PM, Rabl W, Aguilar-Bryan L, Gagel RF, Bryan J (1995) Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. Science 268:426–429

Thornton PS, MacMullen C, Ganguly A, Ruchelli E, Steinkrauss L, Crane A, Aguilar-Bryan L, Stanley CA (2003) Clinical and molecular characterization of a dominant form of congenital hyperinsulinism caused by a mutation in the high-affinity sulfonylurea receptor. Diabetes 52:2403–2410

Togashi K, Hara Y, Tominaga T, Higashi T, Konishi Y, Mori Y, Tominaga M (2006) TRPM2 activation by cyclic ADP-ribose at body temperature is involved in insulin secretion. EMBO J 25:1804–1815

Trapp S, Proks P, Tucker SJ, Ashcroft FM (1998) Molecular analysis of ATP-sensitive K channel gating and implications for channel inhibition by ATP. J Gen Physiol 112:333–349

Trombetta M, Bonetti S, Boselli M, Turrini F, Malerba G, Trabetti E, Pignatti P, Bonora E, Bonadonna RC (2012) CACNA1E variants affect β cell function in patients with newly diagnosed type 2 diabetes. The Verona newly diagnosed type 2 diabetes study (VNDS) 3. PLoS One 7:e32755

Trube G, Rorsman P, Ohno-Shosaku T (1986) Opposite effects of tolbutamide and diazoxide on the ATP-dependent K^+ channel in mouse pancreatic β-cells. Pflugers Arch 407:493–499

Tschritter O, Stumvoll M, Machicao F, Holzwarth M, Weisser M, Maerker E, Teigeler A, Haring H, Fritsche A (2002) The prevalent Glu23Lys polymorphism in the potassium inward rectifier 6.2 (KIR6.2) gene is associated with impaired glucagon suppression in response to hyperglycemia. Diabetes 51:2854–2860

Tsuura Y, Ishida H, Hayashi S, Sakamoto K, Horie M, Seino Y (1994) Nitric oxide opens ATP-sensitive K^+ channels through suppression of phosphofructokinase activity and inhibits glucose-induced insulin release in pancreatic β cells. J Gen Physiol 104:1079–1098

Tucker SJ, Gribble FM, Zhao C, Trapp S, Ashcroft FM (1997) Truncation of Kir6.2 produces ATP-sensitive K^+ channels in the absence of the sulphonylurea receptor. Nature 387:179–183

Ueda K, Inagaki N, Seino S (1997) MgADP antagonism to Mg^{2+}-independent ATP binding of the sulfonylurea receptor SUR1. J Biol Chem 272:22983–22986

Ullrich S, Wollheim CB (1988) GTP-dependent inhibition of insulin secretion by epinephrine in permeabilized RINm5F cells. Lack of correlation between insulin secretion and cyclic AMP levels. J Biol Chem 263:8615–8620

Ullrich S, Wollheim CB (1989) Galanin inhibits insulin secretion by direct interference with exocytosis. FEBS Lett 247:401–404

Ullrich S, Prentki M, Wollheim CB (1990) Somatostatin inhibition of $Ca2^+$-induced insulin secretion in permeabilized HIT-T15 cells. Biochem J 270:273–276

Vanoye CG, MacGregor GG, Dong K, Tang L, Buschmann AS, Hall AE, Lu M, Giebisch G, Hebert SC (2002) The carboxyl termini of K_{ATP} channels bind nucleotides. J Biol Chem 277:23260–23270

Vaxillaire M, Dechaume A, Busiah K, Cave H, Pereira S, Scharfmann R, de Nanclares GP, Castano L, Froguel P, Polak M (2007) New ABCC8 mutations in relapsing neonatal diabetes and clinical features. Diabetes 56:1737–1741

Vaxillaire M, Veslot J, Dina C, Proenca C, Cauchi S, Charpentier G, Tichet J, Fumeron F, Marre M, Meyre D, Balkau B, Froguel P (2008) Impact of common type 2 diabetes risk polymorphisms in the DESIR prospective study. Diabetes 57:244–254

Vignali S, Leiss V, Karl R, Hofmann F, Welling A (2006) Characterization of voltage-dependent sodium and calcium channels in mouse pancreatic α- and β-cells. J Physiol 572:691–706

Vogalis F, Zhang Y, Goyal RK (1998) An intermediate conductance K^+ channel in the cell membrane of mouse intestinal smooth muscle. Biochim Biophys Acta 1371:309–316

Wagner TF, Loch S, Lambert S, Straub I, Mannebach S, Mathar I, Düfer M, Lis A, Flockerzi V, Philipp SE, Oberwinkler J (2008) Transient receptor potential M3 channels are ionotropic steroid receptors in pancreatic β cells. Nat Cell Biol 10:1421–1430

Wagner R, Kaiser G, Gerst F, Christiansen E, Due-Hansen ME, Grundmann M, Machicao F, Peter A, Kostenis E, Ulven T, Fritsche A, Haring HU, Ullrich S (2013) Reevaluation of fatty acid receptor 1 as a drug target for the stimulation of insulin secretion in humans. Diabetes 62:2106–2111

Wendt A, Birnir B, Buschard K, Gromada J, Salehi A, Sewing S, Rorsman P, Braun M (2004) Glucose inhibition of glucagon secretion from rat α-cells is mediated by GABA released from neighboring β-cells. Diabetes 53:1038–1045

Wesslen N, Pipeleers D, Van de Winkel M, Rorsman P, Hellman B (1987) Glucose stimulates the entry of Ca^{2+} into the insulin-producing β cells but not into the glucagon-producing α 2 cells. Acta Physiol Scand 131:230–234

Wiser O, Trus M, Hernandez A, Renstrom E, Barg S, Rorsman P, Atlas D (1999) The voltage sensitive Lc-type Ca^{2+} channel is functionally coupled to the exocytotic machinery. Proc Natl Acad Sci USA 96:248–253

Woll KH, Lonnendonker U, Neumcke B (1989) ATP-sensitive potassium channels in adult mouse skeletal muscle: different modes of blockage by internal cations, ATP and tolbutamide. Pflugers Arch 414:622–628

Wollheim CB, Winiger BP, Ullrich S, Wuarin F, Schlegel W (1990) Somatostatin inhibition of hormone release: effects on cytosolic Ca^{2+} and interference with distal secretory events. Metabolism 39:101–104

Worley JF 3rd, McIntyre MS, Spencer B, Dukes ID (1994) Depletion of intracellular Ca^{2+} stores activates a maitotoxin-sensitive nonselective cationic current in β-cells. J Biol Chem 269:32055–32058

Xia F, Gao X, Kwan E, Lam PP, Chan L, Sy K, Sheu L, Wheeler MB, Gaisano HY, Tsushima RG (2004) Disruption of pancreatic β-cell lipid rafts modifies $K_v2.1$ channel gating and insulin exocytosis. J Biol Chem 279:24685–24691

Xu E, Kumar M, Zhang Y, Ju W, Obata T, Zhang N, Liu S, Wendt A, Deng S, Ebina Y, Wheeler MB, Braun M, Wang Q (2006) Intra-islet insulin suppresses glucagon release via GABA-GABAA receptor system. Cell Metab 3:47–58

Yan L, Figueroa DJ, Austin CP, Liu Y, Bugianesi RM, Slaughter RS, Kaczorowski GJ, Kohler MG (2004) Expression of voltage-gated potassium channels in human and rhesus pancreatic islets. Diabetes 53:597–607

Yanagihara K, Irisawa H (1980) Inward current activated during hyperpolarization in the rabbit sinoatrial node cell. Pflugers Arch 385:11–19

Yang SN, Berggren PO (2005) β-cell Ca_v channel regulation in physiology and pathophysiology. Am J Physiol Endocrinol Metab 288:E16–E28

Yang SN, Berggren PO (2006) The role of voltage-gated calcium channels in pancreatic β-cell physiology and pathophysiology. Endocr Rev 27:621–676

Yang SN, Larsson O, Branstrom R, Bertorello AM, Leibiger B, Leibiger IB, Moede T, Kohler M, Meister B, Berggren PO (1999) Syntaxin 1 interacts with the L(D) subtype of voltage-gated Ca^{2+} channels in pancreatic β cells. Proc Natl Acad Sci USA 96:10164–10169

Yorifuji T, Nagashima K, Kurokawa K, Kawai M, Oishi M, Akazawa Y, Hosokawa M, Yamada Y, Inagaki N, Nakahata T (2005) The C42R mutation in the Kir6.2 (KCNJ11) gene as a cause of transient neonatal diabetes, childhood diabetes, or later-onset, apparently type 2 diabetes mellitus. J Clin Endocrinol Metab 90:3174–3178

Yoshimoto Y, Fukuyama Y, Horio Y, Inanobe A, Gotoh M, Kurachi Y (1999) Somatostatin induces hyperpolarization in pancreatic islet α cells by activating a G protein-gated K^+ channel. FEBS Lett 444:265–269

Zawalich WS, Zawalich KC (1997) Influence of pyruvic acid methyl ester on rat pancreatic islets. Effects on insulin secretion, phosphoinositide hydrolysis, and sensitization of the β cell. J Biol Chem 272:3527–3531

Zerangue N, Schwappach B, Jan YN, Jan LY (1999) A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K_{ATP} channels. Neuron 22:537–548

Zhang M, Houamed K, Kupershmidt S, Roden D, Satin LS (2005) Pharmacological properties and functional role of K_{slow} current in mouse pancreatic β-cells: SK channels contribute to K_{slow} tail current and modulate insulin secretion. J Gen Physiol 126:353–363

Zhang Q, Bengtsson M, Partridge C, Salehi A, Braun M, Cox R, Eliasson L, Johnson PR, Renstrom E, Schneider T, Berggren PO, Gopel S, Ashcroft FM, Rorsman P (2007) R-type Ca^{2+}-channel-evoked CICR regulates glucose-induced somatostatin secretion. Nat Cell Biol 9:453–460

Zhang Y, Zhang N, Gyulkhandanyan AV, Xu E, Gaisano HY, Wheeler MB, Wang Q (2008) Presence of functional hyperpolarisation-activated cyclic nucleotide-gated channels in clonal α cell lines and rat islet α cells. Diabetologia 51:2290–2298

Zhang Y, Liu Y, Qu J, Hardy A, Zhang N, Diao J, Strijbos PJ, Tsushima R, Robinson RB, Gaisano HY, Wang Q, Wheeler MB (2009) Functional characterization of hyperpolarization-activated cyclic nucleotide-gated channels in rat pancreatic β cells. J Endocrinol 203:45–53

Zhang HY, Liao P, Wang JJ, de Yu J, Soong TW (2010) Alternative splicing modulates diltiazem sensitivity of cardiac and vascular smooth muscle $Ca_v1.2$ calcium channels. Br J Pharmacol 160:1631–1640

Zhao Y, Fang Q, Straub SG, Sharp GW (2008) Both G i and G o heterotrimeric G proteins are required to exert the full effect of norepinephrine on the β-cell K ATP channel. J Biol Chem 283:5306–5316

Zhou H, Zhang T, Harmon JS, Bryan J, Robertson RP (2007) Zinc, not insulin, regulates the rat α-cell response to hypoglycemia in vivo. Diabetes 56:1107–1112

Zünkler BJ, Lins S, Ohno-Shosaku T, Trube G, Panten U (1988) Cytosolic ADP enhances the sensitivity to tolbutamide of ATP-dependent K^+ channels from pancreatic β-cells. FEBS Lett 239:241–244

ATP-Sensitive Potassium Channels in Health and Disease

11

Peter Proks and Rebecca Clark

Contents

Abstract

The ATP-sensitive potassium (K_{ATP}) channel plays a crucial role in insulin secretion and thus glucose homeostasis. K_{ATP} channel activity in the pancreatic β-cell is finely balanced; increased activity prevents insulin secretion, whereas reduced activity stimulates insulin release. β-cell metabolism tightly regulates K_{ATP} channel gating, and if this coupling is perturbed, two distinct disease states

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_6,

can result. Diabetes occurs when the K_{ATP} channel fails to close in response to increased metabolism, whereas congenital hyperinsulinism results when K_{ATP} channels remain closed even at very low blood glucose levels. In general there is a good correlation between the magnitude of K_{ATP} current and disease severity. Mutations that cause a complete loss of K_{ATP} channels in the β-cell plasma membrane produce a severe form of congenital hyperinsulinism, whereas mutations that partially impair channel function produce a milder phenotype. Similarly mutations that greatly reduce the ATP sensitivity of the K_{ATP} channel lead to a severe form of neonatal diabetes with associated neurological complications, while mutations that cause smaller shifts in ATP sensitivity cause neonatal diabetes alone. This chapter reviews our current understanding of the pancreatic β-cell K_{ATP} channel and highlights recent structural, functional, and clinical advances.

Keywords

ATP-sensitive potassium channel • Neonatal diabetes • Congenital hyperinsulinism • Insulin secretion • Pancreatic β-cell

Abbreviations

ABC	ATP-binding cassette
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
CHI	Congenital hyperinsulinism
CL3	3rd cytosolic loop in the sulfonylurea receptor connecting TMD0 to TMD1
DEND	Developmental delay epilepsy and neonatal diabetes
GCK	Glycolytic enzyme glucokinase
GIP	Gastrointestinal peptide
GIRK	G protein-coupled inwardly rectifying potassium channel
GLP-1	Glucagon-like-peptide-1
GLUD1	Mitochondrial glutamate dehydrogenase
HbA1C	Glycosylated (or glycated) hemoglobin
i-DEND	Intermediate DEND syndrome
K_{ATP}	ATP-sensitive potassium
MRP	Multidrug-resistant protein
NBD	Nucleotide-binding domain
NBS	Nucleotide-binding site
NDM	Neonatal diabetes mellitus
PNDM	Permanent neonatal diabetes mellitus
SCHAD	Short-chain l-3-hydroxyacyl-CoA dehydrogenase
SUR	Sulfonylurea receptor
TMD	Transmembrane domain
TNDM	Transient neonatal diabetes mellitus

Introduction

Insulin, as the only hormone able to lower blood glucose concentration, is of great importance in glucose homeostasis. Insulin is released from the β-cells of the pancreatic islets of Langerhans in response to changes in nutrient, hormone, and transmitter levels (Ashcroft and Rorsman 1989). Electrical activity of the β-cell is central to the secretion of insulin. The extent of insulin release and electrical activity is directly correlated: in the absence of β-cell electrical activity, no insulin is secreted (Ashcroft and Rorsman 2004).

The ATP-sensitive potassium (K_{ATP}) channel is a key component of stimulus-secretion coupling in the pancreatic β-cell. The resting membrane potential in β-cells is principally determined by the activity of the K_{ATP} channel (a small depolarizing inward current of unknown origin is also present, but it must be extremely small, as it has proved difficult to measure) (Ashcroft and Rorsman 1989). The K_{ATP} channel is responsible for the initiation of electrical activity and regulates its extent at suprathreshold glucose concentrations (Ashcroft et al. 1984; Kanno et al. 2002a). The electrical resistance of the β-cell membrane is also determined by the K_{ATP} channel, which is low when K_{ATP} channels are open and high when they are closed. Therefore, when K_{ATP} channels are closed and membrane resistance is high, small changes in the K_{ATP} current can lead to membrane depolarization, electrical activity, and insulin secretion (Ashcroft 2005).

Given the critical role of the K_{ATP} channel in insulin secretion and glucose homeostasis, it is not surprising that K_{ATP} channel mutations can lead to diseases of both hypo- and hyperglycemia (Gloyn et al. 2004a; Thomas et al. 1995, 1996). This chapter focuses on the role of the β-cell K_{ATP} channels in health and disease, taking into account recent genetic, clinical, structural and functional advances.

Role of K_{ATP} Channels in the Pancreas and Other Tissues

K_{ATP} channels act as metabolic sensors, coupling the metabolism of a cell to its membrane potential and electrical excitability. They are expressed in many tissues including the pancreas, skeletal and smooth muscle and the brain (Seino and Miki 2003). They link cell metabolism to electrical activity by sensing changes in adenine nucleotide concentrations and regulating membrane K^+ fluxes (Seino and Miki 2004). A decrease in metabolism opens K_{ATP} channels, causing K^+ efflux, membrane hyperpolarization and reduced electrical activity. An increase in metabolism closes K_{ATP} channels and prevents K^+ efflux, which triggers membrane depolarization. The resulting electrical activity stimulates responses such as the release of neurotransmitter at brain synapses, insulin exocytosis or muscle contraction (Ashcroft and Rorsman 2004).

The physiological role of the K_{ATP} channel has been best characterized in the pancreatic β-cell. The pancreatic K_{ATP} channel was discovered 25 years ago by Cook and Hales (1984); its closure by glucose metabolism was first demonstrated by Ashcroft et al. (1984). The link between glucose metabolism and insulin release

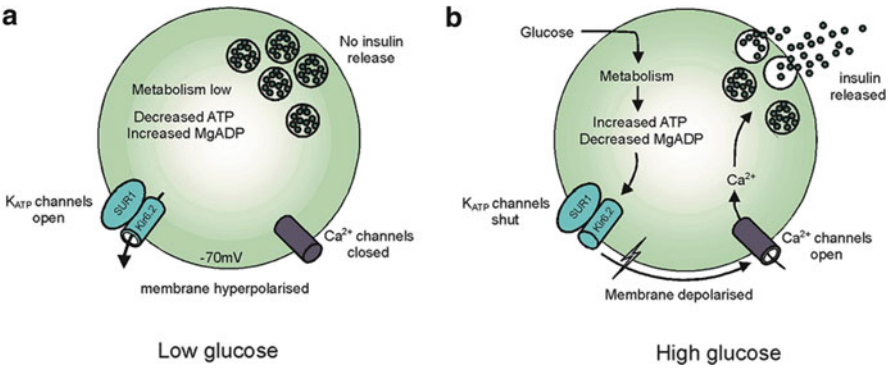

Fig. 1 Stimulus-secretion coupling in pancreatic β-cells. (**a**) When extracellular glucose, and thus β-cell metabolism, is low, K_{ATP} channels are open. As a result, the cell membrane is hyperpolarized. This keeps voltage-gated Ca^{2+} channels closed, so that Ca^{2+} influx remains low and no insulin is released. (**b**) When extracellular glucose concentration rises, glucose is taken up by the β-cell and metabolized. Metabolism generates ATP at the expense of MgADP, thereby closing K_{ATP} channels. This causes membrane depolarization, opening of voltage-gated Ca^{2+} channels, Ca^{2+} influx, and insulin secretion

in the β-cell is illustrated in Fig. 1. At substimulatory glucose concentrations, the β-cell K_{ATP} channel is open. Hence, the cell membrane is hyperpolarized and voltage-gated calcium channels are closed (Ashcroft and Rorsman 1989). Insulin secretion is therefore prevented. In response to an increase in the blood glucose concentration, insulin release from the β-cell is initiated. Glucose is transported into pancreatic β-cells and metabolized, thereby increasing the ATP/ADP ratio. This closes the K_{ATP} channel, producing a membrane depolarization that opens voltage-gated calcium channels: the influx of calcium into the β-cell triggers insulin exocytosis (Ashcroft 2007). K_{ATP} channel activity in the β-cell is finely balanced – increased activity leads to reduced insulin secretion, whereas reduced K_{ATP} channel activity decreases insulin release. Thus, loss-of-function mutations in K_{ATP} channel genes cause oversecretion of insulin and result in hyperinsulinemia. Conversely, gain-of-function mutations result in undersecretion of insulin, hyperglycemia, and a condition known as neonatal diabetes (Gloyn et al. 2004a; Thomas et al. 1995, 1996). Similarly, impaired metabolic regulation of K_{ATP} channels, resulting from mutations in genes that influence β-cell metabolism, can cause both hyperinsulinemia and diabetes.

K_{ATP} channels are also expressed in pancreatic α-cells where they have been proposed to play a role in glucagon secretion (Gopel et al. 2000a). Unlike insulin secretion from β-cells, glucagon secretion exhibits dual dependency on K_{ATP} channel activity: intermediate K_{ATP} channel currents stimulate glucagon release, while both high and low activity has an inhibitory effect (MacDonald et al. 2007). Since the resting activity of K_{ATP} channels in healthy α-cells is low, this would imply that inhibition of K_{ATP} channels due to rise in glucose concentration would inhibit glucagon release. It has been hypothesized that diabetic α-cells have

increased resting activity of K_{ATP} channels, above the value optimal for glucagon release, so an increase in glucose metabolism would result in stimulation of glucagon secretion (Rorsman et al. 2008). Consequently, glucose has opposite effects on glucagon secretion in normal and diabetic α-cells.

Göpel et al. have demonstrated the presence of K_{ATP} channels in pancreatic δ-cells (Gopel et al. 2000b). Stimulus-secretion coupling in pancreatic δ-cells is expected to work in the same way as in pancreatic β-cells, with glucose stimulation leading to closure of K_{ATP} channels and the resulting membrane depolarization triggering somatostatin release (Kanno et al. 2002b).

The K_{ATP} channel further contributes to glucose homeostasis by controlling glucose uptake in skeletal muscle (Miki et al. 2002) and GLP-1 secretion from L-cells in the gut (Gribble et al. 2003). In the hypothalamus it is involved in the counter-regulatory response to glucose (Miki et al. 2001) and modulates neurotransmitter release in the hippocampus and substantia nigra (Wang et al. 2004; Liss et al. 1999; Avshalumov and Rice 2003; Zawar et al. 1999; Griesemer et al. 2002; Amoroso et al. 1990; Schmid-Antomarchi et al. 1990). The K_{ATP} channel is also thought to play important roles in altered metabolic states of tissues, for example, hyperglycemia, cardiac stress, ischemia and hypoxia (Zingman et al. 2002; Yamada et al. 2001; Hernandez-Sanchez et al. 2001; Heron-Milhavet et al. 2004; Suzuki et al. 2002; Gumina et al. 2003).

Molecular Structure and Functional Properties of the β-Cell K_{ATP} Channel

The K_{ATP} channel is a hetero-octameric complex (Shyng and Nichols 1997; Clement et al. 1997) comprising four Kir6.x subunits and four sulfonylurea receptor (SUR) subunits (Fig. 2). Kir6.x is an inwardly rectifying K-channel (Inagaki et al. 1995a, b; Sakura et al. 1995) that forms the potassium-selective pore. Inward rectifiers conduct positive charge more easily in the inward direction across the membrane. This is due to the high-affinity block by endogenous polyamines and magnesium ions at positive membrane potentials. There are two isoforms: Kir6.1, which is expressed in vascular smooth muscle (Inagaki et al. 1995b), and Kir6.2, which is expressed more widely, including in the β-cell (Sakura et al. 1995). ATP binding to the Kir6.2 subunit causes K_{ATP} channel closure (Tucker et al. 1997).

The sulfonylurea receptor is a member of the ABC (ATP-binding cassette) superfamily (Aguilar-Bryan et al. 1995). Its major regulatory role is conferring sensitivity to stimulation by Mg-nucleotides via two nucleotide-binding domains – NBD1 and NBD2 (Gribble et al. 1997; Nichols et al. 1996). Each of the NBDs contains sequence motifs called Walker A and Walker B that are essential for binding the phosphate groups of nucleotides. ATP binding to SUR1 causes head-to-tail dimerization of the NBDs and formation of two nucleotide-binding sites (NBS1 and NBS2) within the dimer interface. NBS2 possesses greater ATPase activity than NBS1 and its occupancy by MgADP stimulates K_{ATP} channel activity (Zingmann et al. 2001). In addition, SUR1 also mediates (i) activation by

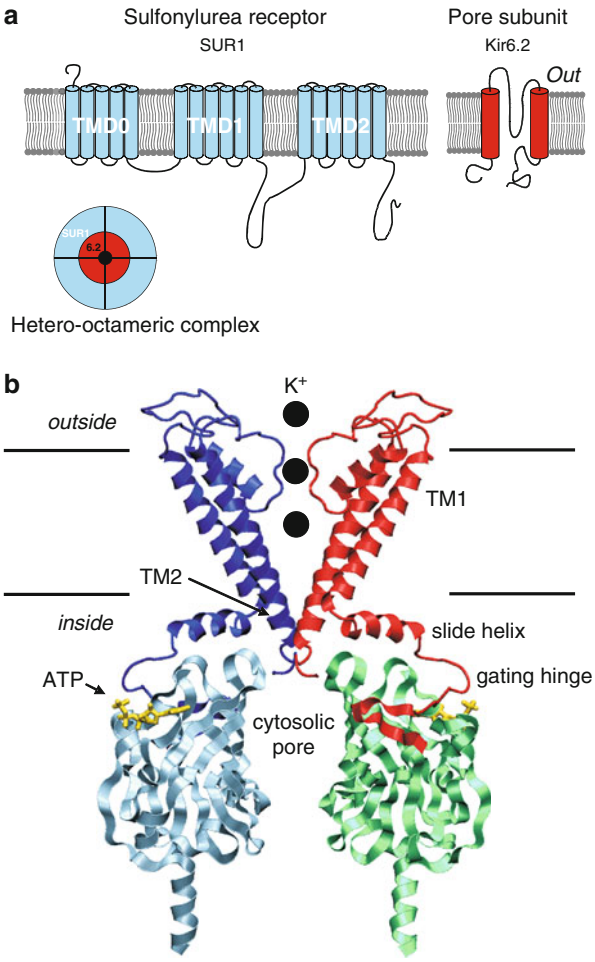

Fig. 2 The structure of the K_{ATP} channel. (**a**) Membrane topology of the sulfonylurea receptor (*left*) and Kir6.2 subunit (*right*). These subunits associate in a 4:4 octamer (*below left*). (**b**) Homology model of the Kir6.2 tetramer viewed from the side (Antcliff et al. 2005). For clarity, the intracellular domains of two subunits and the transmembrane domains of two separate subunits are shown. ATP (denoted by *arrow* and shown in *stick* representation) is docked into its binding sites

K-channel openers such as diazoxide and (ii) inhibition by sulfonylureas such as tolbutamide and glibenclamide (Tucker et al. 1997; Aguilar-Bryan et al. 1995). There are three isoforms of the sulfonylurea receptor. SUR1 is expressed in β-cells and neurons (Aguilar-Bryan et al. 1995) and to some extent in heart and skeletal muscle (Flagg et al. 2010), SUR2A in skeletal and cardiac muscle (Chutkow et al. 1996; Inagaki et al. 1996), and SUR2B in smooth muscle and brain (Shi et al. 2005; Miki and Seino 2005; Isomoto et al. 1996). The K_{ATP} channel found in β-cells is made up of four Kir6.2 subunits and four SUR1 subunits. Current evidence indicates that pancreatic α-cells and δ-cells also possess the β-cell type of K_{ATP} channel (Rorsman et al. 2008; Gopel et al. 2000b).

Kir6.2 is unable to reach the membrane surface in the absence of SUR1 and vice versa. Both Kir6.2 and SUR1 contain an endoplasmic reticulum retention motif (RKR). This ensures that only fully functional K_{ATP} channels are trafficked to the

plasma membrane, as these motifs are only masked when the two subunits associate together (Zerangue et al. 1999). However, truncation at the C-terminus of Kir6.2 at residue 355 (Kir6.2ΔC) deletes the ER retention signal and allows independent surface expression of Kir6.2 (Tucker et al. 1997; Zerangue et al. 1999). This allows the intrinsic properties of Kir6.2 to be assessed in the absence of SUR1.

Studies of Kir6.2ΔC have allowed specific functions to be assigned to Kir6.2 and SUR1. It is now clear that metabolic regulation of K_{ATP} channel activity is mediated by both Kir6.2 and SUR1 and that the two subunits are able to influence the function of each other. The ATP-binding site responsible for channel closure lies on Kir6.2 (Tucker et al. 1997), whereas MgADP binding to NBS2 of SUR1 opens the channel (Tucker et al. 1997; Gribble et al. 1997; Nichols et al. 1996; Aittoniemi et al. 2009). MgATP can also stimulate K_{ATP} channel activity via SUR1, but it must first be hydrolyzed to MgADP (Zingman et al. 2001).

SUR1 therefore functions as a second metabolic sensor and, when combined with Kir6.2, creates a channel with exquisite sensitivity to changes in adenine nucleotide concentrations (Ashcroft 2007).

The activatory effect of MgADP is thought to involve two mechanisms: (i) increase in channel activity (P_O) and (ii) reduction of nucleotide binding at Kir6.2 (Nichols et al. 1996; Shyng et al. 1997; Matsuo et al. 2000; John et al. 2001; Abraham et al. 2002; Proks et al. 2010). A recent study of channels with a mutation in the Kir6.2 subunit of the channel that renders K_{ATP} channels insensitive to nucleotide block (G334D, Drain et al. 1998) allowed to characterize in detail the former mechanism (Proks et al. 2010).

SUR1 has several other effects on Kir6.2 (Tucker et al. 1997; Nichols 2006; Proks and Ashcroft 2009): it increases the channel ATP sensitivity approximately tenfold, and it decreases the ATP concentration required to half-maximally close the channel (IC_{50}) from ~100 μM to ~10 μM in the presence of SUR1 (Tucker et al. 1997) and also enhances the open probability of the channel in the absence of nucleotides in excised membrane patches ($P_O[0]$) from 0.1 to around 0.4. It appears that Kir6.2 also alters the function of SUR1. In the presence of Kir6.2, the K_m for ATP hydrolysis is greater, suggesting a lower affinity for the K_{ATP} channel complex compared to SUR1 alone (Mikhailov et al. 2005; de Wet et al. 2007a). The K_{ATP} channel complex also has a higher turnover rate compared to SUR1 alone, which suggests that Kir6.2 may have an effect similar to substrate activation seen in other ABC transporters such as MRP1 (Aittoniemi et al. 2009; Mao et al. 1999).

The IC_{50} for ATP inhibition of K_{ATP} channels in excised patches is ~10 μM, yet cytoplasmic ATP concentrations are millimolar, thus predicting that K_{ATP} channels are ~99 % inhibited at physiological nucleotide concentrations. In contrast, estimates of the percentage of open channels at substimulatory glucose concentrations from whole-cell experiments appear to be much greater, ~5–25 % (Proks and Ashcroft 2009). Recently, the open-cell configuration was used to estimate the ATP sensitivity of K_{ATP} channels in intact cells (Tarasov et al. 2006a). It was found that channel sensitivity is substantially shifted to higher ATP concentrations, indicating that the excised patch data are not a reliable indicator of the ATP sensitivity of K_{ATP} channels in intact β-cells.

Recent Structural Advances

In order to understand where exactly the nucleotide and drug-binding sites are located on the channel, and how ligand binding leads to changes in channel gating, an atomic resolution structure of the K_{ATP} channel is required. Unfortunately at present, the only published structure of the K_{ATP} channel is an electron microscopy map of the purified complex at 18 Å resolution (Mikhailov et al. 2005). The channel is viewed as a tightly packed complex 13 nm in height and 18 nm in diameter. As expected, the K_{ATP} channel assembles as a central tetrameric Kir6.2 pore surrounded by four SUR1 subunits. However, at this resolution, little, if any, information can be gleaned about ligand-binding sites. A high-resolution structure of either the individual K_{ATP} channel subunits or the entire K_{ATP} channel complex is now essential to bridge the gap between structure and function.

Figure 2b shows a Kir6.2 homology model based on the crystal structures of the transmembrane domain of the bacterial KirBac1.1 channel (Kuo et al. 2003) and the cytosolic domain of the eukaryotic GIRK1 channel (Nishida and MacKinnon 2002). The model lends some insight into the location of nucleotide and drug-binding sites on the K_{ATP} channel (Antcliff et al. 2005). When combined with mutagenesis studies, this constitutes a powerful tool in the study of interaction sites on the K_{ATP} channel. The ATP-binding site was elucidated via automated docking. In agreement with a large body of mutagenesis data (Ashcroft 2005; Gloyn et al. 2004a; Masia et al. 2007a; Shimomura et al. 2006; Tammaro et al. 2005), the ATP-binding pocket was predicted to lie at the interface between the cytosolic domains of adjacent Kir6.2 subunits. The residues in the C-terminus of one subunit form the main binding pocket, and residues from the N-terminus of the adjacent subunit also contribute.

Information on the nucleotide-binding sites of SUR1 is also available. Similar to other ABC proteins, SUR1 has two cytosolic domains that contain consensus sequences for ATP binding and hydrolysis. Mutations of residues in the nucleotide-binding domains (NBDs) impair radiolabeled ATP binding and channel activation by Mg-nucleotides (Gribble et al. 1997). Homology modeling of the complete SUR1 protein is not yet possible, due to a lack of high-resolution structures from the ABCC subfamily of ABC proteins, which could be used as a template. However, several models of the NBDs have been generated using other ABC protein structures as a template (Campbell et al. 2003; de Wet et al. 2008; Babenko 2008). The high sequence conservation and overall folds of NBDs between ABC proteins suggest that homology models of the NBDs of SUR1 may be a good approximation to reality. Despite this, the transmembrane domains of SUR1 are too divergent from other ABC proteins to model accurately at present.

Congenital Hyperinsulinism of Infancy

Following cloning of the Kir6.2 and SUR1 genes in 1995, it was discovered that mutations in the two K_{ATP} channel subunits could cause congenital hyperinsulinism of infancy (CHI). This disorder is a clinically heterogeneous disease characterized

by continuous, unregulated insulin secretion despite severe hypoglycemia (Dunne et al. 2004; Gloyn et al. 2006). Patients usually present with this disorder at birth or shortly afterwards. In the absence of treatment, blood glucose levels can fall so low that irreversible brain damage results. Most cases of CHI are sporadic, but well-documented familial forms also exist. Sporadic forms have an incidence of around one in 50,000 live births (Glaser et al. 2000), but in some isolated communities the incidence is higher (Aguilar-Bryan et al. 1995; Glaser et al. 2000).

CHI is a heterogeneous disorder with mutations recorded in the K_{ATP} channel genes (*ABCC8* and *KCNJ11*), glycolytic enzyme glucokinase (GCK), mitochondrial glutamate dehydrogenase (GLUD1), and short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD) (Thomas et al. 1995, 1996; Nestorowicz et al. 1997; Stanley et al. 1998; Glaser et al. 1998). CHI is also histologically heterogeneous; both diffuse and focal forms of CHI have been reported. The diffuse form affects all of the β-cells within the islets of Langerhans, whereas in the focal form, only an isolated region of β-cells is affected and the surrounding tissue appears normal (Gloyn et al. 2006).

Mechanistically, CHI mutations in K_{ATP} channels can be divided into those that lead to a total or near-total loss of channels in the plasma membrane (Class I), those that impair the ability of Mg-nucleotides to stimulate channel activity (Class II), and those that decrease the intrinsic (i.e., in the absence of nucleotides) channel open probability, $P_O(0)$ (Class III).

ABCC8 and CHI

All CHI mutations are loss-of-function mutations that lead to permanent depolarization of the β-cell membrane. This results in continuous Ca^{2+} influx and insulin secretion, irrespective of the blood glucose level. The most common cause of CHI is mutation of the gene encoding SUR1 (*ABCC8*). SUR1 is located within a region of chromosome 11p15.1 to which a severe form of persistent hyperinsulinemic hypoglycemia of infancy was initially mapped (Thomas et al. 1995). Over 20 years after the first mutation was discovered, more than 100 CHI-causing mutations in SUR1, distributed throughout the gene, have now been described.

Many *ABCC8* mutations lead to reduced surface expression of K_{ATP} channels due to abnormal gene expression, protein synthesis, maturation and assembly or membrane trafficking (Dunne et al. 2004; Taschenberger et al. 2002; Partridge et al. 2001; Yan et al. 2004). Such mutations are distributed throughout the protein and in general produce a severe phenotype. Other mutations act by reducing the ability of MgADP to activate the channel, so the channels remain closed in response to metabolic inhibition (Nichols et al. 1996; Dunne et al. 2004; Huopio et al. 2000). These mutations cluster within the NBDs of SUR1 where they impair nucleotide binding/hydrolysis. They have also been reported in other regions of SUR1 (Abdulhadi-Atwan et al. 2008), where they could interfere with Kir6.2-SUR1 coupling or affect MgATP binding/hydrolysis allosterically. In general, mutations of this type result in a less severe phenotype, due to a residual response to MgADP,

and some patients can be treated by the K-channel opener diazoxide (Dunne et al. 2004; Huopio et al. 2000; Magge et al. 2004). However, there is no definite genotype-phenotype correlation and the same mutation can result in CHI of differing severity in different patients.

KCNJ11 and CHI

In contrast to SUR1, relatively few CHI mutations have been reported in *KCNJ11* (Thomas et al. 1996; Nestorowicz et al. 1997; Henwood et al. 2005; Lin et al. 2008; Marthinet et al. 2005). The mutations that have been reported act by reducing or abolishing K_{ATP} channel activity in the surface membrane (Thomas et al. 1996; Nestorowicz et al. 1997; Henwood et al. 2005; Lin et al. 2008; Shimomura et al. 2007). Interestingly, an H259R mutation has been described that affects both the trafficking and function of the K_{ATP} channel (Marthinet et al. 2005). Recent functional analysis of CHI mutation E282K in Kir6.2 also revealed that Kir6.2 contains a di-acidic endoplasmic reticulum exit signal (^{280}DLE^{282}) (Taneja et al. 2009).

Therapeutic Implications

In general, mutations in Kir6.2 and SUR1 cause a severe form of CHI that does not respond to diazoxide (Dunne et al. 2004; Henwood et al. 2005) and requires subtotal pancreatectomy. This occurs due to the absence of K_{ATP} channels. CHI caused by mutations in GCK, GLUD1, or SCHAD respond well to diazoxide (Dunne et al. 2004), as K_{ATP} channel properties are normal. In these patients, diazoxide is able to open K_{ATP} channels, which hyperpolarizes the β-cell membrane and reduces electrical activity and insulin secretion. Genotyping of CHI patients is therefore important in determining the correct therapy.

Interestingly, sulfonylureas and K-channel openers can act as chaperones and rectify trafficking defects associated with some SUR1 mutations (Partridge et al. 2001; Yan et al. 2004). Sulfonylureas restored surface expression of SUR1-A116P and SUR1-V187D (Yan et al. 2004), and diazoxide corrected trafficking of SUR-R1349H (Partridge et al. 2001). The resulting K_{ATP} channels have normal nucleotide sensitivity, so drugs with similar chaperone properties, but without channel blocking activity, could be useful in treating some cases of CHI.

Neonatal Diabetes Mellitus

Neonatal diabetes mellitus (NDM) is defined as hyperglycemia that presents within the first 3 months of life. Around 50 % of cases resolve within 18 months and are named transient neonatal diabetes mellitus (TNDM). The remaining cases require insulin treatment for life and are termed permanent neonatal diabetes

mellitus (PNDM) (Polak and Shield 2004). The estimated incidence of PNDM is approximately one in 100,000 live births (Gloyn et al. 2004b). The majority (~80 %) of cases of TNDM are caused by abnormalities of an imprinted locus on chromosome 6q24 that results in the overexpression of a paternally expressed gene (Temple et al. 1995). However, heterozygous mutations in Kir6.2 can produce a form of neonatal diabetes that resembles TNDM, which remits but may subsequently relapse (Flanagan et al. 2007; Girard et al. 2006; Hattersley and Ashcroft 2005).

Until recently little was known about the genetic causes of PNDM, and indeed some clinicians denied that it existed at all (Ashcroft 2007). It is now known that PNDM does exist and is caused by mutations in a number of genes. Homozygous and compound heterozygous mutations in glucokinase (GCK) have been reported to cause PNDM (Njolstad et al. 2001, 2003; Porter et al. 2005). These are thought to act indirectly by a reduced metabolic generation of ATP, which therefore impairs K_{ATP} channel closure. Several rare syndromes that feature PNDM also exist, including X-linked diabetes mellitus, Wolcott-Rallison syndrome due to mutations in the EIF2AK3 gene, pancreatic agenesis due to mutations in IPF-1 (insulin promoter factor-1), and neonatal diabetes with cerebellar agenesis due to mutations in the PTF-1A gene (Delepine et al. 2000; Sellick et al. 2004; Stoffers et al. 1997; Wildin et al. 2001).

KCNJ11 and NDM

It is now well established that the most common cause of PNDM is heterozygous activating mutations in the *KCNJ11* gene encoding Kir6.2 (Ashcroft 2005). The majority of these mutations arise spontaneously. One class of mutations, such as R50P and R201H (Gloyn et al. 2004a; Shimomura et al. 2006) causes PNDM alone. Other mutations, such as Q52R and I296L, cause a severe phenotype in which PNDM is accompanied by neurological features such as developmental delay, muscle weakness and epilepsy; a condition known as DEND syndrome (Gloyn et al. 2004a; Polak and Shield 2004; Edghill et al. 2004; Sagen et al. 2004; Vaxillaire et al. 2004; Proks et al. 2004, 2005a; Shimomura et al. 2007). Intermediate DEND (i-DEND) is a less severe clinical syndrome in which patients show neonatal diabetes, developmental delay and/or muscle weakness, but not epilepsy (Gloyn et al. 2004a; Polak and Shield 2004; Edghill et al. 2004; Sagen et al. 2004; Vaxillaire et al. 2004; Proks et al. 2004).

Early evidence for the role of Kir6.2 in PNDM came from the generation of a mouse model that overexpressed a mutant K_{ATP} channel in the pancreatic β-cells (Koster et al. 2000). When the N-terminal deletion mutant Kir6.2[ΔN2-30] is expressed in COSm6 cells, it results in a channel with tenfold lower ATP sensitivity than wild-type K_{ATP} channels. Transgenic mice expressing this mutation in β-cells showed severe hyperglycemia, hypoinsulinemia and ketoacidosis within 2 days of birth and died within 5 days.

To date, over 40 gain-of-function mutations in Kir6.2 associated with PNDM have been identified, the most common being at residues R201 and V59

(Flanagan et al. 2007; Hattersley and Ashcroft 2005). Strikingly, these mutations cluster around the predicted ATP-binding site or are located in regions of the protein thought to be involved in channel gating such as the slide helix, the cytosolic mouth of the channel, or gating loops linking the ATP-binding site to the slide helix. They may also affect residues involved in interaction with SUR1.

A strong, but not absolute, correlation between genotype and phenotype appears to exist for Kir6.2 mutations. For example, of 24 patients with mutations at R201, all but 3 have non-remitting neonatal diabetes without neurological features. Of 13 patients with the V59M mutation, 10 have developmental delay and symptoms consistent with i-DEND syndrome (Hattersley and Ashcroft 2005). Mutations that are associated with full DEND syndrome are not found in less severely affected patients. Conversely, two of four patients with the C42R mutation did not develop diabetes until early adulthood, one patient developed transient neonatal diabetes and one exhibited diabetes at 3 years of age (Yorifuji et al. 2005). Therefore, as observed for other types of monogenic diabetes, genetic background and environmental factors may influence the clinical phenotype (Klupa et al. 2002; Bingham and Hattersley 2004).

Location of NDM Mutations in the Kir6.2 Subunit

Residues in Kir6.2 that, when mutated, cause neonatal diabetes cluster in several distinct locations: (i) the putative ATP-binding site of Kir6.2 (R50, I192, R201, F333, G334); (ii) the interfaces between Kir6.2 subunits (F35, C42, and E332); (iii) the interface between Kir6.2 and SUR1 subunits (Q52, G53); and (iv) parts of the channel implicated in channel gating (V59, F60, W68, L164, C166, I197, I296). Most (but not all) mutations that cause additional neurological complications are located further away from the ATP-binding site. For example, Q52 lies within the cytosolic part of the N-terminal domain, which is thought to be involved in the coupling of SUR1 to Kir6.2 (Proks et al. 2004; Reimann et al. 1999; Babenko and Bryan 2002). Residue G53 has been proposed to form a gating hinge, which permits flexibility of the N-terminus of the protein, allowing the induced fit of ATP at the ATP-binding site (Koster et al. 2008). Residue V59 lies within the slide helix, a region of the protein implicated in the gating of the pore (Kuo et al. 2003; Antcliff et al. 2005; Proks et al. 2004, 2005b). C166 lies close to the helix bundle crossing, which is suggested to form an inner gate to the channel (Doyle et al. 1998), and I197 is located within the permeation pathway, in an area thought to be involved in channel gating (Antcliff et al. 2005; Pegan et al. 2005). A gating mutation at residue I296, which causes DEND syndrome, suggested the existence of a novel gate within the cytosolic pore of Kir6.2 (Proks et al. 2005a). This was further supported by recent structural data (Nishida et al. 2007). Mutations of the same residue may result in different phenotypes; for example, the R50Q mutation causes neonatal diabetes alone, while R50P causes DEND syndrome (Shimomura et al. 2006).

Functional Effects of Kir6.2 Mutations Causing NDM

The effects of more than 20 Kir6.2 NDM mutations on the properties of the K_{ATP} channel have been investigated by heterologous expression of recombinant channels, in systems such as *Xenopus* oocytes (Ashcroft 2005; Masia et al. 2007a; Shimomura et al. 2006, 2007, 2009, 2010; Girard et al. 2006; Hattersley and Ashcroft 2005; Proks et al. 2004, 2005a, b, 2006a; Koster et al. 2008; Tarasov et al. 2007, 2006b; Tammaro et al. 2005, 2008; Männikkö et al. 2010, 2011a). All NDM mutations are gain-of-function mutations that decrease the ability of ATP to block the K_{ATP} channel. This reduction in ATP sensitivity means there is an increased K_{ATP} current at physiological concentrations of ATP (~1–5 mM). In β-cells, such an increase in K_{ATP} current is predicted to produce hyperpolarization, which suppresses electrical activity, calcium influx, and insulin secretion. The greater the increase in K_{ATP} current, the more severely insulin secretion will be impaired.

Functional analysis reveals that all Kir6.2 mutations studied to date act by reducing the ATP sensitivity of Kir6.2 via two major mechanisms. These are schematically depicted using a simple allosteric channel-gating scheme in Fig. 3b. Mutations at residues within the Kir6.2 ATP-binding site are expected to reduce the inhibitory effect of nucleotides by impairing binding directly. Pure binding defects will reduce the binding constants of both open (K_O) and closed states (K_C) of the channel by equal factors (Fig. 3b, left) and produce a parallel shift of the ATP dose-response curve to the right of wild type. Such mutations will have no effect on channel gating in the absence of the nucleotide (Fig. 3a; compare top and middle traces). Conversely, mutations in gating regions of the channel reduce the inhibitory effect of ATP indirectly, by biasing the channel towards the open state and impairing its ability to close both in the absence (E_O) and presence (E_A) of bound ATP (Fig. 3b right, Proks et al. 2004, 2005a). A decrease in E_O enhances the open probability of the channel, $P_O(0)$ (Fig. 3a, bottom trace). ATP inhibition is diminished by both a decrease in E_A, which reduces the destabilizing effect of ATP on the open state, and a decrease in E_O, which reduces the availability of closed states to which ATP binds with higher affinity ($K_C > K_O$).

Kir6.2 mutations can also reduce ATP inhibition via a third mechanism. They could alter the transduction of conformational changes in the ATP-binding pocket to the channel gate (in Fig. 3b, these mutations will alter the E_A/E_O and K_C/K_O ratios). For gain-of-function mutations, this would mean a relative increase in ATP binding to the open state of the channel, resulting in a detectable fraction of ATP-resistant current at very high ATP concentrations in Mg-free solutions. This effect has indeed been observed, for example, with mutations at K185, which is predicted to lie within the putative ATP-binding site (e.g. K185E, John et al. 2003).

Most mutations that impair channel inhibition by ATP without altering channel open probability in nucleotide-free solutions ($P_O[0]$) are associated with neonatal diabetes alone (Fig. 4). These mutations lie within the predicted ATP-binding site of Kir6.2 (Gloyn et al. 2004a, 2005; Masia et al. 2007a; Shimomura et al. 2006; Shimomura et al. 2010). The electrophysiological data are consistent with this view,

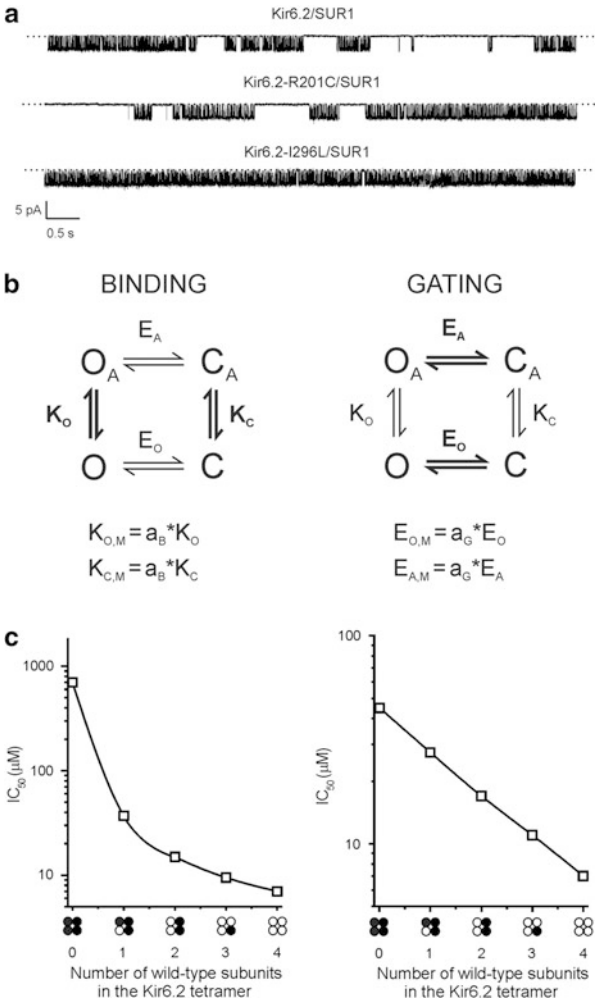

Fig. 3 Molecular mechanisms of NDM mutations in the Kir6.2 subunit. (**a**) Single K_{ATP} channel currents recorded from an inside-out patch at −60 mV in nucleotide-free solution (*top trace*) of wild type (*top*), a mutant channel with a point mutation R201C that is predicted to lie within the ATP-binding site (*middle*), and a mutant channel with a gating mutation I296L that dramatically increases channel open probability $P_O(0)$ (*bottom*). Channel openings are facing downwards; the *dotted line* represents closed-channel level. The open states are clustered into bursts of openings, separated by long closed interburst intervals. Transitions between states within bursts are thought to be governed by a "fast gate" of the channel and are little affected by nucleotides (Li et al. 2002). Transitions between burst and interburst states are thought to be governed by a separate "slow gate" (or gates) and are strongly modulated by nucleotides (Trapp et al. 1998). (**b**) Allosteric scheme for "slow" K_{ATP} channel gating. For simplicity, all interburst closed states are lumped in a single closed state C and all burst states into a single open state, O. In the absence of the nucleotide, the channel alternates between open and closed states with a gating constant E_O. Both O and C states can bind ATP with corresponding binding constants K_O and K_C. In the ATP-bound form, the channel alternates between open and closed states with an altered gating

but biochemical studies are required for confirmation. Most mutations associated with neurological features affect ATP inhibition indirectly by altering channel gating (Girard et al. 2006; Proks et al. 2004, 2005a; Shimomura et al. 2007). It is worth noting that some of these mutations may have additional effects to those on gating (i.e., on ATP binding or on transduction); however, since the mechanism of channel gating is quite complex, it has not yet been determined whether this is the case.

So far we have only considered the effects of NDM mutations on ATP inhibition in Mg-free solutions. Functional studies in the presence of Mg^{2+} demonstrated that a reduction of ATP inhibition due to Mg-nucleotide activation is much more pronounced in NDM mutant channels than in wild-type channels (Proks et al. 2005b). It is not clear whether this enhancement of the Mg-nucleotide activatory effect results from impaired ATP inhibition caused by NDM mutations or whether these mutations also have a direct effect on channel activation by MgATP/ADP. For mutations that are predicted to cause defects in ATP binding, the addition of Mg^{2+} predominantly produced a parallel shift of the ATP dose-response curve to the right (Proks et al. 2005b), unless the channel was completely ATP insensitive in Mg-free solutions as seen for G334D and R50P (Masia et al. 2007a; Shimomura et al. 2006). In contrast, for gating mutations, the addition of Mg^{2+} could also dramatically increase the fraction of channel current insensitive to ATP (Proks et al. 2005b).

Heterozygosity of Kir6.2 Mutations

All NDM patients with mutations in Kir6.2 are heterozygous. In functional studies, the heterozygous state is simulated by coexpression of wild-type and mutant Kir6.2 subunits with SUR1. Since Kir6.2 is a tetramer (Shyng and Nichols 1997), a mixed population of channels will exist, containing between zero and four mutant subunits.

Fig. 3 (continued) constant E_A ($E_A = K_C \times E_O/K_O$). A binding mutation (*left*) affects binding constants for ATP to open (K_O) and closed states (K_C) by the same factor, a_B (for a decrease in ATP binding, $a_B < 1$). A gating mutation affects gating constants in the absence (E_O) and presence (E_A) of ATP by the same factor, a_G (for increase in $P_O(0)$, $a_G < 1$). Index M in all equations refers to mutant channels. (**c**) *Left*: Relationship between the IC_{50} for ATP inhibition and the number of wild-type subunits for heteromeric K_{ATP} channels composed of wild-type subunits or mutant subunits with impaired ATP binding ($a_B = 0.01$ in b) in the absence of Mg^{2+} using a simple concerted gating model (Monod-Wyman-Changeux, Proks and Ashcroft 2009). $P_O(0)$ of all channels is 0.4. The corresponding tetrameric channel species are shown schematically below (*open circles*, wild-type Kir6.2 subunits, *filled circles*, mutant Kir6.2 subunits). *Right*: Relationship between the IC_{50} for ATP inhibition and the number of wild-type subunits for heteromeric K_{ATP} channels composed of wild-type subunits ($P_O(0)$ of the wild type was set to 0.4) or subunits of a gating mutant ($P_O(0)$ of the homomeric mutant was set to 0.82) in the absence of Mg^{2+} using a simple concerted gating model (Monod-Wyman-Changeux, Proks and Ashcroft 2009). The corresponding tetrameric channel species are shown schematically below (*open circles*, wild-type Kir6.2 subunits, *filled circles*, mutant Kir6.2 subunits). In all simulations, the K_O for ATP binding to the open state was 0.003 μM^{-1} (Craig et al. 2008); for the closed states, K_C was determined from the IC_{50} of wild-type channels (7 μM) with $P_O(0) = 0.4$ ($K_C = 0.05\ \mu M^{-1}$)

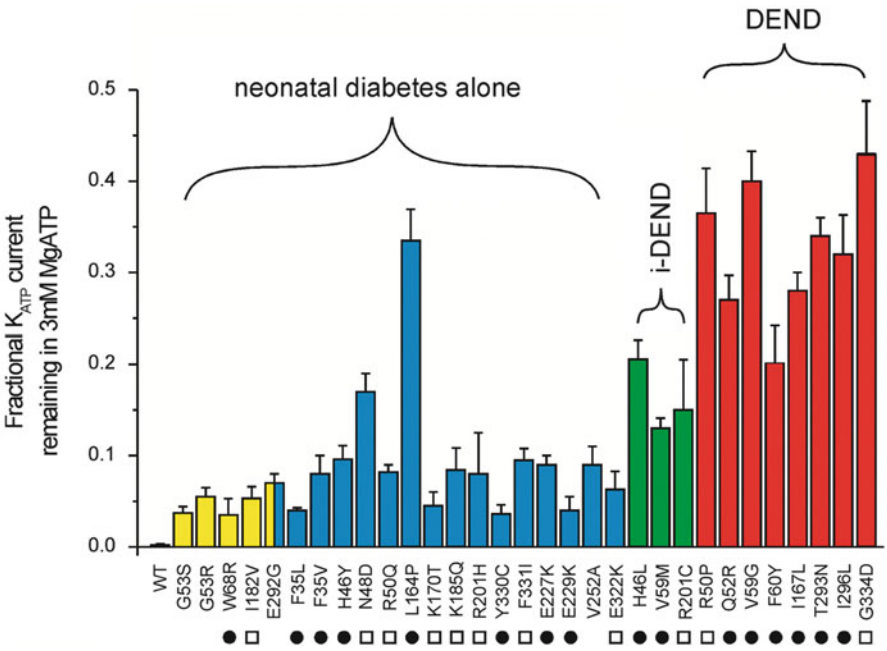

Fig. 4 ATP sensitivity correlates with disease severity but not molecular mechanism. Macroscopic current in 3 mM MgATP in excised patches expressed as a fraction of that in nucleotide-free solution of wild-type (WT) K_{ATP} channels and heterozygous K_{ATP} channels containing the indicated Kir6.2 mutations. With one exception (mutation L164P), disease severity correlates with the extent of unblocked K_{ATP} current. Different phenotypes can be produced by the same molecular mechanism: i.e., impaired ATP binding (*open squares*) or changes in gating (*filled circles*). For those mutations without symbols, no single-channel kinetics have been measured and the molecular mechanism is unclear. *Yellow bars* indicate mutations associated with TNDM, *blue bars* mutations causing PNDM, *green bars* mutations causing i-DEND and *red bars* mutations producing DEND syndrome (Data are taken from Shimomura et al. (2006, 2007, 2009, 2010), Tammaro et al. (2005, 2008), Girard et al. (2006), Proks et al. (2004, 2005a, 2006a), Männikkö et al. (2010, 2011a), Tarasov et al. (2007), Mlynarski et al. (2007))

Assuming equal expression levels of wild-type and mutant Kir6.2 subunits and random mixing between them, the various channel species in the heterozygous mixture will follow a binomial distribution.

Functional studies have shown that for binding mutations, the ATP sensitivity of the heterozygous population is close to that of the wild-type channel. In contrast, for gating mutations, the ATP sensitivity tends to be more intermediate between that of wild-type and homomeric mutant channels. This is consistent with our current understanding of the gating mechanism of the channel that assumes one ATP molecule is able to close the channel (Markworth et al. 2000) and that during gating the four Kir6.2 subunits move simultaneously in a concerted manner (Craig et al. 2008; Drain et al. 2004). Figure 3c shows predicted ATP inhibition IC_{50} values for heteromeric channel species composed of wild-type and mutant subunits

with impaired binding (left) and gating (right) using a simple concerted model. It is clear that if a mutation affects ATP binding alone, only channels with four mutant subunits will have a markedly reduced ATP sensitivity (Fig. 3c, left). Homomeric mutant channels will account for only one-sixteenth of the channel population, thus the shift in ATP sensitivity compared to wild-type will be small. Heteromeric channels containing subunits with impaired gating have more evenly distributed IC_{50} values between that of the wild-type and homomeric mutant channel (Fig. 3c, right). Accordingly, the corresponding heterozygous mixture would have larger shift in ATP sensitivity with regard to that of the wild type.

In the presence of Mg^{2+}, IC_{50} values for ATP inhibition of heterozygous channels with binding mutations are more dramatically increased (~tenfold) than those of the wild-type channel (~twofold; Proks et al. 2005b). Since the mechanism of channel activation by Mg-nucleotides and its interaction with the inhibitory action of ATP is poorly understood, this effect has not been addressed with modeling. In addition to an increase in the IC_{50}, heterozygous channels containing Kir6.2 subunits with impaired gating also show a substantial fraction of ATP-insensitive current (Proks et al. 2005b; Shimomura et al. 2007). A similar effect is observed for heterozygous channels with defects in ATP binding that render homomeric mutant channels completely ATP insensitive (Antcliff et al. 2005; Masia et al. 2007a).

As illustrated in Fig. 4, all NDM-Kir6.2 mutations increase the current of heterozygous channels at 3 mM MgATP at least 20-fold, with DEND mutations having the greatest effect. There is no obvious correlation between the magnitude of the K_{ATP} current and whether the mutation causes permanent or relapsing-remitting neonatal diabetes. There is also no correlation between the phenotype and the molecular mechanism, as mutations causing defects in gating and binding can result in both NDM alone or more severe forms of NDM with neurological complications.

The importance of heterozygosity in determining the severity of a mutation appears to be a novel feature of K_{ATP} channelopathies. It is also worth noting that if mutant and wild-type Kir6.2 subunits were to express at different levels, or if they did not assemble in a random fashion to form heteromers, the composition of the heterozygous population would deviate from a binomial distribution and thus influence the channel ATP sensitivity in a less quantitatively predictable fashion.

ABCC8 and PNDM

Activating mutations in SUR1 have also been shown to cause neonatal diabetes. In contrast to mutations in Kir6.2 which are all dominant heterozygous, SUR1 mutations can be either dominant or recessively inherited (Ellard et al. 2007). Recessive mutations could be homozygous, mosaic due to segmental uniparental isodisomy, or compound heterozygous for another activating mutation or if the second allele is inactivated. Approximately 50 % of SUR1 mutations are spontaneous, arising *de novo* during embryogenesis (Edghill et al. 2010). To date, over 60 mutations in SUR1 have been identified; they are scattered throughout the protein sequence but are particularly concentrated in the first five transmembrane helices (TMD0)

and their connecting loops, in the CL3 linker, which is a long cytosolic loop connecting TMD0 to TMD1, and NBD2 (Aittoniemi et al. 2009). SUR1 mutations can act in two main ways: (i) reducing the inhibition produced by ATP binding at Kir6.2 (Proks et al. 2006b, 2007; Lin et al. 2012; Babenko and Vaxillaire 2011; Zhou et al. 2010) and (ii) enhancing channel activation by Mg-nucleotides (de Wet et al. 2007b, 2008; Lin et al. 2012). Both lead to a greater K_{ATP} current at a particular MgATP concentration (de Wet et al. 2007b, 2008; Babenko and Vaxillaire 2011).

Mutations in SUR1 that decrease the amount of inhibition at Kir6.2 may do so in one of two ways. Firstly, they could reduce ATP binding directly. It is well established that the presence of SUR1 enhances ATP inhibition at Kir6.2, which suggests that SUR1 either contributes to the ATP-binding site itself or influences it allosterically (Tucker et al. 1997). Mutations that are likely to reduce ATP binding directly include A30V in TMD0 and G296R in TMD1 which together form a compound heterozygous mutation that results in PNDM (Lin et al. 2012).

Alternatively, SUR1 mutations could disrupt ATP inhibition indirectly by increasing the channel open probability (Babenko and Bryan 2003). Examples include F132L (Proks et al. 2006b, 2007) in TMD0, L213R in the CL3 linker between TMD0 and TMD1 (Babenko and Vaxillaire 2011) and V324M in TMD1 (Zhou et al. 2010).

K_{ATP} channel activity is determined by both the extent of ATP block at Kir6.2 and Mg-nucleotide activation at the NBDs of SUR1. Hence, gain-of-function SUR1 mutations may act to reduce the overall ATP inhibition by enhancing Mg-nucleotide activation. Many SUR1 mutations that lead to PNDM are found in NBD2 (Ellard et al. 2007; de Wet et al. 2007b; Männikkö et al. 2011b; Ortiz et al. 2012; Babenko et al. 2006). Only one SUR1-PNDM mutation is found in NBD1, and interestingly this mutation lies in the linker that is predicted to form part of NBS2 (de Wet et al. 2008). As predicted from their locations in NBD1 and NBD2, respectively, R826W and R1380L alter ATPase activity (de Wet et al. 2007b, 2008). The former reduces ATPase activity, whereas the latter increases it, yet they both increase MgATP activation of the K_{ATP} channel. How can this be resolved? It appears that both mutations increase the probability of SUR1 being in an MgADP-bound state, which enhances channel activity. R1380L appears to accelerate the catalytic cycle, so that the protein spends less time in the pre-hydrolytic ATP-bound state (de Wet et al. 2007b). R826W acts differently by slowing the rate at which P_i dissociates following ATP hydrolysis and thus halting the cycle in the MgADP-bound post-hydrolytic state (de Wet et al. 2008).

Some NDM mutations which enhance Mg-nucleotide activation are located outside the nucleotide-binding domains (Zhou et al. 2010; Babenko et al. 2006; Masia et al. 2007b). These mutations may exert their effect via enhancing the transduction of the Mg-nucleotide stimulation from the NBSs of SUR1 to the channel pore at Kir6.2; alternatively, they may also allosterically affect nucleotide handling at the NBDs. The former effect is also predicted for mutation G1401R which is located within NBD2 but does not form part of the nucleotide-binding sites (de Wet et al. 2012).

In addition to affecting channel activity, some SUR1 mutations can also affect channel expression at the plasma membrane. An example of such mutation is V324M, of which the activating effect (enhanced Mg-nucleotide activation, enhanced stability of channel open state) is dampened by reduced channel expression at the cell surface (Zhou et al. 2010). The interplay between the two types of opposing defects may be responsible for the fact that the V324M mutation results only in the transient form of the disease.

Mouse Models of PNDM

Mouse models often yield important insights into the molecular mechanisms of human disorders. Neonatal diabetes is no exception, and both gain- and loss-of-function K_{ATP} channel mouse models have been generated. These have allowed PNDM and CHI to be understood in far greater detail than is possible through expression of mutant K_{ATP} channels in heterologous systems.

As mentioned earlier, the first evidence that gain-of-function K_{ATP} channel mutations cause severe neonatal diabetes came from the generation of a mouse model that overexpresses the N-terminal deletion mutant Kir6.2[ΔN2-30] in β-cells (Koster et al. 2000). In these mice, no change in islet architecture, β-cell number, or insulin content was observed. Nevertheless serum insulin levels were extremely low, as expected from the decreased ATP sensitivity of this mutant K_{ATP} channel (Koster et al. 2000). As a result these mice show severe hypoglycemia and typically die within 2 days. Intriguingly, mice in which the mutant gene was expressed in the heart had no obvious cardiac symptoms (Koster et al. 2001), as was subsequently found for human patients with gain-of-function K_{ATP} channel mutations (Ashcroft 2005; Gloyn et al. 2004a). Additionally mice in which the mutant gene was expressed at a lower level did not develop PNDM, but instead had impaired glucose tolerance (Koster et al. 2006). This provides evidence that mice, as well as humans, develop a spectrum of diabetes phenotypes that correlate with the extent of K_{ATP} channel activity.

In another mouse model, a dominant-negative Kir6.2 mutation (Kir6.2-G132S) was introduced into pancreatic β-cells under the control of the human insulin promoter (Miki et al. 1997). Animals in which K_{ATP} channel was functionally inactivated by this mutation initially exhibited hyperinsulinemia, despite severe hypoglycemia, indicating unregulated insulin secretion, which produces a phenotype resembling CHI. Subsequently, adult mice developed hyperglycemia, and glucose-induced insulin secretion was reduced due to substantial β-cell loss (Miki et al. 1997). In the β-cells of transgenic mice, the resting membrane potential and basal intracellular calcium concentration were significantly higher than in wild-type mice. Transgenic mice also appeared to have abnormal pancreatic islet architecture. In complete contrast, mice expressing a different dominant-negative Kir6.2 mutation ($Kir6.2^{132}A^{133}A^{134}A$) showed no β-cell loss and developed hyperinsulinism as adults (Koster et al. 2002). It was suggested that the opposite phenotype of these mice might arise because K_{ATP} channel activity was only partially suppressed

(30 % of β-cells were unaffected). Patients with CHI usually undergo subtotal pancreatectomy as infants to control hyperinsulinism; however, nonsurgically treated patients often progress to glucose intolerance or diabetes (Huopio et al. 2003). The mouse models suggest that this may reflect a gradual β-cell loss.

Both Kir6.2 and SUR1 knockout mice have been generated (Miki et al. 1998; Seghers et al. 2000). In the case of Kir6.2$^{-/-}$ mice, electrophysiological recordings showed that K_{ATP} channel activity was completely absent in pancreatic β-cells (Miki et al. 1998). These mice showed transient hypoglycemia as neonates, but adult mice had reduced insulin secretion in response to glucose and were normoglycemic. It was suggested that the normoglycemia could be due to an increased glucose lowering effect of insulin in these animals, but the precise mechanism remains unclear. SUR1$^{-/-}$ mice also had markedly reduced glucose-induced insulin secretion but normoglycemia (Seghers et al. 2000). Both Kir6.2$^{-/-}$ and SUR1$^{-/-}$ mice showed a graded glucose-induced rise in intracellular Ca^{2+} and insulin exocytosis, indicating the presence of a K_{ATP}-independent amplifying pathway in glucose-induced insulin secretion (Ravier et al. 2009; Rosario et al. 2008; Nenquin et al. 2004; Szollosi et al. 2007).

Recently, a novel mouse model (β-V59M), which expresses one of the most common Kir6.2 mutations found in PNDM patients, was created (Girard et al. 2008). In human patients, the V59M mutation is the most common cause of i-DEND syndrome (Hattersley and Ashcroft 2005). Importantly, these mice express the V59M Kir6.2 subunit specifically in their pancreatic β-cells. They appear to express comparable levels of WT and V59M Kir6.2 mRNA in pancreatic islets, which is key, when considering the heterozygosity of human patients. The β-V59M mice develop severe diabetes soon after birth, and by 5 weeks of age, blood glucose levels are increased and insulin levels are undetectable. Islets isolated from these mice secreted less insulin and showed smaller increases in intracellular calcium concentrations in response to glucose, compared to wild-type mice. The data also showed that the pancreatic islets had a reduced percentage of β-cell mass, an abnormal morphology, and lower insulin content.

A set of similar mouse models were generated by Remedi et al. in which an ATP-insensitive Kir6.2 mutant, K185Q-ΔN30, was expressed specifically in pancreatic β-cells either from birth or following induction by tamoxifen (Remedi et al. 2009, 2011). These mice develop severe glucose intolerance around 3 weeks of age or within 2 weeks of tamoxifen injection and progress to severe diabetes. The disease state can be avoided by islet transplantation or early-onset sulfonylurea therapy.

While the generation of mouse models of PNDM has provided insights into the pathophysiology of the pancreas in this disease, relatively little is known about the extra-pancreatic symptoms associated with i-DEND and DEND syndrome. It is clear that the neurological features associated with K_{ATP} channel mutations constitute a distinct syndrome rather than a secondary consequence of diabetes. Evidence for this includes the fact that developmental delay is not a feature of neonatal diabetes from other causes (Njolstad et al. 2003; Stoffers et al. 1997; Temple et al. 2000), that there is a strong genotype-phenotype relationship between the functional severity of mutations and the clinical phenotype observed, and that

the neurological features are consistent with the tissue distribution of the K_{ATP} channel in muscle, neurons and the brain (Seino and Miki 2003; Inagaki et al. 1995a). Recent studies on mice expressing the V59M mutation indicate that muscle dysfunction caused by this mutation is neuronal in origin (Clark et al. 2010, 2012). Further work using animal models is needed to understand precisely how mutations in the K_{ATP} channel lead to muscle weakness as well as epilepsy and developmental delay.

Implications for Therapy

Prior to the discovery that PNDM can be caused by mutations in Kir6.2 and SUR1, many patients were assumed to be suffering from early-onset type 1 diabetes. Accordingly they were treated with insulin injections. Recognition that PNDM patients actually possess gain-of-function mutations in K_{ATP} channel genes rapidly led to a switch to sulfonylurea treatment. Sulfonylureas are drugs such as tolbutamide or glibenclamide that specifically block the K_{ATP} channel and thus stimulate insulin secretion. Fortunately, since sulfonylureas had been used to safely treat patients with type 2 diabetes for many years, no clinical trials were required.

To date, most patients with *KCNJ11* and *ABCC8* mutations (>90 %) have successfully transferred from insulin injections to sulfonylurea therapy (Pearson et al. 2006; Rafiq et al. 2008). Not only does this improve their quality of life, it also appears to enhance their blood glucose control. Fluctuations in blood glucose are reported to be reduced (UK Prospective Diabetes Study Group 1998) and there is a decrease in the HbA1C levels, which provide a measure of the average blood glucose level during the preceding weeks (Pearson et al. 2006; Rafiq et al. 2008; Zung et al. 2004). This improvement in glycemic control is predicted to reduce the risk of diabetic complications (UK Prospective Diabetes Study Group 1998; The Diabetes Control and Complications Trial Research Group 1993). Interestingly, oral glucose is more effective than intravenous glucose at eliciting insulin secretion in nondiabetics and patients treated with sulfonylureas alike (Pearson et al. 2006). Oral glucose triggers the release of incretins such as gastrointestinal peptide (GIP) and glucagon-like peptide-1 (GLP-1) from the gut. These hormones do not augment insulin secretion directly through K_{ATP} channel closure, instead they exert their effect through binding to specific receptors present in the β-cell. If, however, intracellular calcium levels are elevated by prior closure of K_{ATP} channels, they are able to amplify insulin secretion (Ashcroft and Rorsman 1989). Prior to sulfonylurea therapy, incretins have no effects in PNDM patients with K_{ATP} channel mutations, as their mutant K_{ATP} channels remain open at very high blood glucose levels (Pearson et al. 2006). Following treatment with sulfonylureas, the mutant K_{ATP} channels close and incretins are able to amplify insulin secretion.

Sulfonylureas are very successful at treating patients with K_{ATP} channel mutations that cause PNDM without neurological complications. With one exception (L164P; Tammaro et al. 2008), these mutations have little or no effect

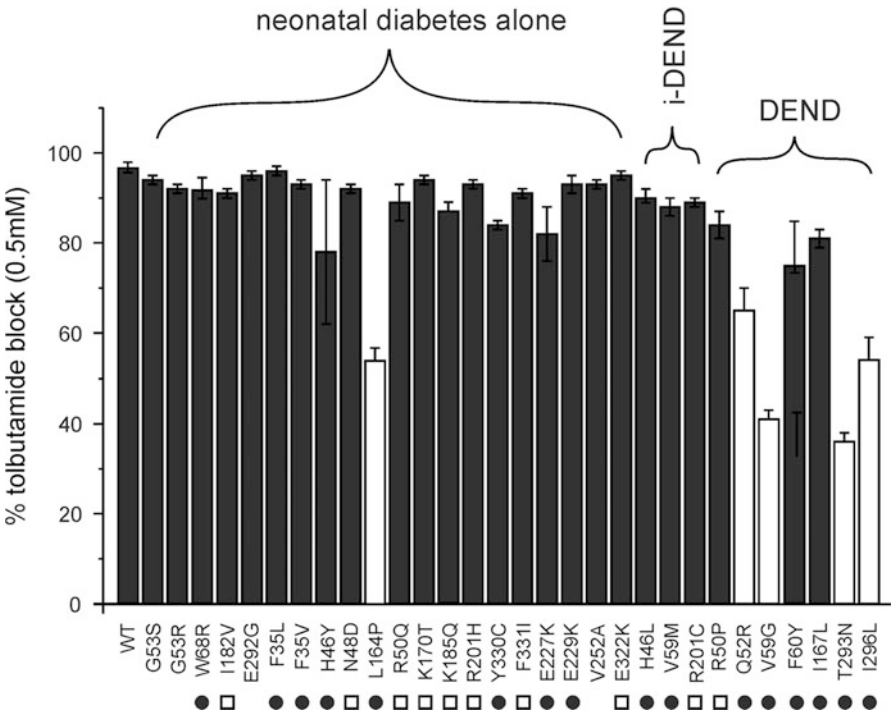

Fig. 5 The efficiency of sulfonylurea block of K_{ATP} channels with NDM mutations in the Kir6.2 subunit. An estimate of the percentage of the tolbutamide block (0.5 mM) of the whole-cell current of wild-type (WT) K_{ATP} channels and heterozygous K_{ATP} channels containing the indicated Kir6.2 mutations. Data were estimated by expressing the block in the presence of tolbutamide and 3 mM azide as a percentage of the current in azide alone. *Black bars* indicate mutant channels without significantly reduced sensitivity to tolbutamide. *White bar*s indicate mutant channels with significantly impaired sulfonylurea sensitivity; patients carrying these mutations were unable to switch to treatment with sulfonylureas (Data are taken from Shimomura et al. (2006, 2007, 2009, 2010), Tammaro et al. (2005), Girard et al. (2006), Proks et al. (2004, 2005a, 2006a), Tammaro et al. (2008), Männikkö et al. (2010, 2011a), Tarasov et al. (2007), Mlynarski et al. (2007))

on sulfonylurea block of the K_{ATP} channel (Pearson et al. 2006; Zung et al. 2004). As summarized in Fig. 5, in functional studies, most heterozygous channels with Kir6.2 mutations remain almost as sensitive to tolbutamide inhibition as wild-type channels, being inhibited between 72 % and 96 % by 0.5 mM of the drug (Pearson et al. 2006). In contrast, patients with mutations that were blocked by <65 % by tolbutamide (Fig. 5, open bars) did not respond to drug therapy. In most cases, sulfonylureas are not effective in DEND patients with Kir6.2 mutations that greatly enhance $P_O(0)$, because of the inability of sulfonylureas to sufficiently block the K_{ATP} channel. Similar to the effect on ATP block, K_{ATP} channel mutations that enhance the channel $P_O(0)$ also impair block by sulfonylureas, and patients with mutations that greatly enhance $P_O(0)$ are thus less likely to be able to transfer to sulfonylurea treatment (Pearson et al. 2006; Rafiq et al. 2008).

There is increasing evidence that sulfonylureas may be able to improve the muscle weakness found in patients with i-DEND syndrome (Shimomura et al. 2007; Slingerland et al. 2006). They may also be able to improve their motor and mental developmental delay. This is significant, since insulin cannot ameliorate the extra-pancreatic symptoms of i-DEND and DEND patients. Sulfonylureas may be able to do so by closing overactive K_{ATP} channels in the brain and muscle as well as the pancreas.

K_{ATP} Channel and Type 2 Diabetes

Given that mutations in Kir6.2 cause neonatal diabetes by decreasing insulin release, it follows that common genetic variations in the same gene, which produce smaller functional effects, may lead to type 2 diabetes later in life. In fact the common Kir6.2 variant, E23K, is strongly linked to an increased risk of type 2 diabetes (Hani et al. 1998; Gloyn et al. 2003; Barroso et al. 2003). The increase in risk is modest, the odds ratio is 1.2, but the high prevalence of the K allele (34 %) makes this a significant population risk. While the genetics is clear, the functional effects of this variant both *in vivo* and *in vitro* are controversial. Both increases and decreases in the ATP sensitivity of Kir6.2/SUR1 channels with E23K mutation have been reported (Schwanstecher et al. 2002; Riedel et al. 2003; Villareal et al. 2009). Others have argued that it is not the K23 variant that is causal but the A1369 variant in SUR1 (Hamming et al. 2009; Fatehi et al. 2012). Considerable variability has also been reported for the effects of the E23K polymorphism on insulin secretion in humans; however, a larger study showed reduced insulin secretion (Villareal et al. 2009). Such result would be compatible with overactivity of the K_{ATP} channel.

Conclusions and Future Directions

Despite over 25 years of intense research into the K_{ATP} channel, many mysteries remain. Where exactly nucleotides and therapeutic drugs bind, and how this binding modulates K_{ATP} channel gating, is still unclear. Insight into this requires high-resolution structural information on Kir6.2 and SUR1 and more detailed functional analyses. Further studies of naturally occurring mutations will be valuable in highlighting key K_{ATP} channel residues. Electrophysiological studies, in combination with biochemical experiments, on the intact K_{ATP} complex are required to understand how exactly these mutations function. The structure of the entire K_{ATP} channel complex would be even more valuable in elucidating how the interaction of nucleotides and drugs with SUR1 is communicated to Kir6.2.

At the clinical level, the discovery that Kir6.2 mutations cause neonatal diabetes has resulted in a major change in treatment for PNDM patients. Most patients are able to successfully transfer from insulin injections to sulfonylurea tablets, with the additional bonus of improving their glycemic control upon doing so. It remains to be determined the extent to which sulfonylureas can improve the extra-pancreatic

symptoms of patients with i-DEND and DEND syndrome and it is unclear why older patients respond less well to sulfonylurea treatment. Tying in with this, the mechanism by which severe Kir6.2 mutations cause muscle weakness, developmental delay and epilepsy remains to be elucidated. These questions are likely to require intense investigation and will not be easy to achieve. Nonetheless the quest for answers about the K_{ATP} channel promises to be an exciting and fruitful adventure.

Acknowledgements The authors would like to thank Dr. Heidi de Wet for valuable comments about the manuscript.

References

Abdulhadi-Atwan M, Bushman J, Tornovsky-Babaey S et al (2008) Novel *de novo* mutation in sulfonylurea receptor 1 presenting as hyperinsulinism in infancy followed by overt diabetes in early adolescence. Diabetes 57:1935–1940

Abraham MR, Selivanov VA, Hodgson DM et al (2002) Coupling of cell energetics with membrane metabolic sensing. Integrative signaling through creatine kinase phosphotransfer disrupted by M-CK gene knock-out. J Biol Chem 277:24427–24434

Aguilar-Bryan L, Nichols CG, Wechsler SW et al (1995) Cloning of the β cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science 268:423–426

Aittoniemi J, Fotinou C, Craig TJ et al (2009) Review. SUR1: a unique ATP-binding cassette protein that functions as an ion channel regulator. Philos Trans R Soc Lond 364:257–267

Amoroso S, Schmid-Antomarchi H, Fosset M et al (1990) Glucose, sulfonylureas, and neurotransmitter release: role of ATP-sensitive K^+ channels. Science 247:852–854

Antcliff JF, Haider S, Proks P et al (2005) Functional analysis of a structural model of the ATP-binding site of the K_{ATP} channel Kir6.2 subunit. EMBO J 24:229–239

Ashcroft FM (2005) ATP-sensitive potassium channelopathies: focus on insulin secretion. J Clin Invest 115:2047–2058

Ashcroft FM (2007) The Walter B. Cannon physiology in perspective lecture, 2007. ATP-sensitive K^+ channels and disease: from molecule to malady. Am J Physiol Endocrinol Metab 293: E880–E889

Ashcroft FM, Rorsman P (1989) Electrophysiology of the pancreatic β-cell. Prog Biophys Mol Biol 54:87–143

Ashcroft FM, Rorsman P (2004) Type 2 diabetes mellitus: not quite exciting enough? Hum Mol Genet 13(Spec No 1):R21–R31

Ashcroft FM, Harrison DE, Ashcroft SJ (1984) Glucose induces closure of single potassium channels in isolated rat pancreatic β-cells. Nature 312:446–448

Avshalumov MV, Rice ME (2003) Activation of ATP-sensitive K^+(K_{ATP}) channels by H_2O_2 underlies glutamate-dependent inhibition of striatal dopamine release. Proc Natl Acad Sci 100:11729–11734

Babenko AP (2008) A novel *ABCC8* (SUR1)-dependent mechanism of metabolism-excitation uncoupling. J Biol Chem 283:8778–8782

Babenko AP, Bryan J (2002) SUR-dependent modulation of K_{ATP} channels by an N-terminal KiR6.2 peptide. Defining intersubunit gating interactions. J Biol Chem 277:43997–44004

Babenko AP, Bryan J (2003) Sur domains that associate with and gate K_{ATP} pores define a novel gatekeeper. J Biol Chem 278:41577–41580

Babenko AP, Vaxillaire M (2011) Mechanism of K_{ATP} hyperactivity and sulfonylurea tolerance due to a diabetogenic mutation in L0 helix of sulfonylurea receptor 1 (*ABCC8*). FEBS Lett 585:3555–3559

Babenko AP, Polak M, Cave H et al (2006) Activating mutations in the *ABCC8* gene in neonatal diabetes mellitus. N Engl J Med 355:456–466

Barroso I, Luan J, Middelberg RP et al (2003) Candidate gene association study in type 2 diabetes indicates a role for genes involved in β-cell function as well as insulin action. PLoS Biol 1:E20

Bingham C, Hattersley AT (2004) Renal cysts and diabetes syndrome resulting from mutations in hepatocyte nuclear factor-1β. Nephrol Dial Transplant 19:2703–2708

Campbell JD, Sansom MS, Ashcroft FM (2003) Potassium channel regulation. EMBO Rep 4:1038–1042

Chutkow WA, Simon MC, Le Beau MM et al (1996) Cloning, tissue expression, and chromosomal localization of SUR2, the putative drug-binding subunit of cardiac, skeletal muscle, and vascular K_{ATP} channels. Diabetes 45:1439–1445

Clark RH, McTaggart JS, Webster R et al (2010) Muscle dysfunction caused by a K_{ATP} channel mutation in neonatal diabetes is neuronal in origin. Science 329:458–461

Clark R, Männikkö R, Stuckey DJ, Iberl M, Clarke K, Ashcroft FM (2012) Mice expressing a human K_{ATP} channel mutation have altered channel ATP sensitivity but no cardiac abnormalities. Diabetologia 55:1195–1204

Clement JP, Kunjilwar K, Gonzalez G et al (1997) Association and stoichiometry of K_{ATP} channel subunits. Neuron 18:827–838

Cook DL, Hales CN (1984) Intracellular ATP directly blocks K^+ channels in pancreatic β-cells. Nature 311:271–273

Craig TJ, Ashcroft FM, Proks P (2008) How ATP inhibits the open K_{ATP} channel. J Gen Physiol 132:131–144

de Wet H, Mikhailov MV, Fotinou C et al (2007a) Studies of the ATPase activity of the ABC protein SUR1. FEBS J 274:3532–3544

de Wet H, Rees MG, Shimomura K et al (2007b) Increased ATPase activity produced by mutations at arginine-1380 in nucleotide-binding domain 2 of *ABCC8* causes neonatal diabetes. Proc Natl Acad Sci 104:18988–18992

de Wet H, Proks P, Lafond M et al (2008) A mutation (R826W) in nucleotide-binding domain 1 of *ABCC8* reduces ATPase activity and causes transient neonatal diabetes. EMBO Rep 9:648–654

de Wet H, Shimomura K, Aittoniemi J et al (2012) A universally conserved residue in the SUR1 subunit of the K_{ATP} channel is essential for translating nucleotide binding at SUR1 into channel opening. J Physiol 590:5025–5036

Delepine M, Nicolino M, Barrett T et al (2000) EIF2AK3, encoding translation initiation factor 2-α kinase 3, is mutated in patients with Wolcott-Rallison syndrome. Nat Genet 25:406–409

Doyle DA, Morais Cabral J, Pfuetzner RA et al (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science 280:69–77

Drain P, Li LH, Wang J (1998) K_{ATP} channel inhibition by ATP requires distinct functional domains of the cytoplasmic C terminus of the pore-forming subunit. Proc Natl Acad Sci 95:13953–13958

Drain P, Geng X, Li L (2004) Concerted gating mechanism underlying K_{ATP} channel inhibition by ATP. Biophys J 86:2101–2112

Dunne MJ, Cosgrove KE, Shepherd RM et al (2004) Hyperinsulinism in infancy: from basic science to clinical disease. Physiol Rev 84:239–275

Edghill EL, Gloyn AL, Gillespie KM (2004) Activating mutations in the *KCNJ11* gene encoding the ATP-sensitive K^+ channel subunit Kir6.2 are rare in clinically defined type 1 diabetes diagnosed before 2 years. Diabetes 53:2998–3001

Edghill EL, Flanagan SE, Ellard S (2010) Permanent neonatal diabetes due to activating mutations in *ABCC8* and *KCNJ11*. Rev Endocr Metab Disord 11:193–198

Ellard S, Flanagan SE, Girard CA et al (2007) Permanent neonatal diabetes caused by dominant, recessive, or compound heterozygous SUR1 mutations with opposite functional effects. Am J Hum Genet 81:375–382

Fatehi M, Raja M, Carter C et al (2012) The ATP-sensitive K^+ channel *ABCC8* S1369A type 2 diabetes risk variant increases MgATPase activity. Diabetes 61:241–249

Flagg TP, Enkvetchakul D, Koster JC, Nichols CG (2010) Muscle K_{ATP} channels: recent insights to energy sensing and myoprotection. Physiol Rev 90:799–829

Flanagan SE, Patch AM, Mackay DJ et al (2007) Mutations in ATP-sensitive K^+ channel genes cause transient neonatal diabetes and permanent diabetes in childhood or adulthood. Diabetes 56:1930–1937

Girard CA, Shimomura K, Proks P et al (2006) Functional analysis of six Kir6.2 (*KCNJ11*) mutations causing neonatal diabetes. Pflugers Arch 453:323–332

Girard CA, Wunderlich FT, Shimomura K (2008) Expression of an activating mutation in the gene encoding the K_{ATP} channel subunit Kir6.2 in mouse pancreatic β-cells recapitulates neonatal diabetes. J Clin Invest 119:80–90

Glaser B, Kesavan P, Heyman M et al (1998) Familial hyperinsulinism caused by an activating glucokinase mutation. N Engl J Med 338:226–230

Glaser B, Thornton P, Otonkoski T et al (2000) Genetics of neonatal hyperinsulinism. Arch Dis Child 82:F79–F86

Gloyn AL, Weedon MN, Owen KR et al (2003) Large-scale association studies of variants in genes encoding the pancreatic β-cell K_{ATP} channel subunits Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) confirm that the *KCNJ11* E23K variant is associated with type 2 diabetes. Diabetes 52:568–572

Gloyn AL, Pearson ER, Antcliff J et al (2004a) Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med 350:1838–1849

Gloyn AL, Cummings EA, Edghill EL et al (2004b) Permanent neonatal diabetes due to paternal germline mosaicism for an activating mutation of the *KCNJ11* gene encoding the Kir6.2 subunit of the β-cell potassium adenosine triphosphate channel. J Clin Endocrinol Metab 89:3932–3935

Gloyn AL, Reimann F, Girard C et al (2005) Relapsing diabetes can result from moderately activating mutations in *KCNJ11*. Hum Mol Genet 14:925–934

Gloyn AL, Siddiqui J, Ellard S (2006) Mutations in the genes encoding the pancreatic β-cell K_{ATP} channel subunits Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) in diabetes mellitus and hyperinsulinism. Hum Mutat 27:220–231

Gopel SO, Kanno T, Barg S et al (2000a) Regulation of glucagon release in mouse α-cells by K_{ATP} channels and inactivation of TTX-sensitive Na^+ channels. J Physiol 528:509–520

Gopel SO, Kanno T, Barg S et al (2000b) Patch-clamp characterisation of somatostatin-secreting δ-cells in intact mouse pancreatic islets. J Physiol 528:497–507

Gribble FM, Tucker SJ, Ashcroft FM (1997) The essential role of the Walker A motifs of SUR1 in K-ATP channel activation by Mg-ADP and diazoxide. EMBO J 16:1145–1152

Gribble FM, Williams L, Simpson AK et al (2003) A novel glucose-sensing mechanism contributing to glucagon-like peptide-1 secretion from the GLU Tag cell line. Diabetes 52:1147–1154

Griesemer D, Zawar C, Neumcke B (2002) Cell-type specific depression of neuronal excitability in rat hippocampus by activation of ATP-sensitive potassium channels. Eur Biophys J 31:467–477

Gumina RJ, Pucar D, Bast P et al (2003) Knockout of Kir6.2 negates ischemic preconditioning-induced protection of myocardial energetics. Am J Physiol 284:H2106–H2113

Hamming KS, Soliman D, Matemisz LC et al (2009) Coexpression of the type 2 diabetes susceptibility gene variants *KCNJ11* E23K and *ABCC8* S1369A alter the ATP and sulfonylurea sensitivities of the ATP-sensitive K^+ channel. Diabetes 58:2419–2424

Hani EH, Boutin P, Durand E et al (1998) Missense mutations in the pancreatic islet β cell inwardly rectifying K^+ channel gene (KIR6.2/BIR): a meta-analysis suggests a role in the polygenic basis of Type II diabetes mellitus in Caucasians. Diabetologia 41:1511–1515

Hattersley AT, Ashcroft FM (2005) Activating mutations in Kir6.2 and neonatal diabetes: new clinical syndromes, new scientific insights, and new therapy. Diabetes 54:2503–2513

Henwood MJ, Kelly A, Macmullen C et al (2005) Genotype-phenotype correlations in children with congenital hyperinsulinism due to recessive mutations of the adenosine triphosphate-sensitive potassium channel genes. J Clin Endocrinol Metab 90:789–794

Hernandez-Sanchez C, Basile AS, Fedorova I et al (2001) Mice transgenically overexpressing sulfonylurea receptor 1 in forebrain resist seizure induction and excitotoxic neuron death. Proc Natl Acad Sci 98:3549–3554

Heron-Milhavet L, Xue-Jun Y, Vannucci SJ et al (2004) Protection against hypoxic-ischemic injury in transgenic mice overexpressing Kir6.2 channel pore in forebrain. Mol Cell Neurosci 25:585–593

Huopio H, Reimann F, Ashfield R et al (2000) Dominantly inherited hyperinsulinism caused by a mutation in the sulfonylurea receptor type 1. J Clin Invest 106:897–906

Huopio H, Otonkoski T, Vauhkonen I et al (2003) A new subtype of autosomal dominant diabetes attributable to a mutation in the gene for sulfonylurea receptor 1. Lancet 361:301–307

Inagaki N, Gonoi T, Clement JP (1995a) Reconstitution of IK_{ATP}: an inward rectifier subunit plus the sulfonylurea receptor. Science 270:1166–1170

Inagaki N, Tsuura Y, Namba N et al (1995b) Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle, and heart. J Biol Chem 270:5691–5694

Inagaki N, Gonoi T, Clement JP et al (1996) A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. Neuron 16:1011–1017

Isomoto S, Kondo C, Yamada M et al (1996) A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. J Biol Chem 271:24321–24324

John SA, Weiss JN, Ribalet B (2001) Regulation of cloned ATP-sensitive K channels by adenine nucleotides and sulfonylureas: interactions between SUR1 and positively charged domains on Kir6.2. J Gen Physiol 118:391–405

John SA, Weiss JN, Xie LH et al (2003) Molecular mechanism for ATP-dependent closure of the K^+ channel Kir6.2. J Physiol 552:23–34

Kanno T, Rorsman P, Gopel SO (2002a) Glucose-dependent regulation of rhythmic action potential firing in pancreatic β-cells by K_{ATP}-channel modulation. J Physiol 545:501–507

Kanno T, Gopel SO, Rorsman P et al (2002b) Cellular function in multicellular system for hormone-secretion: electrophysiological aspect of studies on α-, β- and δ-cells of the pancreatic islet. Neurosci Res 42:79–90

Klupa T, Warram JH, Antonellis A et al (2002) Determinants of the development of diabetes (maturity-onset diabetes of the young-3) in carriers of HNF-1 α mutations: evidence for parent-of-origin effect. Diabetes Care 25:2292–2301

Koster JC, Marshall BA, Ensor N et al (2000) Targeted overactivity of β cell K_{ATP} channels induces profound neonatal diabetes. Cell 100:645–654

Koster JC, Knopp A, Flagg TP et al (2001) Tolerance for ATP-insensitive K_{ATP} channels in transgenic mice. Circ Res 89:1022–1029

Koster JC, Remedi MS, Flagg TP et al (2002) Hyperinsulinism induced by targeted suppression of β cell K_{ATP} channels. Proc Natl Acad Sci 99:16992–16997

Koster JC, Remedi MS, Masia R et al (2006) Expression of ATP-insensitive K_{ATP} channels in pancreatic β-cells underlies a spectrum of diabetic phenotypes. Diabetes 55:2957–2964

Koster JC, Kurata HT, Enkvetchakul D et al (2008) A DEND mutation in Kir6.2 (*KCNJ11*) reveals a flexible N-terminal region critical for ATP-sensing of the K_{ATP} channel. Biophys J 95:4689–4697

Kuo A, Gulbis JM, Antcliff JF et al (2003) Crystal structure of the potassium channel KirBac1.1 in the closed state. Science 300:1922–1926

Li L, Geng X, Drain P (2002) Open state destabilization by ATP occupancy is mechanism speeding burst exit underlying K_{ATP} channel inhibition by ATP. J Gen Physiol 119:105–116

Lin YW, Bushman JD, Yan FF et al (2008) Destabilization of ATP-sensitive potassium channel activity by novel *KCNJ11* mutations identified in congenital hyperinsulinism. J Biol Chem 283:9146–9156

Lin YW, Akrouh A, Hsu Y et al (2012) Compound heterozygous mutations in the SUR1 (ABCC 8) subunit of pancreatic K_{ATP} channels cause neonatal diabetes by perturbing the coupling between Kir6.2 and SUR1 subunits. Channels 6:133–138

Liss B, Bruns R, Roeper J (1999) Alternative sulfonylurea receptor expression defines metabolic sensitivity of K_{ATP} channels in dopaminergic midbrain neurons. EMBO J 18:833–846

MacDonald PE, De Marinis YZ, Ramracheya R et al (2007) AK $_{ATP}$ channel-dependent pathway within α cells regulates glucagon release from both rodent and human islets of Langerhans. PLoS Biol 5:e143

Magge SN, Shyng SL, MacMullen C et al (2004) Familial leucine-sensitive hypoglycemia of infancy due to a dominant mutation of the β-cell sulfonylurea receptor. J Clin Endocrinol Metab 89:4450–4456

Männikkö R, Jefferies C, Flanagan SE, Hattersley A, Ellard S, Ashcroft FM (2010) Interaction between mutations in the slide helix of Kir6.2 associated with neonatal diabetes and neurological symptoms. Hum Mol Genet 19:963–972

Männikkö R, Stansfeld PJ, Ashcroft AS, Hattersley AT, Sansom MS, Ellard S, Ashcroft FM (2011a) A conserved tryptophan at the membrane-water interface acts as a gatekeeper for Kir6.2/SUR1 channels and causes neonatal diabetes when mutated. J Physiol 589:3071–3083

Männikkö R, Flanagan SE, Sim X et al (2011b) Mutations of the same conserved glutamate residue in NBD2 of the sulfonylurea receptor 1 subunit of the K_{ATP} channel can result in either hyperinsulinism or neonatal diabetes. Diabetes 60:1813–1822

Mao Q, Leslie EM, Deeley RG et al (1999) ATPase activity of purified and reconstituted multidrug resistance protein MRP1 from drug-selected H69AR cells. Biochim Biophys Acta 1461:69–82

Markworth E, Schwanstecher C, Schwanstecher M (2000) ATP^{4-} mediates closure of pancreatic β-cell ATP-sensitive potassium channels by interaction with 1 of 4 identical sites. Diabetes 49:1413–1418

Marthinet E, Bloc A, Oka Y et al (2005) Severe congenital hyperinsulinism caused by a mutation in the Kir6.2 subunit of the adenosine triphosphate-sensitive potassium channel impairing trafficking and function. J Clin Endocrinol Metab 90:5401–5406

Masia R, Koster JC, Tumini S et al (2007a) An ATP-binding mutation (G334D) in *KCNJ11* is associated with a sulfonylurea-insensitive form of developmental delay, epilepsy, and neonatal diabetes. Diabetes 56:328–336

Masia R, De Leon DD, MacMullen C et al (2007b) A mutation in the TMD0-L0 region of sulfonylurea receptor-1 (L225P) causes permanent neonatal diabetes mellitus (PNDM). Diabetes 56:1357–1362

Matsuo M, Tanabe K, Kioka N et al (2000) Different binding properties and affinities for ATP and ADP among sulfonylurea receptor subtypes, SUR1, SUR2A, and SUR2B. J Biol Chem 275:28757–28763

Mikhailov MV, Campbell JD, de Wet H et al (2005) 3-D structural and functional characterization of the purified K_{ATP} channel complex Kir6.2-SUR1. EMBO J 24:4166–4175

Miki T, Seino S (2005) Roles of K_{ATP} channels as metabolic sensors in acute metabolic changes. J Mol Cell Cardiol 38:917–925

Miki T, Tashiro F, Iwanaga T et al (1997) Abnormalities of pancreatic islets by targeted expression of a dominant-negative K_{ATP} channel. Proc Natl Acad Sci 94:11969–11973

Miki T, Nagashima K, Tashiro F et al (1998) Defective insulin secretion and enhanced insulin action in K_{ATP} channel-deficient mice. Proc Natl Acad Sci 95:10402–10406

Miki T, Liss B, Minami K et al (2001) ATP-sensitive K^+ channels in the hypothalamus are essential for the maintenance of glucose homeostasis. Nat Neurosci 4:507–512

Miki T, Minami K, Zhang L et al (2002) ATP-sensitive potassium channels participate in glucose uptake in skeletal muscle and adipose tissue. Am J Physiol Endocrinol Metab 283: E1178–E1184

Mlynarski W, Tarasov AI, Gach A et al (2007) Sulfonylurea improves CNS function in a case of intermediate DEND syndrome caused by a mutation in *KCNJ11*. Nat Clin Pract Neurol 3:640–645

Nenquin M, Szollosi A, Aguilar-Bryan L et al (2004) Both triggering and amplifying pathways contribute to fuel-induced insulin secretion in the absence of sulfonylurea receptor-1 in pancreatic β-cells. J Biol Chem 279:32316–32324

Nestorowicz A, Inagaki N, Gonoi T et al (1997) A nonsense mutation in the inward rectifier potassium channel gene, Kir6.2, is associated with familial hyperinsulinism. Diabetes 46:1743–1748

Nichols CG (2006) K_{ATP} channels as molecular sensors of cellular metabolism. Nature 440:470–476

Nichols CG, Shyng SL, Nestorowicz A et al (1996) Adenosine diphosphate as an intracellular regulator of insulin secretion. Science 272:1785–1787

Nishida M, MacKinnon R (2002) Structural basis of inward rectification: cytoplasmic pore of the G protein-gated inward rectifier GIRK1 at 1.8 A resolution. Cell 111:957–965

Nishida M, Cadene M, Chait BT et al (2007) Crystal structure of a Kir3.1-prokaryotic Kir channel chimera. EMBO J 26:4005–4015

Njolstad PR, Sovik O, Cuesta-Munoz A et al (2001) Neonatal diabetes mellitus due to complete glucokinase deficiency. N Engl J Med 344:1588–1592

Njolstad PR, Sagen JV, Bjorkhaug L et al (2003) Permanent neonatal diabetes caused by glucokinase deficiency: inborn error of the glucose-insulin signaling pathway. Diabetes 52:2854–2860

Ortiz D, Voyvodic P, Gossack L et al (2012) Two neonatal diabetes mutations on transmembrane helix 15 of SUR1 increase affinity for ATP and ADP at nucleotide binding domain 2. J Biol Chem 287:17985–17995

Partridge CJ, Beech DJ, Sivaprasadarao A (2001) Identification and pharmacological correction of a membrane trafficking defect associated with a mutation in the sulfonylurea receptor causing familial hyperinsulinism. J Biol Chem 276:35947–35952

Pearson ER, Flechtner I, Njolstad PR et al (2006) Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. N Engl J Med 355:467–477

Pegan S, Arrabit C, Zhou W et al (2005) Cytoplasmic domain structures of Kir2.1 and Kir3.1 show sites for modulating gating and rectification. Nat Neurosci 8:279–287

Polak M, Shield J (2004) Neonatal and very-early-onset diabetes mellitus. Semin Neonatol 9:59–65

Porter JR, Shaw NJ, Barrett TG et al (2005) Permanent neonatal diabetes in an Asian infant. J Pediatr 146:131–133

Proks P, Ashcroft FM (2009) Modeling K_{ATP} channel gating and its regulation. Prog Biophys Mol Biol 99:7–19

Proks P, Antcliff JF, Lippiat J et al (2004) Molecular basis of Kir6.2 mutations associated with neonatal diabetes or neonatal diabetes plus neurological features. Proc Natl Acad Sci 101:17539–17544

Proks P, Girard C, Haider S et al (2005a) A gating mutation at the internal mouth of the Kir6.2 pore is associated with DEND syndrome. EMBO Rep 6:470–475

Proks P, Girard C, Ashcroft FM (2005b) Functional effects of *KCNJ11* mutations causing neonatal diabetes: enhanced activation by MgATP. Hum Mol Genet 14:2717–2726

Proks P, Girard C, Baevre H et al (2006a) Functional effects of mutations at F35 in the NH2-terminus of Kir6.2 (*KCNJ11*), causing neonatal diabetes, and response to sulfonylurea therapy. Diabetes 55:1731–1777

Proks P, Arnold AL, Bruining J et al (2006b) A heterozygous activating mutation in the sulphonylurea receptor SUR1 (*ABCC8*) causes neonatal diabetes. Hum Mol Genet 15:1793–1800

Proks P, Shimomura K, Craig TJ et al (2007) Mechanism of action of a sulphonylurea receptor SUR1 mutation (F132L) that causes DEND syndrome. Hum Mol Genet 16:2011–2019

Proks P, de Wet H, Ashcroft FM (2010) Activation of the K_{ATP} channel by Mg-nucleotide interaction with SUR1. J Gen Physiol 136:389–405

Rafiq M, Flanagan SE, Patch AM et al (2008) Effective treatment with oral sulfonylureas in patients with diabetes due to sulfonylurea receptor 1 (SUR1) mutations. Diabetes Care 31:204–209

Ravier MA, Nenquin M, Miki T et al (2009) Glucose controls cytosolic Ca^{2+} and insulin secretion in mouse islets lacking adenosine triphosphate-sensitive K^+ channels owing to a knockout of the pore-forming subunit Kir6.2. Endocrinology 150:33–45

Reimann F, Tucker SJ, Proks P et al (1999) Involvement of the n-terminus of Kir6.2 in coupling to the sulphonylurea receptor. J Physiol 518:325–336

Remedi MS, Kurata HT, Scott A et al (2009) Secondary consequences of β cell inexcitability: identification and prevention in a murine model of K_{ATP}-induced neonatal diabetes mellitus. Cell Metab 9:140–151

Remedi MS, Agapova SE, Vyas AK, Hruz PW, Nichols CG (2011) Acute sulfonylurea therapy at disease onset can cause permanent remission of K_{ATP}-induced diabetes. Diabetes 60:2515–2522

Riedel MJ, Boora P, Steckley D et al (2003) Kir6.2 polymorphisms sensitize β-cell ATP-sensitive potassium channels to activation by acyl CoAs: a possible cellular mechanism for increased susceptibility to type 2 diabetes? Diabetes 52:2630–2635

Rorsman P, Salehi SA, Abdulkader F et al (2008) K_{ATP} channels and glucose-regulated glucagon secretion. Trends Endocrinol Metab 19:277–284

Rosario LM, Barbosa RM, Antunes CM et al (2008) Regulation by glucose of oscillatory electrical activity and 5-HT/insulin release from single mouse pancreatic islets in absence of functional K_{ATP} channels. Endocr J 55:639–650

Sagen JV, Raeder H, Hathout E et al (2004) Permanent neonatal diabetes due to mutations in *KCNJ11* encoding Kir6.2: patient characteristics and initial response to sulfonylurea therapy. Diabetes 53:2713–2718

Sakura H, Ammala C, Smith PA et al (1995) Cloning and functional expression of the cDNA encoding a novel ATP-sensitive potassium channel subunit expressed in pancreatic β-cells, brain, heart and skeletal muscle. FEBS Lett 377:338–344

Schmid-Antomarchi H, Amoroso S, Fosset M et al (1990) K^+ channel openers activate brain sulfonylurea-sensitive K^+ channels and block neurosecretion. Proc Natl Acad Sci 87:3489–3492

Schwanstecher C, Meyer U, Schwanstecher M (2002) KIR6.2 polymorphism predisposes to type 2 diabetes by inducing overactivity of pancreatic β-cell ATP-sensitive K^+ channels. Diabetes 51:875–879

Seghers V, Nakazaki M, DeMayo F et al (2000) Sur1 knockout mice. A model for K_{ATP} channel-independent regulation of insulin secretion. J Biol Chem 275:9270–9277

Seino S, Miki T (2003) Physiological and pathophysiological roles of ATP-sensitive K^+ channels. Prog Biophys Mol Biol 81:133–176

Seino S, Miki T (2004) Gene targeting approach to clarification of ion channel function: studies of Kir6.x null mice. J Physiol 554:295–300

Sellick GS, Barker KT, Stolte-Dijkstra I et al (2004) Mutations in PTF1A cause pancreatic and cerebellar agenesis. Nat Genet 36:1301–1305

Shi NQ, Ye B, Makielski JC (2005) Function and distribution of the SUR isoforms and splice variants. J Mol Cell Cardiol 39:51–60

Shimomura K, Girard CA, Proks P et al (2006) Mutations at the same residue (R50) of Kir6.2 (*KCNJ11*) that cause neonatal diabetes produce different functional effects. Diabetes 55:1705–1712

Shimomura K, Horster F, de Wet H et al (2007) A novel mutation causing DEND syndrome: a treatable channelopathy of pancreas and brain. Neurology 69:1342–1349

Shimomura K, Flanagan SE, Zadek B, Lethby M, Zubcevic L, Girard CA, Petz O, Mannikko R, Kapoor RR, Hussain K, Skae M, Clayton P, Hattersley A, Ellard S, Ashcroft FM (2009) Adjacent mutations in the gating loop of Kir6.2 produce neonatal diabetes and hyperinsulinism. EMBO Mol Med 1:166–177

Shimomura K, de Nanclares GP, Foutinou C, Caimari M, Castaño L, Ashcroft FM (2010) The first clinical case of a mutation at residue K185 of Kir6.2 (*KCNJ11*): a major ATP-binding residue. Diabet Med 27:225–229

Shyng S, Nichols CG (1997) Octameric stoichiometry of the K_{ATP} channel complex. J Gen Physiol 110:655–664

Shyng SL, Ferrigni T, Nichols CG (1997) Regulation of K_{ATP} channel activity by diazoxide and MgADP. Distinct functions of the two nucleotide binding folds of the sulfonylurea receptor. J Gen Physiol 110:643–654

Slingerland AS, Nuboer R, Hadders-Algra M et al (2006) Improved motor development and good long-term glycaemic control with sulfonylurea treatment in a patient with the syndrome of intermediate developmental delay, early-onset generalised epilepsy and neonatal diabetes associated with the V59M mutation in the *KCNJ11* gene. Diabetologia 49:2559–2563

Stanley CA, Lieu YK, Hsu B et al (1998) Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. N Engl J Med 338:1352–1357

Stoffers DA, Zinkin NT, Stanojevic V (1997) Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. Nat Genet 15:106–110

Suzuki M, Sasaki N, Miki T et al (2002) Role of sarcolemmal K_{ATP} channels in cardioprotection against ischemia/reperfusion injury in mice. J Clin Invest 109:509–516

Szollosi A, Nenquin M, Aguilar-Bryan L et al (2007) Glucose stimulates Ca^{2+} influx and insulin secretion in 2-week-old β-cells lacking ATP-sensitive K^+ channels. J Biol Chem 282:1747–1756

Tammaro P, Girard C, Molnes J et al (2005) Kir6.2 mutations causing neonatal diabetes provide new insights into Kir6.2-SUR1 interactions. EMBO J 24:2318–2330

Tammaro P, Flanagan SE, Zadek B et al (2008) A Kir6.2 mutation causing severe functional effects *in vitro* produces neonatal diabetes without the expected neurological complications. Diabetologia 51:802–810

Taneja TK, Mankouri J, Karnik R et al (2009) Sar1-GTPase-dependent ER exit of K_{ATP} channels revealed by a mutation causing congenital hyperinsulinism. Hum Mol Genet 18:2400–2413

Tarasov AI, Girard CA, Ashcroft FM (2006a) ATP sensitivity of the ATP-sensitive K^+ channel in intact and permeabilized pancreatic β-cells. Diabetes 55:2446–2454

Tarasov AI, Welters HJ, Senkel S et al (2006b) A Kir6.2 mutation causing neonatal diabetes impairs electrical activity and insulin secretion from INS-1 β-cells. Diabetes 55:3075–3082

Tarasov AI, Girard CA, Larkin B et al (2007) Functional analysis of two Kir6.2 (*KCNJ11*) mutations, K170T and E322K, causing neonatal diabetes. Diabetes Obes Metab 9(Suppl 2):46–55

Taschenberger G, Mougey A, Shen S et al (2002) Identification of a familial hyperinsulinism-causing mutation in the sulfonylurea receptor 1 that prevents normal trafficking and function of K_{ATP} channels. J Biol Chem 277:17139–17146

Temple IK, James RS, Crolla JA et al (1995) An imprinted gene(s) for diabetes? Nat Genet 9:110–112

Temple IK, Gardner RJ, Mackay DJ et al (2000) Transient neonatal diabetes: widening the understanding of the etiopathogenesis of diabetes. Diabetes 49:1359–1366

The Diabetes Control and Complications Trial Research Group (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 329:977–986

Thomas PM, Cote GJ, Wohllk N et al (1995) Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. Science 268:426–429

Thomas PM, Ye Y, Lightner E (1996) Mutation of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. Hum Mol Genet 5:1809–1812

Trapp S, Proks P, Tucker SJ et al (1998) Molecular analysis of ATP-sensitive K channel gating and implications for channel inhibition by ATP. J Gen Physiol 112:333–349

Tucker SJ, Gribble FM, Zhao C et al (1997) Truncation of Kir6.2 produces ATP-sensitive K^+ channels in the absence of the sulphonylurea receptor. Nature 387:179–183

UK Prospective Diabetes Study Group (1998) Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 352:837–853

Vaxillaire M, Populaire C, Busiah K et al (2004) Kir6.2 mutations are a common cause of permanent neonatal diabetes in a large cohort of French patients. Diabetes 53:2719–2722

Villareal DT, Koster JC, Robertson H et al (2009) Kir6.2 variant E23K increases ATP-sensitive K^+ channel activity and is associated with impaired insulin release and enhanced insulin sensitivity in adults with normal glucose tolerance. Diabetes 58:1869–1878

Wang R, Liu X, Hentges ST et al (2004) The regulation of glucose-excited neurons in the hypothalamic arcuate nucleus by glucose and feeding-relevant peptides. Diabetes 53:1959–1965

Wildin RS, Ramsdell F, Peake J et al (2001) X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet 27:18–20

Yamada K, Ji JJ, Yuan H et al (2001) Protective role of ATP-sensitive potassium channels in hypoxia-induced generalized seizure. Science 292:1543–1556

Yan F, Lin CW, Weisiger E et al (2004) Sulfonylureas correct trafficking defects of ATP-sensitive potassium channels caused by mutations in the sulfonylurea receptor. J Biol Chem 279:11096–11105

Yorifuji T, Nagashima K, Kurokawa K, Kawai M, Oishi M, Yoshiharu A, Hosokawa M, Yanada Y, Inagaki N, Nakahete T (2005) The C42R mutation in the Kir6.2 (*KCNJ11*) gene as a cause of transient neonatal diabetes, childhood diabetes, or later-onset, apparently type 2 diabetes mellitus. J Clin Endocrinol Metab 90:3174–3178

Zawar C, Plant TD, Schirra C et al (1999) Cell-type specific expression of ATP-sensitive potassium channels in the rat hippocampus. J Physiol 514(Pt 2):327–341

Zerangue N, Schwappach B, Jan YN et al (1999) A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K_{ATP} channels. Neuron 22:537–548

Zhou Q, Garin I, Castaño L et al (2010) Neonatal diabetes caused by mutations in sulfonylurea receptor 1: interplay between expression and Mg-nucleotide gating defects of ATP-sensitive potassium channels. J Clin Endocrinol Metab 95:E473–E478

Zingman LV, Alekseev AE, Bienengraeber M et al (2001) Signaling in channel/enzyme multimers: ATPase transitions in SUR module gate ATP-sensitive K^+ conductance. Neuron 31:233–245

Zingman LV, Hodgson DM, Bast PH et al (2002) Kir6.2 is required for adaptation to stress. Proc Natl Acad Sci 99:13278–13283

Zung A, Glaser B, Nimri R et al (2004) Glibenclamide treatment in permanent neonatal diabetes mellitus due to an activating mutation in Kir6.2. J Clin Endocrinol Metab 89:5504–5507

β Cell Store-Operated Ion Channels

12

Colin A. Leech, Richard F. Kopp, Louis H. Philipson, and Michael W. Roe

Contents

Abstract

Signaling molecules produced in the pancreatic β-cell following mitochondrial oxidation of glycolytic intermediate metabolites and oxidative phosphorylation trigger Ca^{2+}-dependent signaling pathways that regulate insulin exocytosis. Much is known about ATP-sensitive K^+ and voltage-gated Ca^{2+} currents that contribute to Ca^{2+}-dependent signal transduction in β-cells and insulin secretion, but relatively little is known about other Ca^{2+} channels that regulate β-cell Ca^{2+} signaling dynamics and insulin secretion. In a wide range of eukaryotic cells, store-operated Ca^{2+} entry (SOCE) plays a critical role regulating spatial and temporal changes in cytoplasmic Ca^{2+} concentration, endoplasmic reticulum (ER)

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_40,

Ca^{2+} homeostasis, gene expression, protein biosynthesis, and cell viability. Although SOCE has been proposed to play important roles in β-cell Ca^{2+} signaling and insulin secretion, the underlying molecular mechanisms remain undefined. In this chapter, we provide both an overview of our current understanding of ionic currents regulated by ER Ca^{2+} stores in insulin-secreting cells and a review of studies in other cell systems that have identified the molecular basis and regulation of SOCE.

Keywords

Calcium signaling • Stimulus-secretion coupling • Store-operated ion channels • Store-operated calcium entry • Calcium release-activated calcium channel • Calcium release-activated nonselective cation channel • Stromal interaction molecule • TRP channels • Orai • Insulin secretion

Introduction

Store-operated Ca^{2+} entry (SOCE) plays a critical role in regulating spatial and temporal changes in cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_c$), endoplasmic reticulum (ER) Ca^{2+} homeostasis and protein biosynthesis, mitochondrial function, secretion, and cell viability. The ER regulates Ca^{2+} signaling by two main pathways: [a] the lumen of the ER is a subcellular site of sequestration or store for Ca^{2+} that is rapidly and transiently released into the cytoplasm following exposure of cells to stimuli that open Ca^{2+} channels in the ER membrane (e.g., inositol 1,4,5-*tris*phosphate receptors and ryanodine receptors), and [b] Ca^{2+} concentration in the ER ($[Ca^{2+}]_{er}$) regulates gating of store-operated cation (SOC) channels located in the plasma membrane (PM, Fig. 1).

A cation conductance activated by depletion of intracellular Ca^{2+} stores is present in pancreatic β-cells. Although the SOC channel current (I_{SOC}) has been proposed to regulate glucose-stimulated changes in β-cell membrane potential, $[Ca^{2+}]_c$ oscillations and insulin secretion (Roe et al. 1998), the molecular identity of the SOC channel and the mechanisms that control its activity remain unresolved. The expression of I_{SOC} in β-cells was proposed from studies showing β-cell depolarization and Mn^{2+} quenching of fura-2 fluorescence following the depletion of Ca^{2+} stores (Leech et al. 1994; Worley et al. 1994a, b). I_{SOC} was determined to be a nonselective cation current, rather than a Ca^{2+}-selective current (Worley et al. 1994; Roe et al. 1998). The classical Ca^{2+} release-activated Ca^{2+} current (I_{CRAC}) in non-β-cells is most frequently formed by stromal interaction molecule 1 (STIM1) and Orai1. The nonselective SOC in β-cells suggests a different subunit composition that has yet to be determined but could involve TRPC1, a channel that is expressed in β-cells (Sakura and Ashcroft 1997; Qian et al. 2002; Jacobson and Philipson 2007). TRPC1 is known to associate with STIM1/Orai1 in other cell types

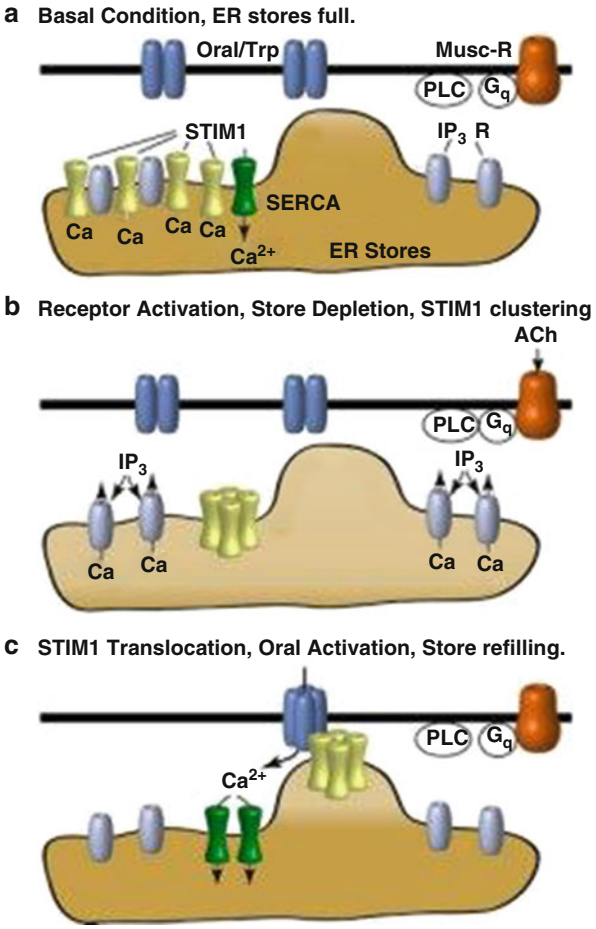

Fig. 1 (**a**) **Basal conditions, ER stores full**. Under resting conditions, the ER Ca^{2+} stores are full, Ca^{2+} binds the EF-hand domains in STIM1, and inositol trisphosphate receptors (IP_3R) are closed. Leakage from the stores is opposed by sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity pumping Ca^{2+} into the store. Only the ER Ca^{2+} store is illustrated for simplicity, other stores may also be important, described in text. Orai channel proteins are present as dimers in the plasma membrane along with TRP family channels. (**b**) **Receptor activation, store depletion, and Stim1 clustering**. Activation of Gq-coupled receptors, illustrated is the muscarinic receptor, activates phospholipase C (PLC) to generate inositol 1,4,5-*tris*phosphate (IP_3) and diacylglycerol (not shown). IP_3 activates IP_3Rs allowing Ca^{2+} to leave the stores and causing Ca^{2+} levels to fall, sensed by STIM1. Store depletion causes clustering of STIM1. (**c**) **STIM1 translocation, Orai activation, and store refilling**. After STIM1 clusters, it translocates to punctae associated with the plasma membrane where it induces dimerization of Orai dimers to form a tetrameric channel that is activated by interaction with cytosolic domains of STIM1. Activation of this channel allows Ca^{2+} influx, and SERCA activity leads to store refilling and inactivation of store-operated Ca^{2+} entry. Further details provided in text

to generate a nonselective cation current that can be activated through both store-dependent and store-independent mechanisms (Beech 2005; Yuan et al. 2009; Lu et al. 2010). The identification of STIM1 as a Ca^{2+} sensor that responds to changes in ER Ca^{2+} levels and Orai1 as a plasma membrane channel-forming protein that is regulated by STIM1 has led to renewed interest in store-operated Ca^{2+} entry in β-cells. The identification of other channel-forming proteins in β-cells from the transient receptor potential (TRP) family has further broadened interest in this field. The relative ease of obtaining gene knockout mice has enabled a rapid surge in our appreciation of the role of these channel-forming proteins, although much remains to be elucidated.

This review will focus on the current state of knowledge regarding store-operated and store-independent currents in β-cells and their role in regulating insulin secretion and β-cell survival. We will also discuss candidate molecules for SOCs in β-cells in light of their functional role in these cells and other cell types.

Biophysical Characteristics of Store-Operated Ion Currents in β-Cells

The expression of SOC channels in β-cells was proposed from biophysical studies showing β-cell depolarization and Mn^{2+} quenching of fura-2 fluorescence following the depletion of Ca^{2+} stores (Leech et al. 1994; Worley et al. 1994a; Worley et al. 1994b). Enhancement of intracellular [Ca^{2+}] responses to pulses of high extracellular [Ca^{2+}] following depletion of acetylcholine-sensitive stores was also reported, consistent with an SOCE mechanism (Silva et al. 1994). Patch clamp electrophysiology has been used to characterize some of the properties of I_{SOC} in β-cell model cell lines and primary β-cells isolated from rodent islets of Langerhans (Worley et al. 1994a; Worley et al. 1994b; Roe et al. 1998). It was proposed that the store-operated current in β-cells is activated by maitotoxin (MTX) (Roe et al. 1998). The MTX-sensitive conductance has a linear current–voltage relation and is carried through a Ca^{2+}-dependent nonselective cation channel (Leech and Habener 1997; Roe et al. 1998), rather than a Ca^{2+}-selective CRAC channel. The identity of the MTX-sensitive conductance is unknown, but it appears to be expressed in nearly every cell studied. The CRAC current normally shows strong inward rectification and is highly selective for Ca^{2+} over monovalent cations, although channel selectivity can be modulated to increase monovalent ion permeability (Zweifach and Lewis 1996; Kerschbaum and Cahalan 1998; Konno et al. 2012). The relation between the store-operated current and the nonselective cation current in β-cells remains to be fully determined. Based on our recent studies, we propose that the nonselective cation current is activated by increased cytosolic Ca^{2+} rather than by store depletion, likely to be carried through TRPM4/5 channels that have been shown to play a role in regulating insulin secretion (Jacobson and Philipson 2007; Enklaar et al. 2010; Islam 2011), whereas the store-operated current is a Ca^{2+}-selective current mediated by STIM/Orai.

Another characteristic of SOCE is that it undergoes slow, Ca^{2+}-dependent inactivation as $[Ca^{2+}]_c$ increases. This mechanism is believed to prevent Ca^{2+} overload and is mediated through a domain near the cytosolic tail of STIM1 (Fig. 2). This inhibitory domain within STIM1 regulates the binding of an ER resident STIM-inhibitory protein, TMEM66/SARAF (SOCE-associated regulatory factor), to the STIM1 Orai1 activation region (SOAR) (Palty et al. 2012; Jha et al. 2013). SARAF responds to elevated $[Ca^{2+}]_c$ and inhibits both STIM1- and STIM2-mediated Ca^{2+} entry, and overexpression of SARAF leads to a decrease in basal levels of cytosolic, ER, and mitochondrial $[Ca^{2+}]$ (Palty et al. 2012). Although SARAF has not yet been shown to play a functional role in β-cells, it is highly expressed in these cells (T1Dbase.org, β-cell gene atlas) and could therefore be an important regulator of β-cell Ca^{2+} dynamics and bioenergetics.

Stromal Interaction Molecule (STIM)

Recently, high-throughput RNA interference-based analyses have identified the molecular basis of SOC channel activation in *Drosophila* and human cells (Berna-Erro et al. 2012). STIM, a 90-kDa single transmembrane-spanning Ca^{2+}-binding phosphoprotein located in the ER membrane, couples changes in $[Ca^{2+}]_{er}$ with activation of SOCE. STIM proteins contain EF-hand Ca^{2+}-binding domains with low affinity for Ca^{2+} and function as Ca^{2+} sensors within the lumen of the ER (Fig. 2) (Fahrner et al. 2009). Cells express two structurally related isoforms of STIM, STIM1, and STIM2, both of which are involved in regulating Ca^{2+} signaling. STIM1 is distributed homogeneously throughout the ER membrane in resting, unstimulated cells, but following depletion of ER Ca^{2+} stores, or certain other stimuli, STIM1 homodimerizes and translocates to discrete punctae located in regions of the ER membrane that are in close proximity to the PM where it interacts with and activates Orai channel proteins (discussed below) involved in mediating SOCE. This interaction occurs through the SOAR/CAD domain of STIM1 (Fig. 2). This activation region of STIM1 is maintained in a folded, inactive conformation until the stores are depleted (Yu et al. 2013a, b). This auto-inhibitory conformation likely explains the ability of cAMP to induce STIM1 punctae without SOCE activation in β-cells (Tian et al. 2012). In contrast to this effect of cAMP on puncta formation without SOCE, SOCE has been shown to stimulate cAMP production in MIN6 cells (Landa et al. 2005; Martin et al. 2009).

An enhanced yellow fluorescent protein (EYFP)-tagged STIM1 expressed in β-cells translocates to punctae near the plasma membrane following depletion of intracellular Ca^{2+} stores (Tamarina et al. 2008), as in other cell types (Cahalan 2009). Translocation of STIM1 in MIN6 insulinoma cells following depletion of stores using thapsigargin is reversibly blocked by 2-aminoethoxy diphenylborate (2-APB), an inhibitor of store-operated Ca^{2+} entry (Tamarina et al. 2008). We have also shown that downregulation of STIM1 using shRNA significantly reduces the amplitude of store-operated currents and Ca^{2+} entry in MIN6 cells

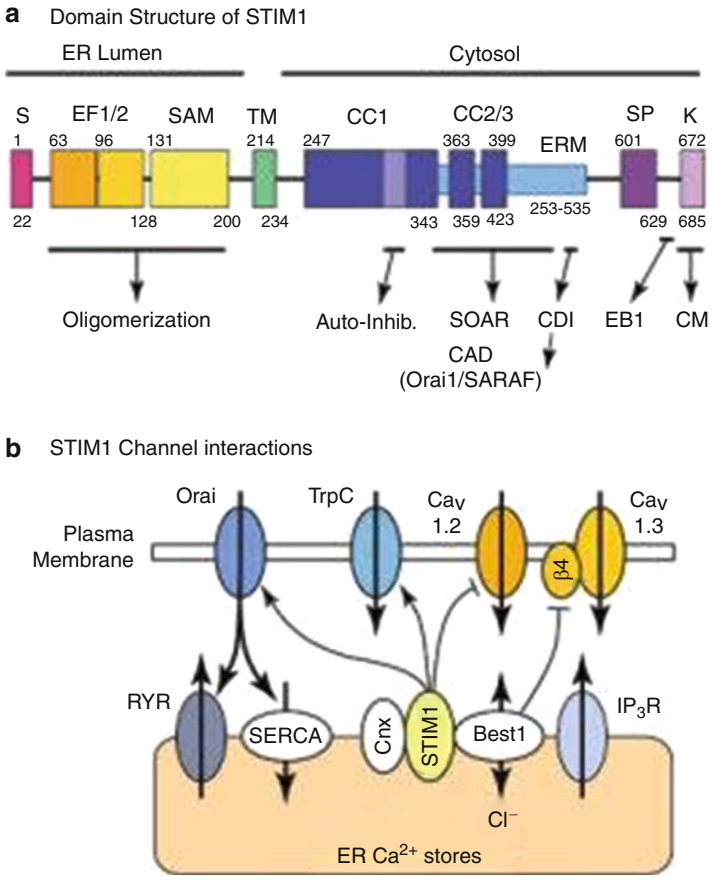

Fig. 2 (**a**) **Domain structure of STIM1**. STIM1 has a single transmembrane (*TM*) segment that spans the ER membrane. A signal peptide (S) is found at the N-terminal of STIM1 located in the ER lumen. A canonical EF hand followed by a "hidden" EF hand (EF1/2) and a sterile α-motif (SAM); the EF-SAM region plays a role in luminal Ca^{2+} sensing and STIM1 oligomerization. The TM region then separates the luminal and cytosolic tails of the molecule. In the cytosol, coiled-coil regions (CC1 and CC2/3) are important for ER localization, protein stability, and stabilizing the oligomeric state of active STIM1. The CC2 region also plays an important role in the Orai1-activating region of STIM1 (SOAR) or CRAC activation domain (CAD) (Park et al. 2009; Yuan et al. 2009). C-terminal to the SOAR/CAD region comes a small segment important for Ca^{2+}-dependent inactivation (CDI) of Orai1, an effect that also requires calmodulin binding to Orai1 (Mullins et al. 2009). STIM1 contains an ERM (ezrin, radixin, moesin) domain (residues 252–535) important for binding specific TRPC channel isoforms (Huang et al. 2006). Further toward the C-terminal is a serine/proline (SP)-rich region followed by a lysine-rich region (K). The K region is involved in the Ca^{2+}-dependent interaction of STIM1 with calmodulin (CM) (Bauer et al. 2008). Between the SP and K regions lies a region involved in an interaction with microtubule end-binding protein-1 (EB1) (Honnappa et al. 2009) (Figure adapted from Stathopulos et al. 2008; Park et al. 2009; Yuan et al. 2009; How et al. 2013). (**b**) **STIM1 channel interactions**. The best characterized interaction of STIM1 is its activation of Orai proteins. Other possible components of store-operated currents are TRPC channels, although the role of STIM1 in

(Leech et al. 2012). These observations establish a role for STIM1 in the regulation of store-operated currents in β-cells, and this role is likely to be as a sensor of stored Ca^{2+} levels. Whether SOC regulation in β-cells is specific to ER stores or is also regulated by acidic stores (Zbidi et al. 2011) and mitochondria (Singaravelu et al. 2011), as demonstrated in other cell types, remains to be determined.

The role of STIM2 in β-cells has not yet been established, but in other cell types it also acts as an ER Ca^{2+} sensor (Oh-Hora et al. 2008; Lopez et al. 2012). STIM2 has been shown to respond to smaller decreases in ER Ca^{2+} than STIM1, and it activates Orai1, playing a role in stabilizing basal cytosolic Ca^{2+} as well as ER Ca^{2+} (Brandman et al. 2007). In contrast, expression of STIM2 can also act as an inhibitor of STIM1-mediated Ca^{2+} entry (Soboloff et al. 2006). Of particular interest for the SOC in β-cells is the observation that in platelets STIM1 associates with both TRPC1 and Orai1 upon depletion of acidic Ca^{2+} stores, whereas STIM2 only associates with Orai1 (Zbidi et al. 2011). Whether this difference plays a functional role in the regulation of basal cytosolic Ca^{2+} in β-cells is unclear, but it is possible that STIM2 might activate a small Ca^{2+}-selective current through Orai1, whereas STIM1 plays a role in activating a larger nonselective current following more extensive store depletion. It is also interesting that different agonists can selectively recruit either STIM1 or STIM2 to support cytosolic Ca^{2+} oscillations in rat basophilic leukemia-1 (RBL-1) cells (Kar et al. 2012). Whether such differential recruitment occurs in the β-cell has not been established.

In addition to interactions with Orai and TRPC channels, STIM proteins also bind to sarco-endoplasmic reticulum Ca^{2+} ATPases (SERCA) isoforms that are important for refilling Ca^{2+} stores. SERCA2β and SERCA3 isoforms are important in regulating β-cell Ca^{2+} oscillations and insulin secretion (Arredouani et al. 2002; Kulkarni et al. 2004; Beauvois et al. 2006). Interestingly, depletion of acidic Ca^{2+} stores induces the association of both STIM1 and STIM2 with SERCA3, whereas the association of STIM1 with SERCA2β was only observed following depletion of the dense tubular system in human platelets, the analog of the ER in other cells, but not following acidic store depletion (Zbidi et al. 2011). Mathematical modeling of β-cells suggests that SERCA2β is important for Ca^{2+} oscillations only after the activation of a store-operated current (Bertram and Arceo 2008), and whether this

Fig. 2 (continued) regulating these channels has been disputed (DeHaven et al. 2009; Lee et al. 2010). STIM1 can also inhibit L-type, $Ca_V1.2$ Ca^{2+} channels (Wang et al. 2010). STIM1 binds calnexin (*Cnx*) and the transport proteins exportin1 and transportin1 (not shown) (Saitoh et al. 2010). Bestrophin1 (*Best1*) is an ER-localized, Ca^{2+}-activated Cl^- channel that interacts with STIM1 and may form the counterion channel that accompanies Ca^{2+} movement in and out of the store (Barro-Soria et al. 2010). Although it is unknown whether Best1 translocates in association with STIM1 into punctae at the plasma membrane, Best1 can inhibit $Ca_V1.3$ channels through association with the β4 subunit (Yu et al. 2008; Reichhart et al. 2010). Although not proven in β-cells, these inhibitory effects of STIM1 and Best1 on $Ca_V1.2$ and $Ca_V1.3$ might be important for the regulation of glucose-induced insulin secretion (see text). STIM1-mediated activation of Orai1 can also provide a source of Ca^{2+} that activates ryanodine receptors (RYR) to maintain the depleted state of the stores and maintain SOCE (Thakur et al. 2012)

relates to the binding of STIM1 to SERCA2β following ER store depletion is speculative at this time. Similarly, the role of acidic Ca^{2+} stores in the regulation of the SOC in β-cells is unknown, and whether the interaction of STIM with SERCA3 plays a role in regulating Ca^{2+} oscillations is also unknown. The role of acidic Ca^{2+} stores in β-cells is not fully understood but glucose, glucagon-like peptide-1 (GLP-1), and insulin signaling are all proposed to involve these stores (Yamasaki et al. 2004; Kim et al. 2008a; Shawl et al. 2009; Arredouani et al. 2010).

The mechanism by which STIM proteins oligomerize and translocate to form punctae (Cahalan 2009) remains uncertain. However, STIM proteins have been shown to bind the transport proteins exportin1 and transportin1, proteins that are associated with nuclear export and import, respectively (Saitoh et al. 2010). Transportin1, also known as importin β2, not only plays a role in nuclear import but also regulates transport of the kinesin motor KIF17 to cilia (Dishinger et al. 2010). Whether transportin1 plays a role in the movement of STIM proteins to aggregate and form punctae remains to be determined. The dynamin-related protein mitofusin 2 inhibits STIM1 translocation in cells with depolarized mitochondria and inhibits store-operated Ca^{2+} entry (Singaravelu et al. 2011). Roles for these proteins in regulating STIM translocation in β-cells have not yet been defined.

At least two mechanisms for STIM proteins to induce activation of plasma membrane channels have been proposed. One model involves the direct conformational coupling of STIM to activate Orai. The direct interaction model proposes that a basic region in the cytoplasmic region of STIM1 is masked by a pseudosubstrate acidic region and is inactive under resting conditions. Activation of STIM1 by store depletion releases the basic region and allows it to interact with an acidic region in the carboxyl-terminus region of Orai1 resulting in channel activation (Korzeniowski et al. 2010).

An alternative model for SOC activation involves the STIM-mediated generation of a soluble Ca^{2+} influx factor (CIF) (Bolotina 2008; Csutora et al. 2008; Gwozdz et al. 2008). CIF is proposed to activate the Ca^{2+}-independent phospholipase A2β (iPLA2β) by displacing inhibitory calmodulin (Bolotina 2004; Smani et al. 2004), as an essential intermediate step in SOC regulation (Csutora et al. 2006). In this regard, it is interesting that STIM proteins bind calnexin, a chaperone protein (Saitoh et al. 2010). Calnexin also binds iPLA2β in β-cells where store depletion with thapsigargin induces arachidonic acid (AA) production (Song et al. 2010). Whether calnexin simultaneously binds STIM and iPLA2β or promotes their functional coupling is unknown. However, the action of iPLA2β to generate AA has regulatory effects on voltage-gated potassium channels (Jacobson et al. 2007) and arachidonate-regulated channels (ARC) (Yeung-Yam-Wah et al. 2010) in β-cells and thus is likely to regulate their electrical excitability independently of any additional effect on SOC activity. The role of iPLA2β in the regulation of SOC has been demonstrated using either molecular knockdown or pharmacological inhibition using bromoenol lactone (BEL) (Smani et al. 2003). It is interesting to note that iPLA2 also plays a role in maintaining the mitochondrial membrane potential (Ma et al. 2011). As described above, mitochondrial depolarization inhibits STIM1 translocation (Singaravelu et al. 2011), and this might contribute to the effects of iPLA2β inhibition on SOC.

It is interesting to note that in addition to activating Orai1, STIM1 is reported to reciprocally inhibit L-type $Ca_V1.2$ channels (Fig. 2) (Wang et al. 2010). Pancreatic β-cells express a range of voltage-gated Ca^{2+} channels that are important regulators of insulin secretion (Seino et al. 1992; Braun et al. 2008). Thus the regulation of Ca^{2+} channel activity by STIM1 could be important because $Ca_V1.2$ is coupled to secretion in INS-1 832/13 cells (Nitert et al. 2008), although in human β-cells $Ca_V1.2$ forms only a small component of the voltage-gated Ca^{2+} current, and $Ca_V1.3$ is the predominant L-type channel isoform (Braun et al. 2008). It is controversial as to whether $Ca_V1.2$ or $Ca_V1.3$ preferentially controls glucose-induced insulin secretion in INS-1 cells (Liu et al. 2003; Nitert et al. 2008); however, in human β-cells, Ca^{2+} influx through P/Q type channels also plays an important role in stimulating exocytosis (Braun et al. 2008). Whether STIM1 regulates $Ca_V1.3$ or other Ca_V isoforms is unknown. It is noteworthy that human bestrophin1, but not mouse isoforms of bestrophin, inhibits $Ca_V1.3$ through interaction with the $Ca_V\beta$ subunit (Yu et al. 2008). Bestrophin1 associates with STIM1 in the ER and augments inositol 1,4,5-*tris*phosphate receptor (IP_3R)-mediated Ca^{2+} release (Barro-Soria et al. 2010). Whether bestrophin-mediated inhibition of $Ca_V1.3$ is relevant in human β-cells or whether bestrophin translocates in association with STIM1 is currently unknown.

STIM1 has also been shown to activate adenylyl cyclase activity in a colonic epithelial cell line (Lefkimmiatis et al. 2009), but it is not known whether this occurs in β-cells. However, previous studies in smooth muscle reported a role for store-operated Ca^{2+} entry in regulating the cAMP response element-binding (CREB) protein transcription factor (Pulver et al. 2004), and it is possible that STIM1-regulated cAMP production might play a role in this response. An alternative possibility is that STIM1-mediated cAMP production might act as a negative-feedback regulator of the SOC through protein kinase A (PKA)-mediated phosphorylation of the channel (Liu et al. 2005a). Cyclic-AMP-elevating hormones that regulate insulin secretion, such as GLP-1, also potentiate Ca^{2+}-induced Ca^{2+} release (CICR) from intracellular stores. The possibility that STIM1 might also control cyclase activity following CICR-mediated store depletion could play an important role in producing localized [cAMP] changes to control β-cell gene expression through CREB or play a role in controlling electrical excitability and exocytosis at the plasma membrane.

SOCE and the Cytoskeleton

Studies on the translocation of EYFP-tagged STIM1 in β-cells following store depletion noted that punctae formed in regions of the plasma membrane that were "actin poor" (Tamarina et al. 2008). It is currently unclear whether STIM1 translocates to regions with preexisting reduced actin levels or whether the translocation process induces displacement of actin. That the cortical actin network in β-cells acts as a barrier that regulates exocytosis has long been appreciated (Orci et al. 1972; Li et al. 1994), but little is known about physiological mechanisms that

might control the actin network. Specific actin-binding proteins can regulate insulin secretion (Bruun et al. 2000), and glucose can reorganize the actin network through cell division control protein 42 homolog (Cdc42), neuronal Wiskott-Aldrich Syndrome protein (N-WASP), and Cofilin (Nevins and Thurmond 2003; Wang et al. 2007; Uenishi et al. 2013). This ability of glucose to regulate the cortical actin network may have been previously underappreciated in view of our emerging understanding of the importance of "newcomer" insulin granules to overall insulin secretion (Nagamatsu et al. 2006; Shibasaki et al. 2007; Xie et al. 2012). At the moment, it remains speculative as to whether the cytoskeleton regulates SOCE in β-cells through effects on STIM1 translocation or SOCE activation. In other cell types, conflicting data have emerged on the role of both the actin filaments and microtubules in regulating translocation of STIM1 and its association with Orai and the activation of SOCE (Galan et al. 2011; Zeng et al. 2012). It has also been reported that disruption of the actin cytoskeleton inhibits the association between calmodulin and Orai1/TRPC1 to enhance SOCE independently of effects on STIM1 translocation and association with Orai1 (Galan et al. 2011). Some of the discrepancies on the role of the cytoskeleton are likely due to cell-specific effects as it was shown in MIN6 cells that the SOCE blocker 2-APB prevented store depletion-dependent translocation of EYFP-STIM1 to the plasma membrane (Tamarina et al. 2008), whereas in HEK293 cells 2-APB induces store-independent clustering of STIM1 (Zeng et al. 2012). Whether store-independent clustering of STIM1 is functionally significant has not been demonstrated, but in β-cells it has been shown that cAMP induces STIM1 punctae formation but not Orai clustering or SOCE activation (Tian et al. 2012). It is possible that this cAMP-dependent puncta formation acts as a priming step to form a coupling mechanism between STIM1 and ryanodine receptor 2 (RYR2). In HEK293 cells, STIM1 and RYR2 co-localize after store depletion such that Ca^{2+} influx through SOCE activates RYR2 and CICR to maintain store depletion and prolong SOCE (Thakur et al. 2012). In view of the fact that in β-cells cAMP induces STIM1 punctae (Tian et al. 2012) and also acts through exchange protein activated by cAMP 2 (Epac2) to sensitize CICR (Dzhura et al. 2010), this coupling mechanism might serve a role in the generation of intracellular Ca^{2+} oscillations, assuming that RYR2 and STIM1 functionally co-localize in β-cells.

An alternative role for the actin cytoskeleton in regulating SOCE comes from its ability to regulate the stability of lipid rafts (Klappe et al. 2013). Lipid rafts have been shown to be important for the formation of STIM1-Orai1-TRPC1 heteromultimers, and disruption of lipid rafts inhibits SOCE (Jardin et al. 2008). Lipid rafts are also known to play a role in insulin secretion through localization of L-type Ca^{2+} channels (Jacobo et al. 2009). Whether lipid rafts are important for the activation of SOCE in β-cells remains to be determined, but it is tempting to speculate that STIM1 puncta formation in β-cells (Tamarina et al. 2008) might be associated with lipid rafts.

An indirect mechanism for actin as a regulator of SOCE comes from its ability to regulate cluster of differentiation 38 (CD38), also known as cyclic ADP-ribose hydrolase or cyclic ADP-ribose cyclase (Shawl et al. 2012). Glucose stimulation of

β-cells induces the internalization of CD38, and this internalization is an essential step for cyclic ADP-ribose (cADPR) production. Inhibition of actin depolymerization prevents CD38 internalization and cADPR production (Shawl et al. 2012). This role of actin depolymerization to permit cADPR production will influence the activation of RYR and release of intracellular Ca^{2+} stores and thus could play a role in SOCE activation. It is therefore possible that glucose acting through Cdc42 to mediate actin remodeling might influence not only insulin granule movement and release but also cADPR production to facilitate store depletion and SOCE activation to modulate Ca^{2+} dynamics in the β-cell.

Orai Channels

While relatively little is known about the expression of Orai channels in β-cells, our preliminary data using PCR and the β-cell gene atlas (t1dbase.org) indicates the expression of all three isoforms. The domain structure of Orai1 is illustrated in Fig. 3. Functional expression of Orai1 and Orai3 is also indicated by the presence of arachidonate-regulated Ca^{2+}(ARC) channels in β-cells (Yeung-Yam-Wah et al. 2010) discussed below.

The channel formed by Orai is Ca^{2+} selective, and homomeric Orai channels might form a component of the SOC in β-cells. Alternatively, Orai might form heteromeric complexes with members of the TRP family to underlie the store-operated current in β-cells (Fig. 3), discussed below. The combination of STIM1 and Orai1 forms the most common type of store-operated Ca^{2+} channel. However, a native store-regulated channel formed by STIM1 and Orai3 was reported in estrogen receptor-positive, but not estrogen receptor-negative, breast cancer cell lines (Motiani et al. 2010). The expression of Orai3 in MCF7 cells is under the control of estrogen receptor α (ERα) (Motiani et al. 2013). Pancreatic β-cells express the α, β and G protein-coupled estrogen receptor (GPR30) isoforms of estrogen receptor that play a role in regulating insulin secretion and preventing β-cell apoptosis (Liu and Mauvais-Jarvis 2010; Nadal et al. 2011). Whether the presence of estrogen receptors in β-cells permits the expression of a store-operated current formed by STIM1-Orai3, or of the Orai3-containing ARC channel, in these cells has yet to be determined.

In addition to the store-dependent regulation of Orai channels by STIM1, Orai channels can be activated by store-independent mechanisms (Fig. 4). A pentameric combination of three Orai1 and two Orai3 subunits forms the store-independent, Ca^{2+}-selective ARC channel (Mignen et al. 2009). The specificity for activation by AA is determined by the cytosolic N-terminal domain of Orai3 and requires the presence of two Orai3 subunits in the channel (Thompson et al. 2010). Despite the store-independence of the ARC, it can be regulated by STIM1 resident in the plasma membrane (Mignen et al. 2007). ARC channel activation occurs downstream of Ca^{2+}-independent AA production by membrane-associated type IV cytosolic phospholipase A2 (cPLA2) and G_q-coupled receptor activation (Osterhout and Shuttleworth 2000). The relative importance of glucose, cPLA2,

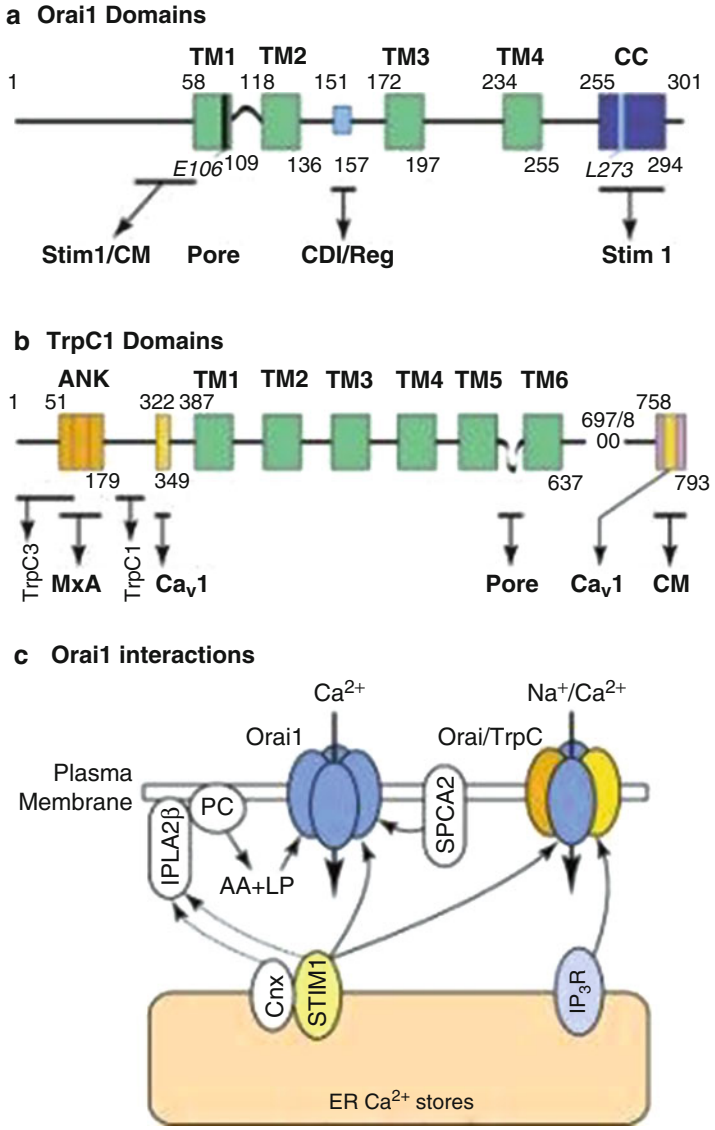

Fig. 3 (**a**) **Orai1 domains**. The Orai proteins contain four transmembrane (TM) domains and most likely form functional channels as tetramers with the TM1 domain lining the pore. The E106 residue forms the Ca^{2+} selectivity filter within the pore, and the extracellular loop between TM1 and TM2 forms an outer vestibule to the pore (McNally et al. 2009; Zhou et al. 2010). A coiled-coil (CC) region in the C-terminal tail and an N-terminal region at the membrane interface form interaction sites with STIM1 (Calloway et al. 2010). The N-terminal domain proximal to TM1 also forms a region that binds calmodulin (CM) (Mullins et al. 2009). Mutation of L273 within the coiled-coil region inhibits binding of Orai1 to STIM1 (Muik et al. 2008). Four amino acids within the TM2-3 intracellular loop V(151)SNV(154) are important for fast Ca^{2+}-dependent inactivation (CDI), and N(153)VHNL(157) play an important regulatory role on channel activity

or iPLA2β for AA production to regulate ARC in β-cells is unknown. ARC forms a small Ca^{2+} current in β-cells (Yeung-Yam-Wah et al. 2010), but the physiological role of the current is uncertain and will not be discussed in detail here. It should also be noted that AA promotes Ca^{2+} mobilization through activation of ryanodine receptors in β-cells (Metz 1988; Woolcott et al. 2006) and thus could indirectly activate SOC through store depletion and also underlie the reported RYR-sensitive activation of TRP-like channels in the plasma membrane of β-cells (Gustafsson et al. 2005).

A second mechanism for store-independent activation of Orai1 has been described for the secretory pathway Ca^{2+}-ATPase isoform 2 (SPCA2) (Feng et al. 2010).

Fig. 3 (continued) (Srikanth et al. 2013). (**b**) **TRPC1 domains**. TRPC channels contain six TM domains with the pore formed by the TM5–6 loop (see review Ambudkar et al. (2006) for more details). At the N-terminal of TRPC1 is a domain involved in heteromerization with TRPC3 (Liu et al. 2005) and another domain required for homomeric interaction (TRPC1) (Engelke et al. 2002). Between these domains is an ankyrin (ANK) repeat that binds other proteins including MxA, a member of the dynamin superfamily (Lussier et al. 2005). Two domains that interact with caveolin 1 (Ca_vI) have been identified (Brazer et al. 2003; Sundivakkam et al. 2009) along with a calmodulin (CM) binding domain (Singh et al. 2002). Two aspartate residues (DD) are reported to mediate an electrostatic interaction with 684KK685 of STIM1 that is important for activation of TRPC1 (Zeng et al. 2008). (**c**) **Orai1 interactions**. Orai1 is present in the plasma membrane as dimers under resting conditions and forms a tetrameric complex following STIM activation (Penna et al. 2008). The tetrameric nature of Orai is consistently reported but estimates of the Orai: STIM stoichiometry for channel activation vary from 4:2 (Ji et al. 2008) to 4:8 (Li et al. 2010). An alternative model for store-operated Ca^{2+} influx involves the activation of type VI iPLA2β that hydrolyzes phosphatidylcholine (*PC*) and generates arachidonic acid (*AA*) and lysophospholipids (*LP*); LP then activates the channel (Smani et al. 2003, 2004). In this model, store depletion generates a Ca^{2+} influx factor (CIF) that relieves the inhibitory effect of calmodulin on iPLA2β; the molecular identity of this CIF is unknown. Whether the calmodulin binding domain of STIM1 (Figure 2) or the increased binding of calnexin (*Cnx*) to iPLA2β after store depletion (Song et al. 2010) plays a role in current activation is unknown. Yet another mechanism has been demonstrated in mammary tumor cells where the secretory pathway Ca^{2+}-ATPase (SPCA2) binds to Orai1 resulting in its activation (Feng et al. 2010). Although this mechanism has not yet been demonstrated in β-cells, SPCA2 is expressed in human β-cells (t1dBase.org) and could play a role in regulating Ca^{2+} influx in these cells.
In addition to forming homomeric channels, Orai can also interact with several members of the TRPC family to form nonselective cation channels that are either store-regulated or store-independent (Liao et al. 2007, 2009; Jardin et al. 2009; Woodard et al. 2010). The presence of Orai1 has been reported to be essential for TRPC channels to be store responsive and confer STIM1 sensitivity (Liao et al. 2007). TRPC1 also interacts with the type 3 inositol 1,4,5-*tris*phosphate receptor (IP_3R3) (Sundivakkam et al. 2009), and TRPC3 interacts with IP_3R1 (Woodard et al. 2010). This interaction with IP_3Rs has been suggested as a conformational coupling gating mechanism for TRPC channels (Zarayskiy et al. 2007).
Store depletion using either thapsigargin or carbachol also induces translocation of Orai1 into the plasma membrane through a SNAP-25-dependent mechanism that is important for the maintenance, but not the initiation, of store-operated Ca^{2+} entry (Woodard et al. 2008). Additionally, α-SNAP regulates SOCE (Miao et al. 2013). Orai1 can also undergo constitutive endosomal recycling through a Rho-dependent pathway with the binding of caveolin-1 (Ca_v1) and dynamin to Orai being important for endocytosis (Yu et al. 2010)

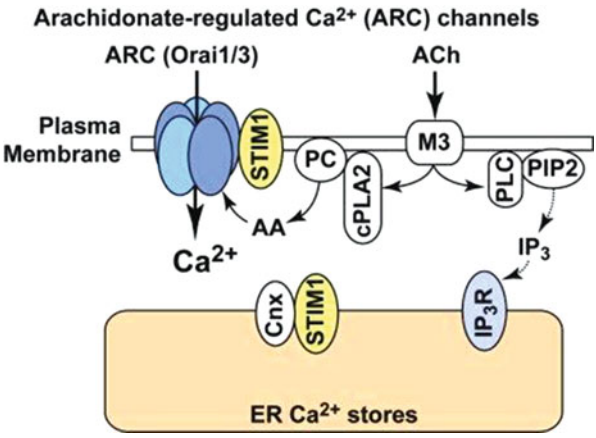

Fig. 4 **Arachidonate-regulated channels (ARC).** ARC channels are highly Ca^{2+} selective and formed as a pentameric assembly of three Orai1 and two Orai3 subunits (Mignen et al. 2009). These channels are functionally expressed in pancreatic β-cells and produce a small current (1.7 pA/pF at −70 mV) (Yeung-Yam-Wah et al. 2010) but could significantly affect the membrane potential in the presence of high glucose, a condition that stimulates arachidonic acid (*AA*) production in β-cells (Turk et al. 1986; Gross et al. 1993). AA activation of ARC channels is conferred by the N-terminal of Orai3 (Thompson et al. 2010). ARC activation is also under the control of GPCR signaling, and low doses of agonist, for example, acetylcholine (*ACh*) at muscarinic M3 receptors – a physiologically important receptor in β-cells (Gilon and Henquin 2001) – are reported to specifically activate a pool of membrane-associated type IV cytosolic PLA2 (*cPLA2*) that hydrolyzes phosphatidylcholine (*PC*) (Osterhout and Shuttleworth 2000), whereas high agonist concentrations activate phospholipase C (*PLC*) and hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP_2) resulting in IP_3 production, store depletion, and activation of CRAC currents. This difference in agonist sensitivity is proposed to be important for the reciprocal regulation of ARC and capacitative currents, where ARC channels are inactivated by sustained $[Ca^{2+}]_c$ elevation at high agonist concentrations where CRAC currents are active (Mignen et al. 2001). It is also interesting to note that forced expression of Orai1 and Orai3 in a 1:1 ratio, unlike the 3:2 ratio for ARC channels, produces a current with a lower selectivity for Ca^{2+} (Schindl et al. 2009), although the reversal potential of these currents is significantly more positive than store-operated currents in β-cells. The ARC channel is also regulated by STIM1, but this regulation is independent of store depletion or STIM1 translocation. This effect of STIM1 on ARC appears to be mediated by STIM1 localized in the plasma membrane and can be inhibited by the application to intact cells of an antibody against the N-terminal of STIM1, with no effect on CRAC channel activation by store depletion and translocation of ER resident STIM1 (Mignen et al. 2007)

This pathway is specific for SPCA2 and is not observed with SPCA1 and may involve plasma membrane resident proteins rather than proteins specifically localized to the Golgi complex, the "normal" location of SPCA2 (Feng et al. 2010). Whether this pathway is relevant in β-cells remains to be established. Expression of a novel transcript of SPCA2 in pancreas is specific to exocrine cells in mice (Garside et al. 2010), but expression of SPCA2 in human β-cells is documented (T1Dbase.org).

TRP Channels in β Cells

TRP family channels were first identified in the *Drosophila* visual system, and there are 28 members in six families (see Clapham et al. 2003; http://iuphar-db.org). Several members of the TRP family of channels have been reported in β-cells (see reviews (Jacobson and Philipson 2007; Islam 2011)), summarized in Table 1 (nomenclature for pre-2003 data has been updated to the IUPHAR standard (Clapham et al. 2003)). As would be predicted, the majority of the TRP channels for which a function has been ascribed are involved in the stimulation of insulin secretion by regulating β-cell excitability or play a role in the induction of β-cell death. TRP channels play a role in β-cell death in response to oxidative stress (Ishii et al. 2006) or in response to human islet amyloid polypeptide (Casas et al. 2008).

TRPM2 knockout mice show modestly impaired insulin secretion in response to glucose and GLP-1 (Uchida et al. 2010). TRPM2 is expressed in human islets in both long (TRPM2-L) and short forms (TRPM2-S) where it mediates H_2O_2-induced Ca^{2+} influx (Bari et al. 2009; Fig. 5). TRPM2-S lacks a pore-forming domain and regulates Ca^{2+} influx through TRPM2-L, reducing cell death in response to H_2O_2 (Zhang et al. 2003). In addition to promoting Ca^{2+} influx across the plasma membrane, TRPM2 also functions as a Ca^{2+} release channel for the lysosomal compartment with both functions playing a role in H_2O_2-induced β-cell death (Lange et al. 2009). TRPM2 can also be activated by cADPR (Kolisek et al. 2005; Togashi et al. 2006), a second messenger involved in glucose-stimulated insulin secretion that also activates ryanodine receptors (Takasawa et al. 1995), and *O*′-acetyl-ADPribose (*O*AADPr), a product of sirtuin activity (Grubisha et al. 2006). Whether this effect on TRPM2 plays a role in the ability of sirtuins to enhance insulin secretion and protect β-cells from

Table 1 Trp channel isoforms in β cells

Isoform	Role in β cells	Reference(s)
TrpC1	Unknown (expression detected)	Sakura and Ashcroft 1997
TrpC4	Expression detected	Qian et al. 2002
	Leptin signaling/CaMKKβ activation	Park et al. 2013
TrpM2-L	Stimulates insulin secretion	Qian et al. 2002; Ishii et al. 2006; Togashi et al. 2006; Bari et al. 2009; Lange et al. 2009
	Role in H_2O_2-induced cell death	
TrpM2-S	Regulates TrpM2-L, inhibits cell death	Zhang et al. 2003; Bari et al. 2009
TrpM4	Stimulates insulin secretion	Cheng et al. 2007; Marigo et al. 2009
TrpM5	Stimulates insulin secretion	Colsoul et al. 2010
TrpV1	Stimulates insulin secretion	Akiba et al. 2004
TrpV2	Stimulates insulin secretion	Hisanaga et al. 2009
	Promotes insulinoma cell growth	
TrpV4	Promotes cell death	Casas et al. 2008
TrpV5	Expression decreases in ZDF rats	Janssen et al. 2002

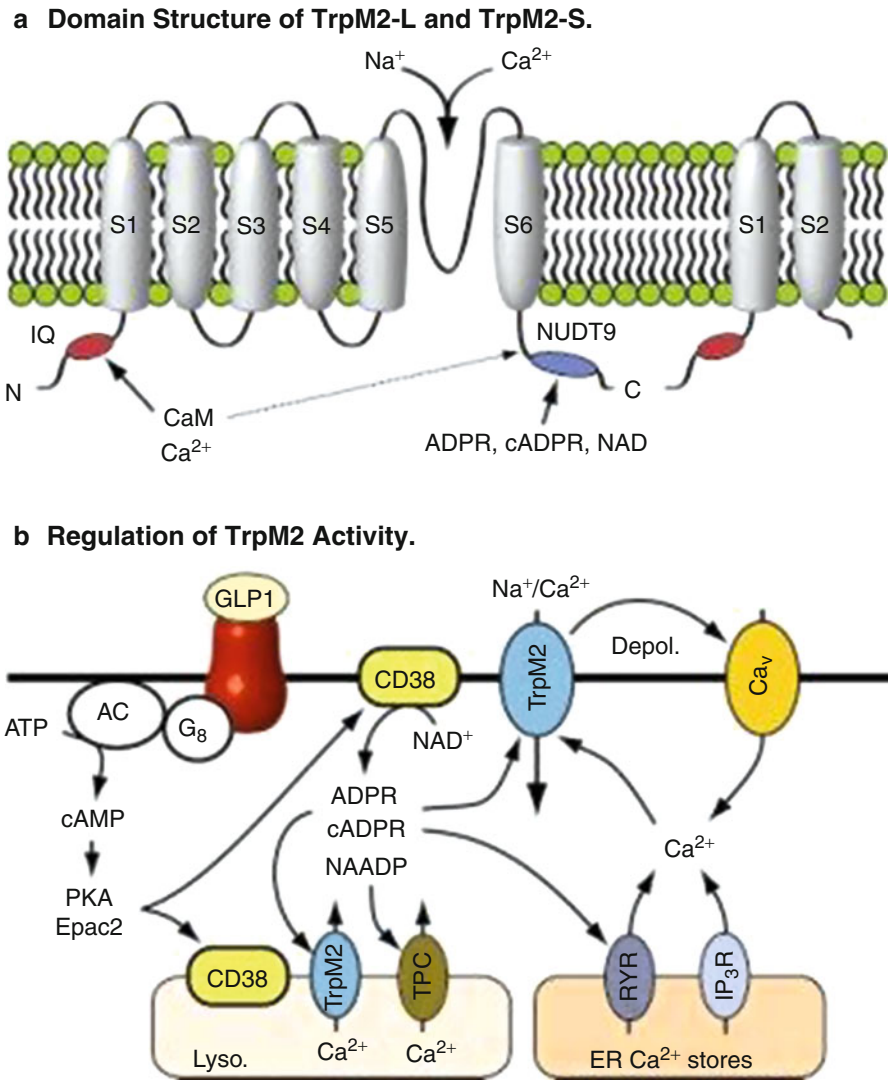

Fig. 5 (**a**) **Domain structure of TRPM2-L and TRPM2-S**. Full-length human TRPM2-L is a 1553-amino-acid protein containing 6 transmembrane domains with the pore-selectivity filter between S5 and S6. An IQ-like domain in the N-terminal binds calmodulin (*CaM*) and confers Ca^{2+}-dependent activation; CaM also can bind weakly to the C-terminal (Tong et al. 2006). The C-terminal contains a nudix homology domain (NUDT9) that binds channel agonists ADPR and cADPR with ADP-ribose pyrophosphatase (ADPRase) activity. In addition to full-length TRPM2, a short, 846-amino-acid variant is expressed that is truncated after the second transmembrane domain and is able to interact with TRPM2-L and regulate its activity (Zhang et al. 2003). Both long and short forms of TRPM2 are expressed in human islets (Bari et al. 2009). Several other splice variants of TRPM2 also occur (Perraud et al. 2003), but their expression in islets is unknown. (**b**) **Regulation of TRPM2 activity**. TRPM2 appears to be expressed in both the plasma membrane and in lysosomal Ca^{2+} stores of β-cells. It is activated by ADPR and cADPR generated

streptozotocin-induced apoptosis (Moynihan et al. 2005; Tang et al. 2011; Vetterli et al. 2011; Luu et al. 2013) is undefined. However, the physiological relevance of sirtuin activation in regulating insulin secretion in human subjects in vivo remains to be determined (Timmers et al. 2013).

TRPM4 and TRPM5 are Ca^{2+}-activated nonselective cation channels, and knockdown of either of these channels in β-cell lines reveals a role in regulating insulin secretion. Data from studies of knockout mice suggest that TRPM4 may be less important than TRPM5 in stimulating insulin secretion *in vivo* (Cheng et al. 2007; Marigo et al. 2009; Colsoul et al. 2010; Enklaar et al. 2010). The physiological pathways that activate TRPM4/5 in β-cells are not fully understood. It is possible that glucose and/or GLP-1 might promote the formation of nicotinic acid adenine nucleotide diphosphate (NAADP) to activate two-pore channels (TPCs) in endo-lysosomal Ca^{2+} stores to promote Ca^{2+} release (Arredouani et al. 2010). This Ca^{2+} release might then directly activate TRPM4/5 in the plasma membrane or act as a trigger for CICR from the ER to provide the source of Ca^{2+} (Arredouani et al. 2010). TRPM4 currents also show a Ca^{2+} dependent increase due to insertion of channels in the plasma membrane by fusion of channel-containing vesicles (Cheng et al. 2007). Kinetic studies suggest that the channel-containing vesicles are not part of the readily releasable pool (Cheng et al. 2007) raising the possibility that they may be present either in the reserve pool or in the small granules of β-cells rather than the large, dense-core insulin-containing vesicles. Whether TRPM2 in the lysosomal stores might also contribute to plasma membrane channel activation is unresolved.

It is additionally interesting that TRPM4 reportedly interacts with sulfonylurea receptor 1 (SUR1) to form an ATP- and sulfonylurea-regulated conductance (Chen et al. 2003; Simard et al. 2010) although this interaction has been disputed (Sala-Rabanal et al. 2012) and may be cell-specific. It is interesting that TRPM4 contains putative ATP binding sites that reverse Ca^{2+}-mediated desensitization of TRPM4, and mutation of the ATP binding sites results in faster and more complete channel desensitization (Nilius et al. 2005). Furthermore, phosphorylation by PKC increases the Ca^{2+} sensitivity of TRPM4, and Ca^{2+} sensitivity is also modulated by calmodulin (Nilius et al. 2005). This raises the possibility that in β-cells, cholinergic stimulation might regulate TRPM4 activity through PLC by inducing IP_3-mediated Ca^{2+} release from the ER and also by activating PKC to increase channel sensitivity to Ca^{2+}. It has also been shown that whereas cholinergic stimulation of β-cells induces a homogeneous rise in diacylglycerol (DAG), the autocrine effect of ATP on $P2Y_1$ receptors produces discrete microdomains of DAG production (Wuttke et al. 2013). Whether these effects of different stimuli produce differential

Fig. 5 (continued) by CD38 from nicotinamide adenine dinucleotide (*NAD^+*) and also by Ca^{2+} that is permissive for activation by cADPR. CD38 activity can be regulated by GLP-1, and it is also involved in the production of NAADP that acts as a ligand for two-pore channels (*TPC*) present in acidic Ca^{2+} stores of β-cells. Ca^{2+} release through the TPC might provide a trigger for TRPM2 activation either directly or by Ca^{2+}-induced Ca^{2+} release (CICR) from ER stores through RYR and IP_3R. TRPM2 plays a role in regulating insulin secretion that may be mediated through inducing cell depolarization and by direct Ca^{2+} influx through these nonselective cation channels

regulation of TRPM4 is unclear. However, hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) by PLC can result in channel desensitization and decreased Ca^{2+} sensitivity (Zhang et al. 2005; Nilius et al. 2006). How these counteracting effects modulate TRPM4 in β-cells is currently unknown.

TRPV1 and TRPV2 channels also play a role in stimulating insulin secretion, and TRPV2 promotes serum- and glucose-induced MIN6 cell growth (Akiba et al. 2004; Hisanaga et al. 2009). In the resting state, the majority of TRPV2 is present in the cytosol and undergoes translocation to the plasma membrane in response to insulin suggesting an autocrine mechanism for channel regulation (Akiba et al. 2004; Hisanaga et al. 2009). A similar channel-trafficking response is also observed with insulin-like growth factor 1 (IGF-1) (Kanzaki et al. 1999). TRPV2 can also be activated by cell swelling (Muraki et al. 2003), an effect that occurs in response to glucose elevation and that activates a volume-sensitive anion channel and insulin secretion in β-cells (Kinard and Satin 1995; Best 2002). However, it has been noted that this anion conductance cannot fully account for swelling-induced insulin secretion (Kinard et al. 2001), and activation of TRPV channels might contribute to the anion channel-independent component of secretion. TRPV4 is also a mechanosensitive channel that is activated by extracellular human islet amyloid polypeptide and is involved in β-cell death (Casas et al. 2008). This channel shows spontaneous activity and is activated by cell swelling with hypotonic media (Strotmann et al. 2000) and therefore could contribute to the regulation of insulin secretion in parallel with volume-sensitive anion channels.

Potentially, the most interesting TRP channels in terms of the SOC are TRPC1 (Fig. 3b), TRPC3, and TRPC6 that can each play a role in SOC activity (Qian et al. 2002; Beech 2005; Liu et al. 2005b; Liao et al. 2007). However, TRPC3 and TRPC6 appear to be absent or only weakly expressed in β-cells (T1Dbase.org) and thus seem less likely to be important in these cells. The TRPC1 isoform was one of the first to be identified in β-cells (Sakura and Ashcroft 1997), but its physiological role in these cells has not yet been characterized. TRPC1 can form a heteromeric channel with TRPV4 that undergoes STIM1-dependent translocation to the plasma membrane upon store depletion (Ma et al. 2010). Whether functional TRPC1/V4 heteromeric channels are present in the β-cell is unclear, although both subunits are expressed.

A functional interaction of TRPC1 with large conductance Ca^{2+}-activated K^+ (BK) channels might also be important in β-cells. BK channels play a role in β-cells by regulating action potential duration and insulin secretion and also by protecting against apoptosis and oxidative stress (Jacobson et al. 2010; Dufer et al. 2011). In vascular smooth muscle cells, TRPC1 interacts with the α subunit of BK channels and is proposed to provide the source of Ca^{2+} required for BK activation (Kwan et al. 2009). It will be interesting to determine whether TRP channels play a functional role in regulating BK activity in β-cells and contribute to the regulation of secretion or cell viability.

Both TRPC1 (Sundivakkam et al. 2009) and TRPC2 (Brann et al. 2002) are reported to interact with the type 3 IP_3 receptor (IP_3R3). TRPC2

co-immunoprecipitates with IP_3R3 (Brann et al. 2002) and for TRPC1, deletion of COOH-terminal residues 781–789 abolishes the interaction with IP_3R3 and inhibits store-operated Ca^{2+} influx (Sundivakkam et al. 2009). It is also possible that disruption of IP_3R3-regulated SOC contributes to defective insulin secretion in Anx7-deficient mice that have very low expression of IP_3R3 (Srivastava et al. 2002). Interestingly, these same C-terminal residues in TRPC1 also mediate interaction with the scaffold domain of caveolin-1 (CSD), and this CSD also interacts with IP_3R3 (Sundivakkam et al. 2009). Deletion of the CSD augments store-operated Ca^{2+} influx (Sundivakkam et al. 2009). TRPC1 undergoes internalization that blocks Ca^{2+} entry in neutrophils (Itagaki et al. 2004). Whether the interaction of TRPC1 with caveolin plays a role in its internalization, similar to the effect of caveolin on Orai1 (Yu et al. 2010), has not been established. Orai1 undergoes active recycling between an endosomal compartment and the plasma membrane in resting egg cells through a Rho-dependent mechanism (Yu et al. 2010). Internalization inhibits SOC, but whether this regulatory mechanism occurs in β-cells is unknown. It is possible that SOC might be regulated by incretin-mediated cAMP elevation through an Epac2/Rap1/Rap1-activated Rho GTPase-activating protein (RA-RhoGAP) pathway (Aivatiadou et al. 2009) to control the plasma membrane level of Orai1.

It is interesting to note that TRPC1 expression is regulated by hepatic nuclear factor 4a (HNF4α), and expression of TRPC1 is reduced in the liver and kidney of Zucker diabetic fatty (ZDF) or streptozotocin-induced diabetic rats (Niehof and Borlak 2008). Mutations in HNF4α are associated with maturity-onset diabetes of the young (MODY1), type 2 diabetes mellitus (T2DM) (Sookoian et al. 2010), and impaired insulin secretion (Hansen et al. 2002). Whether these diabetes-related mutations in HNF4α influence TRPC1 expression in β-cells and contribute to impaired insulin secretion in these subjects is unknown, although knockout of HNF4α produces a 60 % reduction in Kir6.2 expression (Gupta et al. 2005). It should be noted that the SOCE in platelets from type 2 diabetic subjects has been reported to either decrease (Jardin et al. 2011) or increase (Zbidi et al. 2009). This underscores the importance of defining the regulatory mechanisms underlying the expression of SOCE components in β-cells.

Another member of the TRP family expressed in β-cells, TRPC4, is activated by leptin signaling as an important determinant of K_{ATP} channel trafficking to the plasma membrane (Park et al. 2013). Activation of TRPC4 by leptin has a biphasic effect on glucose-induced Ca^{2+} oscillations in INS-1 cells, initially increasing their amplitude and frequency, then subsequently inhibiting the oscillations (Park et al. 2013). Whether this effect of leptin and the activation of TRPC4 influences SOCE or the filling state of intracellular Ca^{2+} stores in β-cells remain unresolved. The leptin signaling pathway also leads to the activation of AMP-activated protein kinase (AMPK) (Park et al. 2013). Since AMPK has been shown to promote the degradation of Orai1 and might also contribute to the downregulation of gene transcription of both STIM1 and Orai1 (Lang et al. 2012), this suggests that leptin might regulate β-cell excitability not only by increasing K_{ATP} channel activity but also through a reduction of SOCE.

Gene Regulation of Store-Operated Channels in β Cells

Although we currently do not understand the genetic regulation of SOCE components in β-cells, several pathways known to be present in the β-cell have been shown to regulate STIM/Orai gene expression in other cell types. STIM1 expression and SOCE amplitude are inhibited by Wilms tumor suppressor 1 (WT1) and increased by early growth response 1 (EGR1) (Ritchie et al. 2010). In β-cells, EGR1 is induced by glucose (Josefsen et al. 1999) and GLP-1R agonists (Kim et al. 2008b) where it regulates insulin gene expression (Eto et al. 2006). Whether glucose and GLP-1, acting through EGR1, also play a role in regulating STIM1 expression in β-cells has not been established.

Serum- and glucocorticoid-inducible kinase-1 (SGK1) upregulates STIM1 and Orai1 expression and also reduces Orai1 degradation (Lang et al. 2012). STIM/Orai expression is also upregulated by NFκB (Lang et al. 2012), a transcription factor known to play a role in the induction of β-cell apoptosis (Cnop et al. 2005). Interestingly, NFκB also leads to depletion of ER Ca^{2+} stores (Cnop et al. 2005) and thus is predicted to increase both channel expression and activity.

SGK3 has also been shown to upregulate expression of STIM2 in dendritic cells (Schmid et al. 2012), and knockdown of SGK3 impairs β-cell function and glucose homeostasis (Yao et al. 2011). Whether SGK3 influences STIM expression in β-cells to contribute to the maintenance of cell function is unknown.

Polyamines also play an interesting role by differentially regulating STIM1 and STIM2 expression; depletion of cellular polyamines decreases STIM1 and increases STIM2 expression (Rao et al. 2012). Polyamines are well-known regulators of K_{ATP} channel activity in β-cells (Nichols and Lopatin 1997). Interestingly, depletion of polyamines has also been suggested to have a protective effect on β-cells in the development of type 1 diabetes mellitus (Tersey et al. 2013). Additional studies will be necessary to determine whether this protective effect involves the regulation of STIM isoforms.

Store-Operated Channels and Diabetes

There is little direct evidence for a role of defects in SOCE in diabetes, either as a potential cause or as a consequence. Platelets from patients with T2DM show increased levels of STIM1/Orai1 and TRPC3 (Zbidi et al. 2009). Changes in expression of SERCA isoforms have also been reported in diabetic mouse islets (Roe et al. 1994). These SERCA defects are predicted to cause SOCE activation due to reduced ER [Ca^{2+}] levels, similar to the effects of NFκB described above. Mutations in SGK1 have also been identified that are associated with the development of T2DM, and increased activity of SGK1 can be induced by hyperglycemia (Lang et al. 2009). As described above, SGK1 increases STIM1/Orai1 expression and also increases the activity of TRPV4 and TRPV5 that are expressed in β-cells. TRPV4 can also be activated by islet amyloid polypeptide causing Ca^{2+} influx and triggering apoptosis (Casas et al. 2008), whereas TRPV5 (ECaC1) expression

decreases in aging ZDF rats (Janssen et al. 2002). These various effects that deplete ER Ca^{2+} levels and increase STIM/Orai expression might lead to Ca^{2+} overload and cytotoxicity contributing to β-cell failure and the development of diabetes.

Antibodies to CD38 have been described in both type 1 and type 2 diabetes patients (Mallone and Perin 2006) although others found similar levels of autoantibodies in diabetic and nondiabetic subjects (Sordi et al. 2005). However, antibodies to CD38 are under development for the treatment of various forms of cancer (Chillemi et al. 2013), and the autoantibodies found in patients often have an acute stimulatory effect on insulin secretion from human islets (Antonelli and Ferrannini 2004), whereas prolonged exposure impairs β-cell function and viability, at least in vitro (Marchetti et al. 2002). While the role of CD38 in β-cells is somewhat controversial and there appears to be interspecies variability and also differences between strains of mice, disruption of CD38 accelerates the development of diabetes in nonobese diabetic (NOD) mice (Chen et al. 2006) as well as induces β-cell apoptosis in C57BL/6 mice (Johnson et al. 2006). Additional work will be needed to determine whether these effects are mediated through effects on Ca^{2+} stores and SOCE.

Summary

Identification and characterization of the molecular components of SOCE in a wide range of eukaryotic cells have provided fundamental new insights into the roles of SOCE in multiple cellular processes. While insulin-secreting cells express a key subset of these molecules, the importance of the SOCE pathway, in addition to the so-called consensus model of metabolic coupling of glucose to K_{ATP} channels for Ca^{2+} influx, remains unresolved. It is clear that much more work will be necessary before we understand the molecular basis of SOCE and its regulation and importance in the β-cell, whether SOCE signaling pathways are affected in diabetes, or whether mutations in the molecular components of SOCE contribute to the development of diabetes.

Acknowledgments Research in the authors' laboratories was supported by the American Diabetes Association Research Award 1-12-BS-109 (CAL) and by the National Institutes of Health R01 grants DK074966 and DK092616 (MWR).

Cross-References

► Calcium Signaling in the Islets
► Electrical, Calcium, and Metabolic Oscillations in Pancreatic Islets
► Electrophysiology of Islet Cells
► Molecular Basis of cAMP Signaling in Pancreatic β Cells
► Pancreatic β Cells in Metabolic Syndrome

References

Aivatiadou E, Ripolone M, Brunetti F, Berruti G (2009) cAMP-Epac2-mediated activation of Rap1 in developing male germ cells: RA-RhoGAP as a possible direct down-stream effector. Mol Reprod Dev 76:407–416

Akiba Y, Kato S, Katsube K, Nakamura M, Takeuchi K, Ishii H, Hibi T (2004) Transient receptor potential vanilloid subfamily 1 expressed in pancreatic islet β cells modulates insulin secretion in rats. Biochem Biophys Res Commun 321:219–225

Ambudkar IS, Bandyopadhyay BC, Liu X, Lockwich TP, Paria B, Ong HL (2006) Functional organization of TRPC-Ca^{2+} channels and regulation of calcium microdomains. Cell Calcium 40:495–504

Antonelli A, Ferrannini E (2004) CD38 autoimmunity: recent advances and relevance to human diabetes. J Endocrinol Invest 27:695–707

Arredouani A, Guiot Y, Jonas JC, Liu LH, Nenquin M, Pertusa JA, Rahier J, Rolland JF, Shull GE, Stevens M, Wuytack F, Henquin JC, Gilon P (2002) SERCA3 ablation does not impair insulin secretion but suggests distinct roles of different sarcoendoplasmic reticulum Ca^{2+} pumps for Ca^{2+} homeostasis in pancreatic β-cells. Diabetes 51:3245–3253

Arredouani A, Evans AM, Ma J, Parrington J, Zhu MX, Galione A (2010) An emerging role for NAADP-mediated Ca^{2+} signaling in the pancreatic β-cell. Islets 2:323–330

Bari MR, Akbar S, Eweida M, Kuhn FJ, Gustafsson AJ, Luckhoff A, Islam MS (2009) H_2O_2-induced Ca^{2+} influx and its inhibition by N-(p-amylcinnamoyl) anthranilic acid in the β-cells: involvement of TRPM2 channels. J Cell Mol Med 13:3260–3267

Barro-Soria R, Aldehni F, Almaca J, Witzgall R, Schreiber R, Kunzelmann K (2010) ER-localized bestrophin 1 activates Ca^{2+}-dependent ion channels TMEM16A and SK4 possibly by acting as a counterion channel. Pflugers Arch 459:485–497

Bauer MC, O'Connell D, Cahill DJ, Linse S (2008) Calmodulin binding to the polybasic C-termini of STIM proteins involved in store-operated calcium entry. Biochemistry 47:6089–6091

Beauvois MC, Merezak C, Jonas JC, Ravier MA, Henquin JC, Gilon P (2006) Glucose-induced mixed $[Ca^{2+}]_c$ oscillations in mouse β-cells are controlled by the membrane potential and the SERCA3 Ca^{2+}-ATPase of the endoplasmic reticulum. Am J Physiol Cell Physiol 290:C1503–C1511

Beech DJ (2005) TRPC1: store-operated channel and more. Pflugers Arch 451:53–60

Berna-Erro A, Redondo PC, Rosado JA (2012) Store-operated Ca^{2+} entry. Adv Exp Med Biol 740:349–382

Bertram R, Arceo RC 2nd (2008) A mathematical study of the differential effects of two SERCA isoforms on Ca^{2+} oscillations in pancreatic islets. Bull Math Biol 70:1251–1271

Best L (2002) Study of a glucose-activated anion-selective channel in rat pancreatic β-cells. Pflugers Arch 445:97–104

Bolotina VM (2004) Store-operated channels: diversity and activation mechanisms. Sci STKE 2004:pe34

Bolotina VM (2008) Orai, STIM1 and iPLA2β: a view from a different perspective. J Physiol 586:3035–3042

Brandman O, Liou J, Park WS, Meyer T (2007) STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca^{2+} levels. Cell 131:1327–1339

Brann JH, Dennis JC, Morrison EE, Fadool DA (2002) Type-specific inositol 1,4,5-trisphosphate receptor localization in the vomeronasal organ and its interaction with a transient receptor potential channel, TRPC2. J Neurochem 83:1452–1460

Braun M, Ramracheya R, Bengtsson M, Zhang Q, Karanauskaite J, Partridge C, Johnson PR, Rorsman P (2008) Voltage-gated ion channels in human pancreatic β-cells: electrophysiological characterization and role in insulin secretion. Diabetes 57:1618–1628

Brazer SC, Singh BB, Liu X, Swaim W, Ambudkar IS (2003) Caveolin-1 contributes to assembly of store-operated Ca^{2+} influx channels by regulating plasma membrane localization of TRPC1. J Biol Chem 278:27208–27215

Bruun TZ, Hoy M, Gromada J (2000) Scinderin-derived actin-binding peptides inhibit Ca^{2+}- and GTPgammaS-dependent exocytosis in mouse pancreatic β-cells. Eur J Pharmacol 403:221–224

Cahalan MD (2009) STIMulating store-operated Ca^{2+} entry. Nat Cell Biol 11:669–677

Calloway N, Holowka D, Baird B (2010) A basic sequence in STIM1 promotes Ca^{2+} influx by interacting with the C-terminal acidic coiled coil of Orai1. Biochemistry 49:1067–1071

Casas S, Novials A, Reimann F, Gomis R, Gribble FM (2008) Calcium elevation in mouse pancreatic β cells evoked by extracellular human islet amyloid polypeptide involves activation of the mechanosensitive ion channel TRPV4. Diabetologia 51:2252–2262

Chen M, Dong Y, Simard JM (2003) Functional coupling between sulfonylurea receptor type 1 and a nonselective cation channel in reactive astrocytes from adult rat brain. J Neurosci 23:8568–8577

Chen J, Chen YG, Reifsnyder PC, Schott WH, Lee CH, Osborne M, Scheuplein F, Haag F, Koch-Nolte F, Serreze DV, Leiter EH (2006) Targeted disruption of CD38 accelerates autoimmune diabetes in NOD/Lt mice by enhancing autoimmunity in an ADP-ribosyltransferase 2-dependent fashion. J Immunol 176:4590–4599

Cheng H, Beck A, Launay P, Gross SA, Stokes AJ, Kinet JP, Fleig A, Penner R (2007) TRPM4 controls insulin secretion in pancreatic β-cells. Cell Calcium 41:51–61

Chillemi A, Zaccarello G, Quarona V, Ferracin M, Ghimenti C, Massaia M, Horenstein AL, Malavasi F (2013) Anti-CD38 antibody therapy: windows of opportunity yielded by the functional characteristics of the target molecule. Mol Med 19:99–108

Clapham DE, Montell C, Schultz G, Julius D (2003) International Union of Pharmacology. XLIII. Compendium of voltage-gated ion channels: transient receptor potential channels. Pharmacol Rev 55:591–596

Cnop M, Welsh N, Jonas JC, Jorns A, Lenzen S, Eizirik DL (2005) Mechanisms of pancreatic β-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes 54(Suppl 2):S97–S107

Colsoul B, Schraenen A, Lemaire K, Quintens R, Van Lommel L, Segal A, Owsianik G, Talavera K, Voets T, Margolskee RF, Kokrashvili Z, Gilon P, Nilius B, Schuit FC, Vennekens R (2010) Loss of high-frequency glucose-induced Ca^{2+} oscillations in pancreatic islets correlates with impaired glucose tolerance in $TRPM5^{-/-}$ mice. Proc Natl Acad Sci USA 107:5208–5213

Csutora P, Zarayskiy V, Peter K, Monje F, Smani T, Zakharov SI, Litvinov D, Bolotina VM (2006) Activation mechanism for CRAC current and store-operated Ca^{2+} entry: calcium influx factor and Ca^{2+}-independent phospholipase A2β-mediated pathway. J Biol Chem 281:34926–34935

Csutora P, Peter K, Kilic H, Park KM, Zarayskiy V, Gwozdz T, Bolotina VM (2008) Novel role for STIM1 as a trigger for calcium influx factor production. J Biol Chem 283:14524–14531

DeHaven WI, Jones BF, Petranka JG, Smyth JT, Tomita T, Bird GS, Putney JW Jr (2009) TRPC channels function independently of STIM1 and Orai1. J Physiol 587:2275–2298

Dishinger JF, Kee HL, Jenkins PM, Fan S, Hurd TW, Hammond JW, Truong YN, Margolis B, Martens JR, Verhey KJ (2010) Ciliary entry of the kinesin-2 motor KIF17 is regulated by importin-β2 and RanGTP. Nat Cell Biol 12:703–710

Dufer M, Neye Y, Horth K, Krippeit-Drews P, Hennige A, Widmer H, McClafferty H, Shipston MJ, Haring HU, Ruth P, Drews G (2011) BK channels affect glucose homeostasis and cell viability of murine pancreatic β cells. Diabetologia 54:423–432

Dzhura I, Chepurny OG, Kelley GG, Leech CA, Roe MW, Dzhura E, Afshari P, Malik S, Rindler MJ, Xu X, Lu Y, Smrcka AV, Holz GG (2010) Epac2-dependent mobilization of intracellular Ca^{2+} by glucagon-like peptide-1 receptor agonist exendin-4 is disrupted in β-cells of phospholipase C-ε knockout mice. J Physiol 588:4871–4889

Engelke M, Friedrich O, Budde P, Schafer C, Niemann U, Zitt C, Jungling E, Rocks O, Luckhoff A, Frey J (2002) Structural domains required for channel function of the mouse transient receptor potential protein homologue TRP1β. FEBS Lett 523:193–199

Enklaar T, Brixel LR, Zabel B, Prawitt D (2010) Adding efficiency: the role of the CAN ion channels TRPM4 and TRPM5 in pancreatic islets. Islets 2:337–338

Eto K, Kaur V, Thomas MK (2006) Regulation of insulin gene transcription by the immediate-early growth response gene Egr-1. Endocrinology 147:2923–2935

Fahrner M, Muik M, Derler I, Schindl R, Fritsch R, Frischauf I, Romanin C (2009) Mechanistic view on domains mediating STIM1-Orai coupling. Immunol Rev 231:99–112

Feng M, Grice DM, Faddy HM, Nguyen N, Leitch S, Wang Y, Muend S, Kenny PA, Sukumar S, Roberts-Thomson SJ, Monteith GR, Rao R (2010) Store-independent activation of Orai1 by SPCA2 in mammary tumors. Cell 143:84–98

Galan C, Dionisio N, Smani T, Salido GM, Rosado JA (2011) The cytoskeleton plays a modulatory role in the association between STIM1 and the Ca^{2+} channel subunits Orai1 and TRPC1. Biochem Pharmacol 82:400–410

Garside VC, Kowalik AS, Johnson CL, DiRenzo D, Konieczny SF, Pin CL (2010) MIST1 regulates the pancreatic acinar cell expression of Atp2c2, the gene encoding secretory pathway calcium ATPase 2. Exp Cell Res 316:2859–2870

Gilon P, Henquin JC (2001) Mechanisms and physiological significance of the cholinergic control of pancreatic β-cell function. Endocr Rev 22:565–604

Gross RW, Ramanadham S, Kruszka KK, Han X, Turk J (1993) Rat and human pancreatic islet cells contain a calcium ion independent phospholipase A2 activity selective for hydrolysis of arachidonate which is stimulated by adenosine triphosphate and is specifically localized to islet β-cells. Biochemistry 32:327–336

Grubisha O, Rafty LA, Takanishi CL, Xu X, Tong L, Perraud AL, Scharenberg AM, Denu JM (2006) Metabolite of SIR2 reaction modulates TRPM2 ion channel. J Biol Chem 281:14057–14065

Gupta RK, Vatamaniuk MZ, Lee CS, Flaschen RC, Fulmer JT, Matschinsky FM, Duncan SA, Kaestner KH (2005) The MODY1 gene HNF-4α regulates selected genes involved in insulin secretion. J Clin Invest 115:1006–1015

Gustafsson AJ, Ingelman-Sundberg H, Dzabic M, Awasum J, Nguyen KH, Ostenson CG, Pierro C, Tedeschi P, Woolcott O, Chiounan S, Lund PE, Larsson O, Islam MS (2005) Ryanodine receptor-operated activation of TRP-like channels can trigger critical Ca^{2+} signaling events in pancreatic β-cells. FASEB J 19:301–303

Gwozdz T, Dutko-Gwozdz J, Zarayskiy V, Peter K, Bolotina VM (2008) How strict is the correlation between STIM1 and Orai1 expression, puncta formation, and I-CRAC activation? Am J Physiol Cell Physiol 295:C1133–C1140

Hansen SK, Parrizas M, Jensen ML, Pruhova S, Ek J, Boj SF, Johansen A, Maestro MA, Rivera F, Eiberg H, Andel M, Lebl J, Pedersen O, Ferrer J, Hansen T (2002) Genetic evidence that HNF-1α-dependent transcriptional control of HNF-4a is essential for human pancreatic β cell function. J Clin Invest 110:827–833

Hisanaga E, Nagasawa M, Ueki K, Kulkarni RN, Mori M, Kojima I (2009) Regulation of calcium-permeable TRPV2 channel by insulin in pancreatic β-cells. Diabetes 58:174–184

Honnappa S, Gouveia SM, Weisbrich A, Damberger FF, Bhavesh NS, Jawhari H, Grigoriev I, van Rijssel FJ, Buey RM, Lawera A, Jelesarov I, Winkler FK, Wuthrich K, Akhmanova A, Steinmetz MO (2009) An EB1-binding motif acts as a microtubule tip localization signal. Cell 138:366–376

How J, Zhang A, Phillips M, Reynaud A, Lu SY, Pan LX, Ho HT, Yau YH, Guskov A, Pervushin K, Shochat SG, Eshaghi S (2013) Comprehensive analysis and identification of the human STIM1 domains for structural and functional studies. PLoS One 8:e53979

Huang GN, Zeng W, Kim JY, Yuan JP, Han L, Muallem S, Worley PF (2006) STIM1 carboxyl-terminus activates native SOC, I-CRAC and TRPC1 channels. Nat Cell Biol 8:1003–1010

Ishii M, Shimizu S, Hara Y, Hagiwara T, Miyazaki A, Mori Y, Kiuchi Y (2006) Intracellular-produced hydroxyl radical mediates H_2O_2-induced Ca^{2+} influx and cell death in rat β-cell line RIN-5F. Cell Calcium 39:487–494

Islam MS (2011) TRP channels of islets. Adv Exp Med Biol 704:811–830

Itagaki K, Kannan KB, Singh BB, Hauser CJ (2004) Cytoskeletal reorganization internalizes multiple transient receptor potential channels and blocks calcium entry into human neutrophils. J Immunol 172:601–607

Jacobo SM, Guerra ML, Jarrard RE, Przybyla JA, Liu G, Watts VJ, Hockerman GH (2009) The intracellular II-III loops of $Ca_v1.2$ and $Ca_v1.3$ uncouple L-type voltage-gated Ca^{2+} channels from glucagon-like peptide-1 potentiation of insulin secretion in INS-1 cells via displacement from lipid rafts. J Pharmacol Exp Ther 330:283–293

Jacobson DA, Philipson LH (2007) TRP channels of the pancreatic β cell. Handb Exp Pharmacol 179:409–424

Jacobson DA, Weber CR, Bao S, Turk J, Philipson LH (2007) Modulation of the pancreatic islet β-cell-delayed rectifier potassium channel Kv2.1 by the polyunsaturated fatty acid arachidonate. J Biol Chem 282:7442–7449

Jacobson DA, Mendez F, Thompson M, Torres J, Cochet O, Philipson LH (2010) Calcium-activated and voltage-gated potassium channels of the pancreatic islet impart distinct and complementary roles during secretagogue induced electrical responses. J Physiol 588:3525–3537

Janssen SW, Hoenderop JG, Hermus AR, Sweep FC, Martens GJ, Bindels RJ (2002) Expression of the novel epithelial Ca^{2+} channel ECaC1 in rat pancreatic islets. J Histochem Cytochem 50:789–798

Jardin I, Salido GM, Rosado JA (2008) Role of lipid rafts in the interaction between hTRPC1, Orai1 and STIM1. Channels (Austin) 2:401–403

Jardin I, Gomez LJ, Salido GM, Rosado JA (2009) Dynamic interaction of hTRPC6 with the Orai1-STIM1 complex or hTRPC3 mediates its role in capacitative or non-capacitative Ca^{2+} entry pathways. Biochem J 420:267–276

Jardin I, Lopez JJ, Zbidi H, Bartegi A, Salido GM, Rosado JA (2011) Attenuated store-operated divalent cation entry and association between STIM1, Orai1, hTRPC1 and hTRPC6 in platelets from type 2 diabetic patients. Blood Cell Mol Dis 46:252–260

Jha A, Ahuja M, Maleth J, Moreno CM, Yuan JP, Kim MS, Muallem S (2013) The STIM1 CTID domain determines access of SARAF to SOAR to regulate Orai1 channel function. J Cell Biol 202:71–79

Ji W, Xu P, Li Z, Lu J, Liu L, Zhan Y, Chen Y, Hille B, Xu T, Chen L (2008) Functional stoichiometry of the unitary calcium-release-activated calcium channel. Proc Natl Acad Sci USA 105:13668–13673

Johnson JD, Ford EL, Bernal-Mizrachi E, Kusser KL, Luciani DS, Han Z, Tran H, Randall TD, Lund FE, Polonsky KS (2006) Suppressed insulin signaling and increased apoptosis in CD38-null islets. Diabetes 55:2737–2746

Josefsen K, Sorensen LR, Buschard K, Birkenbach M (1999) Glucose induces early growth response gene (Egr-1) expression in pancreatic β cells. Diabetologia 42:195–203

Kanzaki M, Zhang YQ, Mashima H, Li L, Shibata H, Kojima I (1999) Translocation of a calcium-permeable cation channel induced by insulin-like growth factor-I. Nat Cell Biol 1:165–170

Kar P, Bakowski D, Di Capite J, Nelson C, Parekh AB (2012) Different agonists recruit different stromal interaction molecule proteins to support cytoplasmic Ca^{2+} oscillations and gene expression. Proc Natl Acad Sci USA 109:6969–6974

Kerschbaum HH, Cahalan MD (1998) Monovalent permeability, rectification, and ionic block of store-operated calcium channels in Jurkat T lymphocytes. J Gen Physiol 111:521–537

Kim BJ, Park KH, Yim CY, Takasawa S, Okamoto H, Im MJ, Kim UH (2008a) Generation of nicotinic acid adenine dinucleotide phosphate and cyclic ADP-ribose by glucagon-like peptide-1 evokes Ca^{2+} signal that is essential for insulin secretion in mouse pancreatic islets. Diabetes 57:868–878

Kim MJ, Kang JH, Chang SY, Jang HJ, Ryu GR, Ko SH, Jeong IK, Kim MS, Jo YH (2008b) Exendin-4 induction of Egr-1 expression in INS-1 β-cells: interaction of SRF, not YY1, with SRE site of rat Egr-1 promoter. J Cell Biochem 104:2261–2271

Kinard TA, Satin LS (1995) An ATP-sensitive Cl^- channel current that is activated by cell swelling, cAMP, and glyburide in insulin-secreting cells. Diabetes 44:1461–1466

Kinard TA, Goforth PB, Tao Q, Abood ME, Teague J, Satin LS (2001) Chloride channels regulate HIT cell volume but cannot fully account for swelling-induced insulin secretion. Diabetes 50:992–1003

Klappe K, Hummel I, Kok JW (2013) Separation of actin-dependent and actin-independent lipid rafts. Anal Biochem 438:133–135

Kolisek M, Beck A, Fleig A, Penner R (2005) Cyclic ADP-ribose and hydrogen peroxide synergize with ADP-ribose in the activation of TRPM2 channels. Mol Cell 18:61–69

Konno M, Shirakawa H, Miyake T, Sakimoto S, Nakagawa T, Kaneko S (2012) Calumin, a Ca^{2+}-binding protein on the endoplasmic reticulum, alters the ion permeability of Ca^{2+} release-activated Ca^{2+} (CRAC) channels. Biochem Biophys Res Commun 417:784–789

Korzeniowski MK, Manjarres IM, Varnai P, Balla T (2010) Activation of STIM1-Orai1 involves an intramolecular switching mechanism. Sci Signal 3:ra82

Kulkarni RN, Roper MG, Dahlgren G, Shih DQ, Kauri LM, Peters JL, Stoffel M, Kennedy RT (2004) Islet secretory defect in insulin receptor substrate 1 null mice is linked with reduced calcium signaling and expression of sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA)-2b and -3. Diabetes 53:1517–1525

Kwan HY, Shen B, Ma X, Kwok YC, Huang Y, Man YB, Yu S, Yao X (2009) TRPC1 associates with BK_{Ca} channel to form a signal complex in vascular smooth muscle cells. Circ Res 104:670–678

Landa LR Jr, Harbeck M, Kaihara K, Chepurny O, Kitiphongspattana K, Graf O, Nikolaev VO, Lohse MJ, Holz GG, Roe MW (2005) Interplay of Ca^{2+} and cAMP signaling in the insulin-secreting MIN6 β-cell line. J Biol Chem 280:31294–31302

Lang F, Gorlach A, Vallon V (2009) Targeting SGK1 in diabetes. Expert Opin Ther Targets 13:1303–1311

Lang F, Eylenstein A, Shumilina E (2012) Regulation of Orai1/STIM1 by the kinases SGK1 and AMPK. Cell Calcium 52:347–354

Lange I, Yamamoto S, Partida-Sanchez S, Mori Y, Fleig A, Penner R (2009) TRPM2 functions as a lysosomal Ca^{2+}-release channel in β cells. Sci Signal 2:ra23

Lee KP, Yuan JP, So I, Worley PF, Muallem S (2010) STIM1-dependent and STIM1-independent function of transient receptor potential canonical (TRPC) channels tunes their store-operated mode. J Biol Chem 285:38666–38673

Leech CA, Habener JF (1997) Insulinotropic glucagon-like peptide-1-mediated activation of non-selective cation currents in insulinoma cells is mimicked by maitotoxin. J Biol Chem 272:17987–17993

Leech CA, Holz GG, Habener JF (1994) Voltage-independent calcium channels mediate slow oscillations of cytosolic calcium that are glucose dependent in pancreatic β-cells. Endocrinology 135:365–372

Leech CA, Kopp RF, Chepurny OG, Holz GG, Roe MW (2012) Molecular characterization of store-operated Ca^{2+} influx in pancreatic β cells: role of STIM1. Diabetes 61(S1):A31

Lefkimmiatis K, Srikanthan M, Maiellaro I, Moyer MP, Curci S, Hofer AM (2009) Store-operated cyclic AMP signalling mediated by STIM1. Nat Cell Biol 11:433–442

Li G, Rungger-Brandle E, Just I, Jonas JC, Aktories K, Wollheim CB (1994) Effect of disruption of actin filaments by *Clostridium botulinum* C2 toxin on insulin secretion in HIT-T15 cells and pancreatic islets. Mol Biol Cell 5:1199–1213

Li Z, Liu L, Deng Y, Ji W, Du W, Xu P, Chen L, Xu T (2010) Graded activation of CRAC channel by binding of different numbers of STIM1 to Orai1 subunits. Cell Res 21:305–315

Liao Y, Erxleben C, Yildirim E, Abramowitz J, Armstrong DL, Birnbaumer L (2007) Orai proteins interact with TRPC channels and confer responsiveness to store depletion. Proc Natl Acad Sci USA 104:4682–4687

Liao Y, Plummer NW, George MD, Abramowitz J, Zhu MX, Birnbaumer L (2009) A role for Orai in TRPC-mediated Ca^{2+} entry suggests that a TRPC: Orai complex may mediate store and receptor operated Ca^{2+} entry. Proc Natl Acad Sci USA 106:3202–3206

Liu S, Mauvais-Jarvis F (2010) Minireview: Estrogenic protection of β-cell failure in metabolic diseases. Endocrinology 151:859–864

Liu G, Dilmac N, Hilliard N, Hockerman GH (2003) Ca v 1.3 is preferentially coupled to glucose-stimulated insulin secretion in the pancreatic β-cell line INS-1. J Pharmacol Exp Ther 305:271–278

Liu M, Large WA, Albert AP (2005a) Stimulation of β-adrenoceptors inhibits store-operated channel currents via a cAMP-dependent protein kinase mechanism in rabbit portal vein myocytes. J Physiol 562:395–406

Liu X, Bandyopadhyay BC, Singh BB, Groschner K, Ambudkar IS (2005b) Molecular analysis of a store-operated and 2-acetyl-sn-glycerol-sensitive non-selective cation channel. Heteromeric assembly of TRPC1-TRPC3. J Biol Chem 280:21600–21606

Lopez E, Salido GM, Rosado JA, Berna-Erro A (2012) Unraveling STIM2 function. J Physiol Biochem 68:619–633

Lu M, Branstrom R, Berglund E, Hoog A, Bjorklund P, Westin G, Larsson C, Farnebo LO, Forsberg L (2010) Expression and association of TRPC subtypes with Orai1 and STIM1 in human parathyroid. J Mol Endocrinol 44:285–294

Lussier MP, Cayouette S, Lepage PK, Bernier CL, Francoeur N, St-Hilaire M, Pinard M, Boulay G (2005) MxA, a member of the dynamin superfamily, interacts with the ankyrin-like repeat domain of TRPC. J Biol Chem 280:19393–19400

Luu L, Dai FF, Prentice KJ, Huang X, Hardy AB, Hansen JB, Liu Y, Joseph JW, Wheeler MB (2013) The loss of Sirt1 in mouse pancreatic β cells impairs insulin secretion by disrupting glucose sensing. Diabetologia 56:2010–2020

Ma X, Cao J, Luo J, Nilius B, Huang Y, Ambudkar IS, Yao X (2010) Depletion of intracellular Ca^{2+} stores stimulates the translocation of vanilloid transient receptor potential 4-c1 heteromeric channels to the plasma membrane. Arterioscler Thromb Vasc Biol 30:2249–2255

Ma MT, Yeo JF, Farooqui AA, Ong WY (2011) Role of calcium independent phospholipase A2 in maintaining mitochondrial membrane potential and preventing excessive exocytosis in PC12 cells. Neurochem Res 36:347–354

Mallone R, Perin PC (2006) Anti-CD38 autoantibodies in type? Diabetes. Diabetes Metab Res Rev 22:284–294

Marchetti P, Antonelli A, Lupi R, Marselli L, Fallahi P, Nesti C, Baj G, Ferrannini E (2002) Prolonged in vitro exposure to autoantibodies against CD38 impairs the function and survival of human pancreatic islets. Diabetes 51(Suppl 3):S474–S477

Marigo V, Courville K, Hsu WH, Feng JM, Cheng H (2009) TRPM4 impacts on Ca^{2+} signals during agonist-induced insulin secretion in pancreatic β-cells. Mol Cell Endocrinol 299:194–203

Martin AC, Willoughby D, Ciruela A, Ayling LJ, Pagano M, Wachten S, Tengholm A, Cooper DM (2009) Capacitative Ca^{2+} entry via Orai1 and stromal interacting molecule 1 (STIM1) regulates adenylyl cyclase type 8. Mol Pharmacol 75:830–842

McNally BA, Yamashita M, Engh A, Prakriya M (2009) Structural determinants of ion permeation in CRAC channels. Proc Natl Acad Sci USA 106:22516–22521

Metz SA (1988) Exogenous arachidonic acid promotes insulin release from intact or permeabilized rat islets by dual mechanisms. Putative activation of Ca^{2+} mobilization and protein kinase C. Diabetes 37:1453–1469

Miao Y, Miner C, Zhang L, Hanson PI, Dani A, Vig M (2013) An essential and NSF independent role for a-SNAP in store-operated calcium entry. Elife 2:e00802

Mignen O, Thompson JL, Shuttleworth TJ (2001) Reciprocal regulation of capacitative and arachidonate-regulated noncapacitative Ca^{2+} entry pathways. J Biol Chem 276:35676–35683

Mignen O, Thompson JL, Shuttleworth TJ (2007) STIM1 regulates Ca^{2+} entry via arachidonate-regulated Ca^{2+}-selective (ARC) channels without store depletion or translocation to the plasma membrane. J Physiol 579:703–715

Mignen O, Thompson JL, Shuttleworth TJ (2009) The molecular architecture of the arachidonate-regulated Ca^{2+}-selective ARC channel is a pentameric assembly of Orai1 and Orai3 subunits. J Physiol 587:4181–4197

Motiani RK, Abdullaev IF, Trebak M (2010) A novel native store-operated calcium channel encoded by Orai3: selective requirement of Orai3 versus Orai1 in estrogen receptor-positive versus estrogen receptor-negative breast cancer cells. J Biol Chem 285:19173–19183

Motiani RK, Zhang X, Harmon KE, Keller RS, Matrougui K, Bennett JA, Trebak M (2013) Orai3 is an estrogen receptor α-regulated Ca^{2+} channel that promotes tumorigenesis. FASEB J 27:63–75

Moynihan KA, Grimm AA, Plueger MM, Bernal-Mizrachi E, Ford E, Cras-Meneur C, Permutt MA, Imai S (2005) Increased dosage of mammalian Sir2 in pancreatic β cells enhances glucose-stimulated insulin secretion in mice. Cell Metab 2:105–117

Muik M, Frischauf I, Derler I, Fahrner M, Bergsmann J, Eder P, Schindl R, Hesch C, Polzinger B, Fritsch R, Kahr H, Madl J, Gruber H, Groschner K, Romanin C (2008) Dynamic coupling of the putative coiled-coil domain of ORAI1 with STIM1 mediates ORAI1 channel activation. J Biol Chem 283:8014–8022

Mullins FM, Park CY, Dolmetsch RE, Lewis RS (2009) STIM1 and calmodulin interact with Orai1 to induce Ca^{2+}-dependent inactivation of CRAC channels. Proc Natl Acad Sci USA 106:15495–15500

Muraki K, Iwata Y, Katanosaka Y, Ito T, Ohya S, Shigekawa M, Imaizumi Y (2003) TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes. Circ Res 93:829–838

Nadal A, Alonso-Magdalena P, Soriano S, Ripoll C, Fuentes E, Quesada I, Ropero AB (2011) Role of estrogen receptors α, β and GPER1/GPR30 in pancreatic β-cells. Front Biosci 16:251–260

Nagamatsu S, Ohara-Imaizumi M, Nakamichi Y, Kikuta T, Nishiwaki C (2006) Imaging docking and fusion of insulin granules induced by antidiabetes agents: sulfonylurea and glinide drugs preferentially mediate the fusion of newcomer, but not previously docked, insulin granules. Diabetes 55:2819–2825

Nevins AK, Thurmond DC (2003) Glucose regulates the cortical actin network through modulation of Cdc42 cycling to stimulate insulin secretion. Am J Physiol Cell Physiol 285: C698–C710

Nichols CG, Lopatin AN (1997) Inward rectifier potassium channels. Annu Rev Physiol 59:171–191

Niehof M, Borlak J (2008) HNF4α and the Ca-channel TRPC1 are novel disease candidate genes in diabetic nephropathy. Diabetes 57:1069–1077

Nilius B, Prenen J, Tang J, Wang C, Owsianik G, Janssens A, Voets T, Zhu MX (2005) Regulation of the Ca^{2+} sensitivity of the nonselective cation channel TRPM4. J Biol Chem 280:6423–6433

Nilius B, Mahieu F, Prenen J, Janssens A, Owsianik G, Vennekens R, Voets T (2006) The Ca^{2+}-activated cation channel TRPM4 is regulated by phosphatidylinositol 4,5-biphosphate. EMBO J 25:467–478

Nitert MD, Nagorny CL, Wendt A, Eliasson L, Mulder H (2008) $Ca_V1.2$ rather than $Ca_V1.3$ is coupled to glucose-stimulated insulin secretion in INS-1 832/13 cells. J Mol Endocrinol 41:1–11

Oh-Hora M, Yamashita M, Hogan PG, Sharma S, Lamperti E, Chung W, Prakriya M, Feske S, Rao A (2008) Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. Nat Immunol 9:432–443

Orci L, Gabbay KH, Malaisse WJ (1972) Pancreatic β-cell web: its possible role in insulin secretion. Science 175:1128–1130

Osterhout JL, Shuttleworth TJ (2000) A Ca^{2+}-independent activation of a type IV cytosolic phospholipase A(2) underlies the receptor stimulation of arachidonic acid-dependent noncapacitative calcium entry. J Biol Chem 275:8248–8254

Palty R, Raveh A, Kaminsky I, Meller R, Reuveny E (2012) SARAF inactivates the store operated calcium entry machinery to prevent excess calcium refilling. Cell 149:425–438

Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, Raunser S, Walz T, Garcia KC, Dolmetsch RE, Lewis RS (2009) STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. Cell 136:876–890

Park SH, Ryu SY, Yu WJ, Han YE, Ji YS, Oh K, Sohn JW, Lim A, Jeon JP, Lee H, Lee KH, Lee SH, Berggren PO, Jeon JH, Ho WK (2013) Leptin promotes K_{ATP} channel trafficking by AMPK signaling in pancreatic β-cells. Proc Natl Acad Sci USA 110:12673–12678

Penna A, Demuro A, Yeromin AV, Zhang SL, Safrina O, Parker I, Cahalan MD (2008) The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers. Nature 456:116–120

Perraud AL, Schmitz C, Scharenberg AM (2003) TRPM2 Ca^{2+} permeable cation channels: from gene to biological function. Cell Calcium 33:519–531

Pulver RA, Rose-Curtis P, Roe MW, Wellman GC, Lounsbury KM (2004) Store-operated Ca^{2+} entry activates the CREB transcription factor in vascular smooth muscle. Circ Res 94:1351–1358

Qian F, Huang P, Ma L, Kuznetsov A, Tamarina N, Philipson LH (2002) TRP genes: candidates for nonselective cation channels and store-operated channels in insulin-secreting cells. Diabetes 51(Suppl 1):S183–S189

Rao JN, Rathor N, Zhuang R, Zou T, Liu L, Xiao L, Turner DJ, Wang JY (2012) Polyamines regulate intestinal epithelial restitution through TRPC1-mediated Ca^{2+} signaling by differentially modulating STIM1 and STIM2. Am J Physiol Cell Physiol 303:C308–C317

Reichhart N, Milenkovic VM, Halsband CA, Cordeiro S, Strauss O (2010) Effect of bestrophin-1 on L-type Ca^{2+} channel activity depends on the Ca^{2+} channel β-subunit. Exp Eye Res 91:630–639

Ritchie MF, Yue C, Zhou Y, Houghton PJ, Soboloff J (2010) Wilms tumor suppressor 1 (WT1) and early growth response 1 (EGR1) are regulators of STIM1 expression. J Biol Chem 285:10591–10596

Roe MW, Philipson LH, Frangakis CJ, Kuznetsov A, Mertz RJ, Lancaster ME, Spencer B, Worley JF 3rd, Dukes ID (1994) Defective glucose-dependent endoplasmic reticulum Ca^{2+} sequestration in diabetic mouse islets of Langerhans. J Biol Chem 269:18279–18282

Roe MW, Worley JF 3rd, Qian F, Tamarina N, Mittal AA, Dralyuk F, Blair NT, Mertz RJ, Philipson LH, Dukes ID (1998) Characterization of a Ca^{2+} release-activated nonselective cation current regulating membrane potential and $[Ca^{2+}]_i$ oscillations in transgenically derived β-cells. J Biol Chem 273:10402–10410

Saitoh N, Oritani K, Saito K, Yokota T, Ichii M, Sudo T, Fujita N, Nakajima K, Okada M, Kanakura Y (2011) Identification of functional domains and novel binding partners of STIM proteins. J Cell Biochem 112:147–151

Sakura H, Ashcroft FM (1997) Identification of four TRP1 gene variants murine pancreatic β-cells. Diabetologia 40:528–532

Sala-Rabanal M, Wang S, Nichols CG (2012) On potential interactions between non-selective cation channel TRPM4 and sulfonylurea receptor SUR1. J Biol Chem 287:8746–8756

Schindl R, Frischauf I, Bergsmann J, Muik M, Derler I, Lackner B, Groschner K, Romanin C (2009) Plasticity in Ca^{2+} selectivity of Orai1/Orai3 heteromeric channel. Proc Natl Acad Sci USA 106:19623–19628

Schmid E, Bhandaru M, Nurbaeva MK, Yang W, Szteyn K, Russo A, Leibrock C, Tyan L, Pearce D, Shumilina E, Lang F (2012) SGK3 regulates Ca^{2+} entry and migration of dendritic cells. Cell Physiol Biochem 30:1423–1435

Seino S, Chen L, Seino M, Blondel O, Takeda J, Johnson JH, Bell GI (1992) Cloning of the a1 subunit of a voltage-dependent calcium channel expressed in pancreatic β cells. Proc Natl Acad Sci USA 89:584–588

Shawl AI, Park KH, Kim UH (2009) Insulin receptor signaling for the proliferation of pancreatic β-cells: involvement of Ca^{2+} second messengers, IP3, NAADP and cADPR. Islets 1:216–223

Shawl AI, Park KH, Kim BJ, Higashida C, Higashida H, Kim UH (2012) Involvement of actin filament in the generation of Ca^{2+} mobilizing messengers in glucose-induced Ca^{2+} signaling in pancreatic β-cells. Islets 4:145–151

Shibasaki T, Takahashi H, Miki T, Sunaga Y, Matsumura K, Yamanaka M, Zhang C, Tamamoto A, Satoh T, Miyazaki J, Seino S (2007) Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP. Proc Natl Acad Sci USA 104:19333–19338

Silva AM, Rosario LM, Santos RM (1994) Background Ca^{2+} influx mediated by a dihydropyridine- and voltage-insensitive channel in pancreatic β-cells. Modulation by Ni^{2+}, diphenylamine-2-carboxylate, and glucose metabolism. J Biol Chem 269:17095–17103

Simard JM, Kahle KT, Gerzanich V (2010) Molecular mechanisms of microvascular failure in central nervous system injury–synergistic roles of NKCC1 and SUR1/TRPM4. J Neurosurg 113:622–629

Singaravelu K, Nelson C, Bakowski D, Martins de Brito O, Ng SW, Di Capite J, Powell T, Scorrano L, Parekh AB (2011) Mitofusin 2 regulates STIM1 migration from the Ca^{2+} store to the plasma membrane in cells with depolarized mitochondria. J Biol Chem 286:12189–12201

Singh BB, Liu X, Tang J, Zhu MX, Ambudkar IS (2002) Calmodulin regulates Ca^{2+}-dependent feedback inhibition of store-operated Ca^{2+} influx by interaction with a site in the C terminus of TRPC1. Mol Cell 9:739–750

Smani T, Zakharov SI, Leno E, Csutora P, Trepakova ES, Bolotina VM (2003) Ca^{2+}-independent phospholipase A2 is a novel determinant of store-operated Ca^{2+} entry. J Biol Chem 278:11909–11915

Smani T, Zakharov SI, Csutora P, Leno E, Trepakova ES, Bolotina VM (2004) A novel mechanism for the store-operated calcium influx pathway. Nat Cell Biol 6:113–120

Soboloff J, Spassova MA, Hewavitharana T, He LP, Xu W, Johnstone LS, Dziadek MA, Gill DL (2006) STIM2 is an inhibitor of STIM1-mediated store-operated Ca^{2+} entry. Curr Biol 16:1465–1470

Song H, Rohrs H, Tan M, Wohltmann M, Ladenson JH, Turk J (2010) Effects of endoplasmic reticulum stress on group VIA phospholipase A2 in β cells include tyrosine phosphorylation and increased association with calnexin. J Biol Chem 285:33843–33857

Sookoian S, Gemma C, Pirola CJ (2010) Influence of hepatocyte nuclear factor 4α (HNF4α) gene variants on the risk of type 2 diabetes: a meta-analysis in 49,577 individuals. Mol Genet Metab 99:80–89

Sordi V, Lampasona V, Cainarca S, Bonifacio E (2005) No evidence of diabetes-specific CD38 (ADP ribosil cyclase/cyclic ADP-ribose hydrolase) autoantibodies by liquid-phase immunoprecipitation. Diabet Med 22:1770–1773

Srikanth S, Ribalet B, Gwack Y (2013) Regulation of CRAC channels by protein interactions and post-translational modification. Channels (Austin) 7:354–363

Srivastava M, Eidelman O, Leighton X, Glasman M, Goping G, Pollard HB (2002) Anx7 is required for nutritional control of gene expression in mouse pancreatic islets of Langerhans. Mol Med 8:781–797

Stathopulos PB, Zheng L, Li GY, Plevin MJ, Ikura M (2008) Structural and mechanistic insights into STIM1-mediated initiation of store-operated calcium entry. Cell 135:110–122

Strotmann R, Harteneck C, Nunnenmacher K, Schultz G, Plant TD (2000) OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. Nat Cell Biol 2:695–702

Sundivakkam PC, Kwiatek AM, Sharma TT, Minshall RD, Malik AB, Tiruppathi C (2009) Caveolin-1 scaffold domain interacts with TRPC1 and IP3R3 to regulate Ca^{2+} store release-induced Ca^{2+} entry in endothelial cells. Am J Physiol Cell Physiol 296:C403–C413

Takasawa S, Ishida A, Nata K, Nakagawa K, Noguchi N, Tohgo A, Kato I, Yonekura H, Fujisawa H, Okamoto H (1995) Requirement of calmodulin-dependent protein kinase II in cyclic ADP-ribose-mediated intracellular Ca^{2+} mobilization. J Biol Chem 270:30257–30259

Tamarina NA, Kuznetsov A, Philipson LH (2008) Reversible translocation of EYFP-tagged STIM1 is coupled to calcium influx in insulin secreting β-cells. Cell Calcium 44:533–544

Tang MM, Zhu QE, Fan WZ, Zhang SL, Li DZ, Liu LZ, Chen M, Zhang M, Zhou J, Wei CJ (2011) Intra-arterial targeted islet-specific expression of Sirt1 protects β cells from streptozotocin-induced apoptosis in mice. Mol Ther 19:60–66

Tersey SA, Colvin SC, Maier B, Mirmira RG (2014) Protective effects of polyamine depletion in mouse models of type 1 diabetes: implications for therapy. Amino Acids 46:633–642

Thakur P, Dadsetan S, Fomina AF (2012) Bidirectional coupling between ryanodine receptors and Ca^{2+} release-activated Ca^{2+} (CRAC) channel machinery sustains store-operated Ca^{2+} entry in human T lymphocytes. J Biol Chem 287:37233–37244

Thompson JL, Mignen O, Shuttleworth TJ (2010) The N-terminal domain of Orai3 determines selectivity for activation of the store-independent ARC channel by arachidonic acid. Channels (Austin) 4:398–410

Tian G, Tepikin AV, Tengholm A, Gylfe E (2012) cAMP induces stromal interaction molecule 1 (STIM1) puncta but neither Orai1 protein clustering nor store-operated Ca^{2+} entry (SOCE) in islet cells. J Biol Chem 287:9862–9872

Timmers S, Hesselink MK, Schrauwen P (2013) Therapeutic potential of resveratrol in obesity and type 2 diabetes: new avenues for health benefits? Ann N Y Acad Sci 1290:83–89

Togashi K, Hara Y, Tominaga T, Higashi T, Konishi Y, Mori Y, Tominaga M (2006) TRPM2 activation by cyclic ADP-ribose at body temperature is involved in insulin secretion. EMBO J 25:1804–1815

Tong Q, Zhang W, Conrad K, Mostoller K, Cheung JY, Peterson BZ, Miller BA (2006) Regulation of the transient receptor potential channel TRPM2 by the Ca^{2+} sensor calmodulin. J Biol Chem 281:9076–9085

Turk J, Wolf BA, Lefkowith JB, Stump WT, McDaniel ML (1986) Glucose-induced phospholipid hydrolysis in isolated pancreatic islets: quantitative effects on the phospholipid content of arachidonate and other fatty acids. Biochim Biophys Acta 879:399–409

Uchida K, Dezaki K, Damdindorj B, Inada H, Shiuchi T, Mori Y, Yada T, Minokoshi Y, Tominaga M (2010) Lack of TRPM2 impaired insulin secretion and glucose metabolisms in mice. Diabetes 60:119–126

Uenishi E, Shibasaki T, Takahashi H, Seki C, Hamaguchi H, Yasuda T, Tatebe M, Oiso Y, Takenawa T, Seino S (2013) Actin dynamics regulated by the balance of neuronal wiskott-aldrich syndrome protein (N-WASP) and cofilin activities determines the biphasic response of glucose-induced insulin secretion. J Biol Chem 288:25851–25864

Vetterli L, Brun T, Giovannoni L, Bosco D, Maechler P (2011) Resveratrol potentiates glucose-stimulated insulin secretion in INS-1E β-cells and human islets through a SIRT1-dependent mechanism. J Biol Chem 286:6049–6060

Wang Z, Oh E, Thurmond DC (2007) Glucose-stimulated Cdc42 signaling is essential for the second phase of insulin secretion. J Biol Chem 282:9536–9546

Wang Y, Deng X, Mancarella S, Hendron E, Eguchi S, Soboloff J, Tang XD, Gill DL (2010) The calcium store sensor, STIM1, reciprocally controls Orai and $Ca_V1.2$ channels. Science 330:105–109

Woodard GE, Salido GM, Rosado JA (2008) Enhanced exocytotic-like insertion of Orai1 into the plasma membrane upon intracellular Ca^{2+} store depletion. Am J Physiol Cell Physiol 294: C1323–C1331

Woodard GE, Lopez JJ, Jardin I, Salido GM, Rosado JA (2010) TRPC3 regulates agonist-stimulated Ca^{2+} mobilization by mediating the interaction between type I inositol 1,4,5-trisphosphate receptor, RACK1, and Orai1. J Biol Chem 285:8045–8053

Woolcott OO, Gustafsson AJ, Dzabic M, Pierro C, Tedeschi P, Sandgren J, Bari MR, Nguyen KH, Bianchi M, Rakonjac M, Radmark O, Ostenson CG, Islam MS (2006) Arachidonic acid is a physiological activator of the ryanodine receptor in pancreatic β-cells. Cell Calcium 39:529–537

Worley JF 3rd, McIntyre MS, Spencer B, Dukes ID (1994a) Depletion of intracellular Ca^{2+} stores activates a maitotoxin-sensitive nonselective cationic current in β-cells. J Biol Chem 269:32055–32058

Worley JF 3rd, McIntyre MS, Spencer B, Mertz RJ, Roe MW, Dukes ID (1994b) Endoplasmic reticulum calcium store regulates membrane potential in mouse islet β-cells. J Biol Chem 269:14359–14362

Wuttke A, Idevall-Hagren O, Tengholm A (2013) P2Y(1) receptor-dependent diacylglycerol signaling microdomains in β cells promote insulin secretion. FASEB J 27:1610–1620

Xie L, Zhu D, Gaisano HY (2012) Role of mammalian homologue of Caenorhabditis elegans unc-13-1 (Munc13-1) in the recruitment of newcomer insulin granules in both first and second phases of glucose-stimulated insulin secretion in mouse islets. Diabetologia 55:2693–2702

Yamasaki M, Masgrau R, Morgan AJ, Churchill GC, Patel S, Ashcroft SJ, Galione A (2004) Organelle selection determines agonist-specific Ca^{2+} signals in pancreatic acinar and β cells. J Biol Chem 279:7234–7240

Yao LJ, McCormick JA, Wang J, Yang KY, Kidwai A, Colussi GL, Boini KM, Birnbaum MJ, Lang F, German MS, Pearce D (2011) Novel role for SGK3 in glucose homeostasis revealed in SGK3/Akt2 double-null mice. Mol Endocrinol 25:2106–2118

Yeung-Yam-Wah V, Lee AK, Tse FW, Tse A (2010) Arachidonic acid stimulates extracellular Ca^{2+} entry in rat pancreatic β cells via activation of the noncapacitative arachidonate-regulated Ca^{2+} (ARC) channels. Cell Calcium 47:77–83

Yu K, Xiao Q, Cui G, Lee A, Hartzell HC (2008) The best disease-linked Cl^- channel hBest1 regulates Ca_V 1 (L-type) Ca^{2+} channels via src-homology-binding domains. J Neurosci 28:5660–5670

Yu F, Sun L, Machaca K (2010) Constitutive recycling of the store-operated Ca^{2+} channel Orai1 and its internalization during meiosis. J Cell Biol 191:523–535

Yu F, Sun L, Hubrack S, Selvaraj S, Machaca K (2013a) Intramolecular shielding maintains the ER Ca^{2+} sensor STIM1 in an inactive conformation. J Cell Sci 126:2401–2410

Yu J, Zhang H, Zhang M, Deng Y, Wang H, Lu J, Xu T, Xu P (2013b) An aromatic amino acid in the CC1 domain plays a crucial role in the auto-inhibitory mechanism of STIM1. Biochem J 454:401–409

Yuan JP, Kim MS, Zeng W, Shin DM, Huang G, Worley PF, Muallem S (2009a) TRPC channels as STIM1-regulated SOCs. Channels (Austin) 3:221–225

Yuan JP, Zeng W, Dorwart MR, Choi YJ, Worley PF, Muallem S (2009b) SOAR and the polybasic STIM1 domains gate and regulate Orai channels. Nat Cell Biol 11:337–343

Zarayskiy V, Monje F, Peter K, Csutora P, Khodorov BI, Bolotina VM (2007) Store-operated Orai1 and IP3 receptor-operated TRPC1 channel. Channels (Austin) 1:246–252

Zbidi H, Lopez JJ, Amor NB, Bartegi A, Salido GM, Rosado JA (2009) Enhanced expression of STIM1/Orai1 and TRPC3 in platelets from patients with type 2 diabetes mellitus. Blood Cells Mol Dis 43:211–213

Zbidi H, Jardin I, Woodard GE, Lopez JJ, Berna A, Salido GM, Rosado JA (2011) STIM1 and STIM2 are located in the acidic Ca^{2+} stores and associates with Orai1 upon depletion of the acidic stores in human platelets. J Biol Chem 286:12257–12270

Zeng W, Yuan JP, Kim MS, Choi YJ, Huang GN, Worley PF, Muallem S (2008) STIM1 gates TRPC channels, but not Orai1, by electrostatic interaction. Mol Cell 32:439–448

Zeng B, Chen GL, Xu SZ (2012) Store-independent pathways for cytosolic STIM1 clustering in the regulation of store-operated Ca^{2+} influx. Biochem Pharmacol 84:1024–1035

Zhang W, Chu X, Tong Q, Cheung JY, Conrad K, Masker K, Miller BA (2003) A novel TRPM2 isoform inhibits calcium influx and susceptibility to cell death. J Biol Chem 278:16222–16229

Zhang Z, Okawa H, Wang Y, Liman ER (2005) Phosphatidylinositol 4,5-bisphosphate rescues TRPM4 channels from desensitization. J Biol Chem 280:39185–39192

Zhou Y, Ramachandran S, Oh-Hora M, Rao A, Hogan PG (2010) Pore architecture of the ORAI1 store-operated calcium channel. Proc Natl Acad Sci USA 107:4896–4901

Zweifach A, Lewis RS (1996) Calcium-dependent potentiation of store-operated calcium channels in T lymphocytes. J Gen Physiol 107:597–610

Anionic Transporters and Channels in Pancreatic Islet Cells

13

Nurdan Bulur and Willy J. Malaisse

Contents

Abstract

After a brief description of the so-called consensus hypothesis for the mechanism of stimulus-secretion coupling in the process of glucose-induced insulin release, the present chapter, which deals with anionic transporters and channels in pancreatic islet cells, concerns mainly a second modality for the control of

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_41,

insulin secretion by the hexose. In such a perspective, it draws attention to the NBCe1 Na^+/HCO_3^- cotransporters, the volume-regulated anion channel hypothesis, the experimental model of extracellular hypotonicity, the possible role of NAD(P)H oxidase-derived H_2O_2 in the activation of volume-regulated anion channels in β-cells exposed to a hypotonic medium, the identity of the anions concerned by the volume-regulated anion channel hypothesis, the expression and function of anoctamin 1 in rodent and human pancreatic islet cells, the possible role of bicarbonate-activated soluble adenylyl cyclase, the identity and role of aquaporins in insulin-producing cells, and a proposed role for volume-regulated anion channels in glucagon secretion.

Keywords

NBCe1 Na^+/HCO_3^- cotransporter • Volume-regulated anion channels • Extracellular hypoosmolarity • NAD(P)H oxidase-derived H_2O_2 • Anoctamin 1 • Soluble adenylyl cyclase • Aquaporins • Glucagon secretion

Introduction

The so-called consensus hypothesis for the process of glucose-induced insulin secretion postulates that the corresponding mechanism of stimulus-secretion coupling involves a sequence of metabolic, ionic, and motile cellular events.

It had been first proposed that the activation of insulin-producing β-cells in the pancreatic islets in response to a rise in extracellular D-glucose concentration was attributable to the intervention of a stereospecific glucoreceptor possibly located at the level of the β-cell plasma membrane. Such a receptor concept contrasts with the more pedestrian view that the stimulation of insulin release by D-glucose and other nutrient secretagogues is causally linked to their capacity to act as nutrient in the β-cells and, hence, to increase the rate of ATP generation.

The validation of the latter fuel concept emerged inter alia from the following three series of findings. First, the finding that the α-anomer of D-glucose is a more potent insulin secretagogue than its β-anomer, first considered in support of the glucoreceptor theory, was eventually ascribed to the fact that the α-anomer of D-glucose is more efficiently metabolized in isolated pancreatic islets than its β-anomer (Malaisse et al. 1976). Second, a nonmetabolized analog of L-leucine, b(-)-2-amino-bicyclo[2,2,1]heptane-2-carboxylic acid (BCH), which was found to duplicate the insulinotropic action of L-leucine itself, was eventually found to activate pancreatic islet glutamate dehydrogenase and, by doing so, facilitate the catabolism of endogenous amino acids (Sener et al. 1981). Third, the insulinotropic potential of 3-phenylpyruvate, first proposed to be attributable to the intervention of a specific β-cell membrane receptor acting as mediator of the insulin-releasing capacity of 3-phenylpyruvate, was eventually found to coincide with an increased

catabolism of endogenous amino acids acting as partners in transamination reactions leading to the conversion of 3-phenylpyruvate into phenylalanine (Sener et al. 1983; Malaisse et al. 1983).

The coupling between the increased catabolism of exogenous or endogenous nutrients and the remodeling of ionic fluxes in insulin-producing cells soon became, within the framework of the fuel concept for insulin release, a further matter of debate (Malaisse et al. 1979a). For instance, changes in the generation rate or content of high-energy phosphates (e.g., ATP), reducing agents (e.g., NADH and/or NADPH), and protons (H^+) were all taken in due consideration. The consensus hypothesis postulates that the nutrient-induced increase of ATP concentration or ATP/ADP ratio in the cytosolic domain provokes the closing of ATP-sensitive K^+ channels (Cook and Hales 1984), this leading in turn to depolarization of the plasma membrane and subsequent gating of voltage-dependent Ca^{2+} channels, eventually resulting in an increase of Ca^{2+} influx into the β-cell, a rise in the cytosolic concentration of Ca^{2+}, and the activation by Ca^{2+} of an effector system for the translocation and exocytosis of insulin secretory granules.

The third and last step in the stimulus-secretion coupling of glucose-induced insulin release was indeed ascribed to motile events leading to the intracellular translocation of insulin-containing secretory granules and their eventual access to an exocytotic site at the β-cell plasma membrane. The participation of a β-cell microtubular-microfilamentous system in these motile events is supported by a series of ultrastructural, biochemical, functional, and pathophysiological observations (Malaisse and Orci 1979). For instance, the study of motile events in pancreatic endocrine cells by time-lapse cinematography documented that secretory granules underwent back-and-forth saltatory movement along oriented microtubular pathways. A rise in extracellular D-glucose concentration from 2.8 to 16.7 mM resulted in a twofold increase in the frequency of saltatory movements. The second type of motile events consisted in the formation of outward expansions which extend from the cell boundary and, thereafter, retract more or less rapidly. Secretagogues, such as D-glucose, increased the ruffling of the cell membrane in terms of frequency, speed of expansion, duration, and amplitude. Cytochalasin B also dramatically increased the frequency and amplitude of the bleb-like outward expansions of the cell (Somers et al. 1979). These findings support the view that the microtubular apparatus serves as guiding cytoskeleton for the oriented translocation of secretory granules, whereas the microfilamentous cell web may control the eventual access of the granules to exocytotic sites. At these sites, the exocytosis of secretory granules entails the fusion and fission of membranes, followed by the dissolution of the granule core in the interstitial fluid. A chemoosmotic hypothesis was proposed to account for the fission of membranes at the exocytotic site (Somers et al. 1980). It was also proposed that anionic transport at exocytotic sites may account for the phenomenon of chain release, in which two or more secretory granules are discharged, in a row, at the same exocytotic site (Orci and Malaisse 1980).

A Second Modality for the Control of Insulin Secretion by Glucose

More than 20 years ago, Carpinelli and Malaisse (1981) documented the relationship between $^{86}Rb^{+}$ fractional outflow rate from prelabeled and perifused rat pancreatic islets and the concentration of D-glucose at values of zero, 1.7, 2.8, 4.4, 5.6, 8.3, and 16.7 mM. A rise in D-glucose concentration up to about 6–8 mM decreased ^{86}Rb outflow, indicating a decrease in K^{+} conductance, itself attributable to the closing of ATP-sensitive K^{+} channels. However, no further decrease in ^{86}Rb fractional outflow rate and, on the contrary, a modest but significant increase was observed at higher glucose concentrations, namely, in the range of glucidic concentrations provoking the most marked stimulation of insulin release. Thus, it was considered that the progressive increase in K^{+} conductance recorded when the concentration of D-glucose is decreased below 5–6 mM is well suited to prevent undesirable insulin secretion in situations of hypoglycemia, but that another series of cellular events may be responsible for enhancing insulin secretion at high concentrations of D-glucose.

Such a view is supported by later findings, documented among others by Henquin and colleagues, and proposing that, in addition to the closing of ATP-sensitive K^{+} channels located at the plasma membrane of β-cells, a second site may participate in the control of insulin secretion by D-glucose (Henquin et al. 1994). Thus, it was observed that in mouse pancreatic islets exposed to diazoxide (0.2 mM) in order to prevent glucose-induced β-cell plasma membrane depolarization, no increase in cytosolic Ca^{2+} concentrations and no stimulation of insulin release occurred when the concentration of extracellular D-glucose was raised from zero to 6.0 or 20.0 mM. In the presence of diazoxide, depolarization of the plasma membrane was nevertheless provoked by raising the extracellular K^{+} concentration from 4.8 to 30.0 mM. Even in the absence of D-glucose, this resulted in a dramatic increase in cytosolic Ca^{2+} concentration and a modest increase in insulin output. Most importantly, however, under the same experimental conditions, i.e., in the presence of diazoxide and a high extracellular K^{+} concentration (30.0 mM), the rise in D-glucose concentration from zero to 6.0 and 20.0 mM caused a concentration-related further increase in insulin output, despite the fact that the cytosolic Ca^{2+} concentration was significantly lower in the presence of D-glucose than in its absence but coinciding, at the high extracellular K^{+} concentration, with a progressive increase of the ATP/ADP ratio in the islets exposed to increasing concentrations of D-glucose. From these findings, it was indeed concluded that D-glucose is able to affect insulin secretion by acting on a target distinct from the ATP-sensitive K^{+} channels.

The present chapter deals mainly with such a second modality for the control of insulin secretion by glucose.

NBCe1 Na^{+}/HCO_3^{-} Cotransporters

In the search of a complementary mechanism for the stimulus-secretion coupling of glucose-induced insulin release, attention was first paid to the possible role of NBCe1 Na^{+}/HCO_3^{-} cotransporters in such a process.

The NBCe1 Na^+/HCO_3^- cotransporters represent a possible modality for the passage of bicarbonate ions across the cell membrane. Na^+/HCO_3^- cotransporter (NBC) isoform 1 is a member of the SLC4A4 gene family. NBCe1 has two protein variants, which mediate electrogenic Na^+/HCO_3^- cotransport, namely, NBCe1-A (formerly called kNBC1) and NBCe1-B (formerly called pNBC1). They are differentially expressed in a cell- and tissue-specific manner (Parker and Boron 2008). NBCe1-B is the most ubiquitous variant, being expressed, for instance, in the exocrine pancreas, brain, heart, prostate, small and large intestine, stomach, and epididymis. The NBCe1-A variant is more restricted, being most highly expressed in the kidney epithelia and eye. A third NBCe1 variant has also been described in the rat brain and has been named NBCe1-C.

The stoichiometry of the transporter can be altered from $1Na^+/2HCO_3^-$ to $1Na^+/3HCO_3^-$ by phosphorylation of a residue near the carboxyl terminus (Muller-Berger et al. 2001). Functional studies in exocrine pancreatic ducts established that NBCe1-B mediates the influx of one Na^+ with two HCO_3^-. This ion stoichiometry and electrochemical driving forces appear to result, in the pancreas and intestinal tract, in Na^+ and HCO_3^- entry into the cell likely to mediate HCO_3^- uptake across the basolateral membrane to support transepithelial anion secretion (Gawenis et al. 2007). In the kidney, however, the ion stoichiometry and electrochemical driving forces for NBCe1 result in Na^+ and HCO_3^- extrusion across the basolateral membrane, thus participating in HCO_3^- reabsorption in the proximal tubule.

Considering the possible role of changes in Na^+ and HCO_3^- fluxes associated with stimulation of insulin release, e.g., by nutrient secretagogues, attention was recently paid to the expression, variant identity, and function of NBCe1 in rat pancreatic islet cells.

Expression of NBCe1-A and NBCe1-B in Rat Pancreatic Islet Cells

In the first study, Wistar rats were sacrificed under CO_2 anesthesia. The pancreas and kidney were quickly excised and immediately frozen in liquid nitrogen or processed for either microscopy or islet isolation. The methods used for reverse transcription-polymerase chain reaction, Western blot analysis, tissue preparation for immunocytochemistry, immunohistochemistry following the standard ABC-DAB technique, immunofluorescence labeling using universal anti-NBC1 antibody, immunofluorescence labeling using variant-specific anti-NBCe1-A and NBCe1-B antibodies and functional studies including insulin release and D-glucose metabolism in isolated rat islets, intracellular pH measurements and electrophysiological experiments carried out with dispersed rat islet cells, and ^{22}Na net uptake by dispersed cells are all described in detail elsewhere (Soyfoo et al. 2009).

Amplicons corresponding to the expected pair of bases were observed for the NBCe1-A and NBCe1-B and the universal NBCe1 isoforms and β-actin in all specimen tissues, i.e., the kidney, pancreas, and pancreatic islets. Amplification seemed similar in all tissues in the case of both the universal NBCe1 and β-actin.

While pancreatic tissue offered apparently a similar level of amplification for both A and B variants, pancreatic islets yielded a stronger amplification signal for NBCe1-B compared to NBCe1-A.

Immunoblotting with antibodies specific for each variant indicated the expression of both NBCe1-A and NBCe1-B in rat pancreatic islets. The NBCe1-A band in the islets was less intense than that in the kidney despite the fact that the amount of protein used for pancreatic islets was 20 times higher. Using islet and pancreatic homogenates containing equivalent amounts of protein, the staining of the band corresponding to NBCE1-B was more pronounced in islets than in the pancreas. The findings on the expression of NBCe1-A and NBCe1-B in Western blots were superimposable to those obtained by RT-PCR.

In rat pancreatic sections, the antibody recognizing all three variants of the cotransporter stained much more intensely pancreatic islets than the surrounding exocrine tissue. Using the same antibody, NBC1 was localized in an isolated rat pancreatic islet. Both insulin- and glucagon-producing cells appeared to express NBCe1. In order to distinguish whether the labeling observed with this antibody could be attributed to either NBCe1-A or NBCe1-B, variant-specific antibodies were used. NBCe1-A immunolabeling of weak intensity was observed in pancreatic islets, whereas pancreatic acinar cells were completely devoid of NBCe1-A immunoreactivity. Immunoreactivity for NBCe1-B was found in both pancreatic acinar cells and islets, with a labeling intensity considerably stronger in islets than in exocrine pancreas, confirming immunoblotting data. Double labeling using anti-insulin and anti-glucagon antibodies showed partial co-localization of NBCe1-A with insulin, whereas in glucagon-expressing cells NBCe1-A immunoreactivity was absent. The NBCe1-B antibody clearly labeled insulin-producing cells located at the center of the islets, but apparently failed to do so in the glucagon-producing cells located at the periphery of the islets.

Tenidap (3–100 μM) caused a concentration-related inhibition of insulin release evoked over 90-min incubation by D-glucose (16.7 mM) in rat isolated islets, with a half-maximal inhibition close to 50 μM. At a concentration of 100 μM, tenidap failed to affect significantly the basal release of insulin recorded in the presence of 5.6 mM D-glucose, abolished the secretory response at 8.3 mM D-glucose, and severely decreased the insulinotropic action of 16.7 mM D-glucose.

Tenidap (100 μM) also decreased significantly the insulin secretory response to non-glucidic nutrient secretagogues such as 2-ketoisocaproate (10.0 mM) or L-leucine (20.0 mM).

Last, in islets exposed to 8.3 mM D-glucose, tenidap suppressed the enhancing action of non-nutrient secretagogues, including theophylline (1.4 mM), forskolin (5 μM), glibenclamide (5 μM), and cytochalasin B (0.2 mM) upon glucose-stimulated insulin secretion, otherwise obvious in the absence of tenidap.

As judged from the net uptake of $^{22}Na^{+}$, after 10-min incubation, by dispersed islet cells and the apparent distribution space of both L-[1-^{14}C] glucose, used as an extracellular marker, and ^{3}HOH, the net uptake of $^{22}Na^{+}$ corresponded to an estimated intracellular concentration of 35.8 ± 5.7 mM. Whether in the absence or presence of ouabain (1.0–2.0 mM), tenidap (0.1 mM) increased the mean value

for $^{22}Na^+$ net uptake, yielding an overall mean value of 132.5 ± 11.3 % ($n = 66$) as compared ($p < 0.001$) to a mean reference value of 100.0 ± 6.3 % ($n = 64$).

When isolated rat islets were incubated for 90 min in the presence of 16.7 mM D-glucose, tenidap, tested at a 30 μM concentration, inhibited both the utilization of D-[5-^{3}H]glucose and the oxidation of D-[U-^{14}C]glucose. The relative extent of such an inhibition did not differ significantly for the two metabolic variables under consideration, with an overall mean value of 61.0 ± 13.2 % (df = 33).

Tenidap (50–100 μM) provoked a rapid and slowly reversible cellular acidification as judged from the 440 to 480 nm fluorescence ratio in rat islet cells loaded with the pH-sensitive dye BCECF (2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein).

Tenidap was found to provoke within 1–2 min a pronounced hyperpolarization of the β-cell plasma membrane, whether in the presence of 4 or 16 mM D-glucose, this coinciding at the high concentration of the hexose with the suppression of spiking activity.

In the light of the findings so far described, attention should be drawn to the somewhat unexpected increase of $^{22}Na^+$ net uptake caused by tenidap. It could indeed be objected that the prevailing NBCe1-A variant expressed in islet cells is currently considered to work following an influx mode, e.g., in pancreatic duct cells (Parker and Boron 2008). Inter alia, however, the relatively high concentrations of HCO_3^- (alkaline pH) and Na^+ (about 36 mM) in islet cells exposed to 16.7 mM D-glucose could conceivably allow NBCe1-B to cotransport one Na^+ and two HCO_3^- ions from inside the islet cells into the extracellular fluid. This would generate a net inward depolarizing current, consistent with the hyperpolarization accompanying inhibition of the transporter by tenidap. An alternative or complementary modality for the latter hyperpolarization is discussed later in this chapter.

Expression of NBCe1 in Tumoral Insulin-Producing BRIN-BD11 Cells

The methods used for the culture of BRIN-BD11, INS-1, and MIN6 cells, for reverse transcription-polymerase chain reaction, Western blot analysis, immunofluorescence, insulin release, and sodium uptake in the investigations concerning the expression of NBCe1 in tumoral insulin-producing cells are described in detail elsewhere (Bulur et al. 2009).

The BRIN-BD11 cells expressed mainly the NBCe1-B variant, while in INS-1 cells both NBCe1-B and, to a lesser extent, NBCe1-A provided sizeable amplification signals. Such was also the case in the pancreas and kidney.

Western blotting analysis documented the presence, in both BRIN-BD11 cells and the kidney, of a predominant NBCe1 band with a molecular weight close to 130 kDa.

In the 50–100 μM range, tenidap decreased basal insulin output, as measured in the presence of 1.1 mM D-glucose, to 72.2 ± 5.0 % ($n = 5$) of paired control value (26.7 ± 7.7 μU per 30 min; $n = 5$). When the concentration of NaCl was decreased by 50 mM, the release of insulin averaged 215.3 ± 11.6 % ($n = 5$) of paired basal value. In such a hypotonic medium, tenidap lowered insulin secretion down to 85.0 ± 12.2 % ($n = 5$) of paired basal value (recorded in the isoosmotic medium in

the absence of tenidap). The latter percentage was not significantly different from that recorded, also in the presence of tenidap, in the isotonic medium. The relative magnitude of the inhibitory action of tenidap was thus higher in the hypotonic medium than in the isotonic one, resulting in the suppression of the secretory response to extracellular hypoosmolarity.

Incidentally, at higher concentrations (0.5 and 1.0 mM), tenidap provoked a concentration-related augmentation of insulin release in both cells incubated in the isoosmotic and hypotonic medium, this coinciding with the suppression of any significant difference in insulin output from BRIN-BD11 cells exposed to the isotonic or hypotonic medium. A comparable tenidap-induced and concentration-related increase in insulin output was observed in rat pancreatic islets. For instance, in the isotonic medium, tenidap (1.0 mM) increased insulin output from a control value of 42.7 ± 2.0 to 357.2 ± 29.7 μU per 30 min in BRIN-BD11 cells and from 33.5 ± 2.7 to 491.8 ± 24.3 μU/islet per 90 min in isolated rat pancreatic islets. Such dramatic increases in insulin output from either BRIN-BD11 cells or rat isolated pancreatic islets exposed to high concentrations of tenidap might well correspond to an unspecific damaging effect on insulin-producing cells.

At this point, it should be stressed that in a more recent report, tenidap (50–100 μM) was found not only to suppress the regulatory volume decrease otherwise observed in dispersed rat pancreatic islet cells exposed to a hypotonic medium, to hyperpolarize the β-cell membrane potential and suppress glucose-induced electrical activity and to cause a concentration-related inhibition of VRAC currents, whether VRAC activity was provoked by the use of a hypertonic pipette solution in order to induce cell swelling or exposure of the islet cells to 10 mM D-glucose. Indeed, in the same study, tenidap (100 μM) was found to also provoke the activation of K_{ATP} channels and, by doing so, to contribute to the tenidap-induced hyperpolarization (Best et al. 2010a).

Over 5–20 min incubation in the absence of tenidap, the time course for ^{22}Na net uptake by BRIN-BD11 cells was compatible with an apparent Na^+ intracellular concentration of 34.3 ± 10.1 mM. As expected, ouabain (1.0 mM) significantly increased ^{22}Na net uptake. Pooling together results recorded in either the absence or presence of ouabain, tenidap increased ^{22}Na uptake to 143.1 ± 12.6 % ($n = 70$; $p < 0.003$) of the corresponding reference values recorded in the absence of tenidap (100.0 ± 5.9 %; $n = 70$).

Expression of SLC4A4 in Human Pancreatic Islets

In the third and most recent study, the expression of NBCe1 or SLC4A4 was explored in human pancreatic islets. The pancreases were obtained from human cadaveric donors without any primary or secondary quantifiable pathology, from the Transplant Services Foundation of the Hospital Clinic (Barcelona, Spain), after informed consent from their families and approval by the hospital's ethics committee. One part of the tissue was fixed in paraformaldehyde, embedded in paraffin, and sliced for further immunofluorescence studies. From another part of the pancreas,

islets were isolated as previously described (Casas et al. 2007). The last part of the pancreatic gland was utilized as a total pancreatic sample. The techniques used for total RNA isolation, real-time PCR, and immunofluorescent studies are detailed in this recent publication (Hanzu et al. 2012).

After total RNA isolation from isolated islets or total pancreatic sample, qRT-PCR yielded lower gene expression levels of SLC4A4 in isolated islets than in total pancreas, such levels being normalized to the housekeeping gene TBP (TATA box binding protein). The paired pancreas/islet ratio averaged 4.51.

At the protein level, immunostaining of SLC4A4 was as intense in insulin-producing cells as in exocrine pancreatic cells.

Further information concerning the possible participation of NBCe1 in the process of insulin secretion is provided later in this chapter (section "Soluble Adenylyl Cyclase").

The Volume-Regulated Anion Channel Hypothesis

As first proposed in 1997 (Best et al. 1997) and as recently reviewed (Malaisse et al. 2008; Best et al. 2010b), another complementary hypothesis for the stimulus-secretion coupling of glucose-induced insulin release postulates the participation of volume-regulated anion channels in such a process. It is proposed that the entry of D-glucose in insulin-producing cells as mediated by GLUT2, the phosphorylation of the hexose catalyzed mainly by glucokinase and the subsequent acceleration of glucose metabolism lead to the intracellular accumulation of metabolites generated by the catabolism of the hexose, such as lactate and bicarbonate anions. The resulting increase in intracellular osmolarity might then provoke, through increased water uptake, cell swelling and subsequent gating of volume-sensitive anion channels. In the insulin-producing β-cells, the gating of these channels may allow the exit of anions, such as Cl^-, and, hence, provoke a further depolarization of the plasma membrane, with subsequent gating of voltage-sensitive calcium channels. The β-cell volume-regulated anion channel (VRAC) shares several characteristics with that expressed in other tissues and only appears to be distinct from that in other cell types by its halide selectivity (Best et al. 1996).

The postulated activation of VRAC by D-glucose in the pancreatic islet β-cell is supported by a number of observations. This effect was indeed demonstrated at the whole-cell level (Best 1997, 2000) and in single channel recordings (Best 1999, 2002). The increase in β-cell Cl^- permeability provoked by D-glucose will be later discussed in this dissertation. Likewise, the identity of the volume-activated anion channels and the several anionic candidates possibly concerned by such a process are duly considered in the following sections of the present contribution. At this point, it should be stressed that a rise in D-glucose concentration indeed causes a concentration-related increase of β-cell volume and that during sustained exposure to D-glucose, such an increase in β-cell volume persists with often an oscillatory pattern (Miley et al. 1997). Raising the concentration of D-glucose to 20 mM caused

a similar degree of cell swelling in the presence of 2 mM Co^{2+}, a blocker of voltage-sensitive Ca^{2+} channels. This suggests that increase in cell volume in response to glucose is not merely a consequence of an enhanced rate of exocytosis. When 3-*O*-methyl-D-glucose, a non-metabolizable analog which is transported into β-cells in the same manner as D-glucose, was substituted for an equivalent concentration of mannitol, which is relatively impermeant, this only resulted in a modest and transient increase in cell volume probably attributable to the entry of 3-*O*-methyl-D-glucose in the β-cell, as supported by the finding that addition of 3-*O*-methyl-D-glucose with no substitution of mannitol caused no significant increase in β-cell volume. The findings that the glucokinase activator GKA50 causes an increase in cell volume and activation of volume-regulated anion channels in rat pancreatic β-cells (McGlasson et al. 2011) and that the effect of D-glucose to activate the volume-sensitive anion channel is reproduced by 2-ketoisocaproate (Best 1997) supported the view that the increase in β-cell volume evoked by these insulin secretagogues is linked to their capacity to act as nutrient in the β-cell. Last, the abovementioned effect of D-glucose to gate VRAC is suppressed by anion channel inhibitors such as 5-nitro-2(3-phenylpropylamino)benzoic acid.

The Experimental Model of Extracellular Hypotonicity

An acute reduction in the osmolality of the medium bathing isolated pancreatic islets has been recognized as early as in 1975 as a stimulus for insulin release and was found to reproduce the first phase of glucose-induced insulin release (Blackard et al. 1975). In several investigations concerning the volume-regulated anion channel hypothesis, the experimental model of extracellular hypotonicity was often used. In the first extensive study of the stimulus-secretion coupling of hypotonicity-induced insulin release conducted in BRIN-BD11 cells, the following information was gathered (Beauwens et al. 2006).

In the first series of experiments, the secretory response to hypotonicity, as provoked by a decrease in NaCl concentration by 50 mM, was examined in three lines of insulin-producing cells. The findings indicated that the BRIN-BD11 cells, as distinct form either MIN-6 or INS-1 cells, not only display a relatively greater secretory response to hypotonicity but also a positive modulation of such a response by the extracellular concentration of D-glucose. All further experiments were, therefore, conducted only in BRIN-BD11 cells.

The release of insulin recorded in the hypotonic medium averaged 230 ± 17 % ($n = 37$) of the paired basal value measured in the presence of 1.1 mM D-glucose using a salt-balanced iso-osmolar medium. In the latter iso-osmolar medium, a rise in D-glucose concentration from 1.1 to 11.1 mM augmented insulin release by no more than 23 ± 8 % ($n = 15$; $p < 0.01$). The time course for the secretory response to hypoosmolarity was characterized after the peak value recorded over the first 15 min of exposure to the hypotonic medium, by an exponential decrease during the subsequent incubation of 15 min each up to the 90th min of the experiment.

The inhibitor of volume-sensitive anion channels 5-nitro-2-(3-phenylpropylamino)benzoate (NPPB, 0.1 mM) abolished the secretory response to hypotonicity. Such a secretory response represented a Ca^{2+}-dependent process, being inhibited either in the absence of extracellular Ca^{2+} and presence of EGTA (0.5 mM) or in the presence of the organic calcium antagonist verapamil (10.0 μM).

The possible role of ATP-sensitive K^+ channels in the process of hypotonicity-induced insulin release was examined in three series of experiments. First, diazoxide (0.1 mM) decreased to the same relative extent both basal- and hypotonicity-stimulated insulin output, suggesting that the gating of ATP-sensitive K^+ channels by diazoxide played a comparable modulatory role under these two experimental conditions. Second, the hypoglycemic sulfonylurea tolbutamide (10 μM) was found to increase modestly but significantly the release of insulin recorded in the hypotonic medium, possibly by minimizing the fall in insulin secretion otherwise characterizing the secretory response to hypoosmolarity. Last, a rise in extracellular K^+ concentration up to 30 mM while increasing, as expected, insulin release at normal osmolarity decreased the increment in insulin output otherwise attributable to hypoosmolarity. Thus, the rise in K^+ concentration apparently prevented hypoosmolarity to provoke a further depolarization of the plasma membrane. Taken as a whole, these findings suggest that a closing of ATP-sensitive K^+ channels is not involved in hypotonicity-induced insulin release.

The concentration dependency of the response to hypoosmolarity was also examined. For instance, the incorporation of increasing concentrations of sucrose (25–100 mM) to the hypotonic medium provoked in the 25–75 mM range of sucrose concentration a progressive decrease of the hypotonicity-induced increment in insulin output.

Last, two sets of experimental data were consistent with the view that a high intracellular concentration of Cl^- anions, as presumably achieved in β-cells at the intervention of the Na^+-K^+-$2Cl^-$ cotransporter specifically expressed in rat islet β-cell, is required to allow the process of hypotonicity-induced insulin release. First, the Cl^- ionophore tributyltin (1.0 μM), which did not affect significantly basal insulin output, decreased the increment in insulin output attributable to hypoosmolarity. When the concentration of tributyltin was increased to 2.5 μM, the output of insulin was virtually identical at normal osmolarity and in the hypotonic medium. Second, the inhibitor of the Na^+-K^+-$2Cl^-$ cotransporter furosemide (0.1 mM) again did not affect basal insulin output, but severely decreased the output of insulin recorded under hypoosmolar conditions.

In the same study, it was documented that exposure to the hypotonic medium indeed increased the volume of BRIN-BD11 cells, followed by a regulatory volume decrease, itself suppressed in the presence of NPPB. This inhibitor of VRAC also decreased in a rapid and reversible manner both the inward and outward currents provoked by ±100 mV voltage pulses in conventional whole-cell recording with hypertonic intracellular medium to induce BRIN-BD11 cell swelling. NPPB also opposed the effect of hypotonicity to provoke depolarization and induction of spiking activity in the BRIN-BD11 cells. Last, exposure of the BRIN-BD11 cells to a hypotonic medium provoked a rapid increase in the cytosolic Ca^{2+} concentration (Beauwens et al. 2006).

The Possible Role of NAD(P)H Oxidase-Derived H_2O_2 in the Activation of VRAC in β-Cells Exposed to a Hypotonic Medium

It was recently proposed that, in several cell lines, the activation of VRAC under hypotonic extracellular conditions and the ensuing volume regulatory decrease results from NAD(P)H oxidase (NOX)-derived H_2O_2. In a recent study, it was investigated whether a comparable situation prevails in insulin-producing cells, i.e., whether an increase in intracellular H_2O_2 is instrumental in the opening of VRAC in the process of hypotonicity-induced insulin release (Crutzen et al. 2012).

The following findings supported the latter view.

First, exogenous H_2O_2 stimulates insulin release from BRIN-BD11 cells, with a threshold value close to 40 μM and a maximal stimulation at about 100 μM. The secretory response to exogenous H_2O_2, like that evoked by extracellular hypotonicity, was suppressed by 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB; 100 μM).

Second, NAD(P)H oxidase inhibitors, such as diphenylene iodonium chloride (DPI, 10 μM) or plumbagin (30 μM), suppressed in the BRIN-BD11 cells the secretory response to hypotonicity. Such was also the case after preincubation of the BRIN-BD11 cells either with *N*-acetyl-L-cysteine for 24 h or with betulinic acid for 48 h, the latter agent causing a time-related decrease of NOX4 gene expression (as assessed by RT-PCR) in the BRIN-BD11 cells.

Third, exposure of the BRIN-BD11 cells to either exogenous H_2O_2 or extracellular hypotonicity increases their intracellular content in reactive oxygen species. In this respect, the response of the BRIN-BD11 cells to hypotonicity was rapid and sustained, it being abolished by DPI.

Fourth, exogenous H_2O_2 provoked membrane depolarization and electrical activity in the BRIN-BD11 cells, such an effect being opposed by NPPB. Likewise, exogenous H_2O_2 induced the activation of single chloride channels, an effect again opposed by NPPB.

Fifth, hypotonicity provoked cell swelling followed by a regulatory volume decrease in the BRIN-BD11 cells, the latter RVD being suppressed by NPPB, by the NAD(P)H oxidase inhibitors (DPI, plumbagin) and after preincubation of the BRIN-BD11 cells with either *N*-acetyl-L-cysteine or betulinic acid.

Sixth, in dispersed rat islet cells, as distinct from BRIN-BD11 cells, exogenous H_2O_2 again provoked a concentration-related depolarization of the plasma membrane, such an effect being suppressed by NPPB.

Last, in both dispersed rat islet cells and freshly isolated rat pancreatic islets, H_2O_2 (100–200 μM) again stimulated insulin release over 20-min incubation, an effect itself again opposed by NPPB.

Candidate Anions

The volume-regulation anion channel hypothesis here under consideration raises the question as to the identity of the concerned anions under physiological conditions.

Sehlin was the first to report that a rise in D-glucose concentration caused a concentration-related decrease of the $^{36}Cl^-$ content of prelabeled islets prepared from *ob*/*ob* mice, a current model of inherited obesity (Sehlin 1978). From these findings, it was inferred that the hexose stimulates the Cl^- efflux from islet cells and that such an increase in Cl^- permeability may partly mediate the glucose-induced depolarization of insulin-producing cells.

The possible extension of these findings to islets prepared from normal rats was more recently investigated by measuring the changes evoked by increasing concentrations of D-glucose in $^{36}Cl^-$ outflow from prelabeled islets (Malaisse et al. 2004). For such a purpose, after 60-min preincubation at 37 °C in the presence of 3.0 mM D-glucose and $^{36}Cl^-$, the rat islets were incubated for 8–10 min at 37 °C in the presence of increasing concentrations of D-glucose (3–20 mM). After preincubation the $^{36}Cl^-$ content of the islets corresponded to an estimated intracellular Cl^- concentration of 126 ± 13 mM, as compared to 128 mM in islets from *ob*/*ob* mice. The rise in D-glucose concentration during the final incubation period caused a concentration-related increase of $^{36}Cl^-$ efflux from the prelabeled rat islets, with a threshold value close to 5.0 mM D-glucose and a half-maximal response at a D-glucose concentration close to 10.0 mM. These two features are similar to those characterizing the effect of D-glucose upon insulin release from rat islets. The D-glucose concentration-response relationship found in this study was also virtually identical to that obtained by Best when measuring the effect of increasing concentrations of D-glucose upon the channel open probability of a 200 pS anion-selective channel in recordings of cell-attached rat pancreatic β-cells (Best 2000).

Thus, the salient finding in these three series of investigations (Best 2000; Sehlin 1978; Malaisse et al. 2004) consisted in the fact that the concentration-related effect of D-glucose to cause the gating of voltage-sensitive anion channels closely parallels that of the hexose as an insulinotropic agent. This is in sharp contrast to the concentration-related response for the effect of D-glucose to provoke the closing of ATP-sensitive K^+ channels (Carpinelli and Malaisse 1981). Thus, in the latter case, a maximal response is already recorded at a concentration of D-glucose close to 5.0 mM.

Already in 1974, Freinkel et al. reported that a rise in extracellular D-glucose concentration causes a transient increase in inorganic phosphate release from isolated pancreatic islets (Freinkel et al. 1974). Virtually all the radioactive material released from islets prelabeled with ^{32}P-orthophosphate also consists of ^{32}P-orthophosphate. Such a phosphate flush is provoked by nutrient secretagogues, such as D-glucose, D-mannose, D-glyceraldehyde, L-leucine, its non-metabolized analog b(-)2-amino-bicyclo[2,2,1]heptane-2-carboxylic acid (BCH), and 2-ketoisocaproate (Freinkel et al. 1974, 1976; Freinkel 1979; Carpinelli and Malaisse 1980). It coincides with a sizeable decrease in the inorganic phosphate content of the islets (Bukowiecki et al. 1979).

It is only in 2007 that the glucose-induced phosphate flush in pancreatic islets was proposed to be attributable to the gating of volume-sensitive anion channels (Louchami et al. 2007). Thus, it was documented that an increase in D-glucose

concentration from 1.1 to 8.3 mM induces a typical phosphate flush and biphasic stimulation of insulin release. Extracellular hypoosmolarity, as provoked by reducing the NaCl concentration by 50 mM, caused a monophasic increase in both ^{32}P fractional outflow from the islets prelabeled with ^{32}P-orthophosphate and insulin output. The inhibitor of volume-sensitive anion channels 5-nitro-2(3-phenylpropylamino)benzoate, used at a 0.1 mM concentration, inhibited both stimulation of insulin release and phosphate flush induced by either the increase in D-glucose concentration or extracellular hypoosmolarity. It should be underlined that, in these as in previous experiments, the secretory response to D-glucose was biphasic, while that to extracellular hypoosmolarity was monophasic with a rapid exponential return of the secretory rate toward basal value.

The proposed role attributed to the gating of volume-regulated anion channels as a key determinant of the phosphate flush is also compatible with the concentration-response relationship for the stimulation by D-glucose of effluent radioactivity from prelabeled and perifused rat pancreatic islets (Carpinelli and Malaisse 1980). Thus, the threshold concentration of D-glucose for induction of a phosphate flush is close to 4.0 mM with a close-to-maximal response at 16.7 mM.

The findings just mentioned suggest that, in the process of glucose-induced insulin release, another anion or other anions than inorganic phosphate may participate in the second phase of the insulin secretory response, accounting for the oscillation in cell volume recorded during prolonged exposure of islet cells to D-glucose (Miley et al. 1997). For instance, it was proposed that, during the second and sustained phase of insulin secretion evoked by D-glucose or other nutrient secretagogues, the gating of volume-regulated anion channels could provide a route of bicarbonate efflux in insulin-producing cells (Louchami et al. 2007). This proposal takes into account the finding that, in glucose-stimulated islets, the generation of bicarbonate catalyzed by mitochondrial carbonic anhydrase accounts for the majority of CO_2 produced through the oxidative catabolism of the hexose (Sener et al. 2007).

Incidentally, in the study on the possible role of carbonic anhydrase in rat pancreatic islets, acetazolamide, which was used to inhibit the latter enzyme and indeed decreased, when used in the 3.0–10.0 mM range, the production of $H^{14}CO_3^-$ by islets exposed to 16.7 mM D-[U-^{14}C]glucose, was found to slightly decrease intracellular pH and to lower the cytosolic concentration of Ca^{2+}. Whether these ionic effects of acetozolamide could be attributed, in part at least, to an altered cotransport of HCO_3^- and Na^+ by NBC1 remains to be assessed. Nevertheless, it should not be ignored that, in the proximal colon, the NBC1 activity can be increased during carbonic anhydrase inhibition by acetazolamide to maintain maximal levels of HCO_3^- secretion (Gawenis et al. 2007).

In a manner comparable to that just considered in the case of bicarbonate anions, the exit of lactic acid generated by the catabolism of D-glucose may occur at the intervention of volume-regulated anion channels during sustained exposure of the islets to D-glucose. The output of lactic acid from rat islets exposed for 90 min to D-glucose progressively increases from a basal value measured in the absence of the hexose averaging 23 $\pm$ 1 pmol/islet per 90 min ($n = 11$) to a value as high as

218 ± 7 pmol/islet per 90 min ($n = 6$) in the presence of 27.8 mM D-glucose (Sener and Malaisse 1976). At 16.7 mM D-glucose, the intracellular lactate content of the islets reaches a steady-state value not exceeding about 20 pmol/islet (Sener and Malaisse 1976), while the amount of lactate accumulated in the extracellular medium amounts to 181 ± 6 pmol/islet per 90 min (n = 102). The activity and expression of the lactate (monocarboxylate) transporter MCT are low or absent in β-cells (Best et al. 1992; Zhao et al. 2001), potentially leading to intracellular lactate accumulation during glucose stimulation. Thus, such an accumulation may account for both the glucose-induced β-cell swelling and the efflux of lactate via the VRAC, in which β-cells indeed show significant permeability to lactate (Best et al. 2001). This proposal is supported by the finding that the accumulation of D-lactate formed from methylglyoxal leads to β-cells swelling and VRAC activation (Best et al. 1999).

Expression and Function of Anoctamin

The TMEM16 transmembrane protein family consists of 10 different proteins with numerous splice variants that contain 8–9 transmembrane domains. TMEM16A (also called anoctamin1 or ANO1) has been identified as a subunit of activated Cl^- channels that are expressed in epithelial and non-epithelial tissues. All vertebrate cells regulate their volume by activating chloride channels. TMEM16A together with other TMEM16 proteins are activated by cell swelling, leading to a regulatory volume decrease (RVD). As a rule, it is considered that intracellular Ca^{2+} plays a role as a mediator for activation of volume-regulated chloride currents.

Activation of volume-regulated chloride channels is reduced in the colonic epithelium and in salivary acinar cells from mice lacking expression of TMEM16A. Hence, TMEM16 proteins appear to be a crucial component of epithelial volume-regulated Cl^- channels (Almaca et al. 2009). Studies on expression and function of the TMEM16A calcium-activated chloride channel conducted by Huang et al. have contributed to their subcellular location and function in a number of organs, including the epithelial cells, exocrine glands, and trachea, as well as airway and reproductive tract smooth muscle cells (Huang et al. 2009). Anoctamin 6 (or TMEM16F) was proposed as an essential component of the outwardly rectifying chloride channel in airway epithelial cells (Martins et al. 2011).

The expression of all ten members (ANO1–ANO10) in a broad range of murine tissues was also analyzed, each tissue expressing a set of anoctamin that forms cell- and tissue-specific Ca^{2+}-dependent Cl^- channels (Schreiber et al. 2010). In the perspective of the present chapter, two findings merit to be underlined. First, ANO1 produces large and rapidly activating Ca^{2+}-dependent Cl^- current, requiring 10 μM of cytosolic Ca^{2+} for full activation, while being inhibited at higher Ca^{2+} concentrations. Second, among some 26 organs examined for such a purpose, the pancreatic gland was found to express large amounts of ANO1 (Schreiber et al. 2010).

Two recent studies were devoted to the possible role of TMEM16A, also called anoctamin 1, as a volume-regulated anion channel in insulin-producing cells.

Expression and Role of Anoctamins in Rodent Insulin-Producing Cells

The methods used in the first of these two studies for reverse transcription-polymerase chain reaction, immunohistochemistry, measurement of BRIN-BD11 cell volume, insulin release and D-glucose metabolism in rat isolated pancreatic islets, and the monitoring of mouse β-cell membrane potential are detailed elsewhere (Malaisse et al. 2012, 2013).

Anoctamin Expression

Screening of anoctamin mRNA expression by RT-PCR documented its presence in both rat and human pancreas, isolated rat pancreatic islets, and kidney. In rat islets, anoctamin 1 and anoctamin 6 were predominant, with a lower level of anoctamin 10, while in BRIN-BD11 cells, anoctamin 6 predominated with much lower levels of either anoctamin 1 or anoctamin 10. The expression of anoctamin 1 was documented by immunohistochemistry, in mouse and rat and pancreas, with a more intense staining of pancreatic islets, as compared to exocrine pancreas.

BRIN-BD11 Cell Volume

Tannic acid (100 μM) suppressed the regulatory volume decrease otherwise occurring in BRIN-BD11 cells exposed to a hypotonic extracellular medium in the presence of 5.0 mM D-glucose.

Insulin Release

Tannic acid (100 μM) abolished the secretory response to extracellular hypoosmolarity in rat pancreatic islets incubated for 30 min in the presence of 2.8 mM D-glucose. As judged from the effects of increasing concentrations of tannic acid upon insulin output evoked by 16.7 mM D-glucose in rat islets incubated for 90 min and taking into account the basal value for insulin release, as measured in the presence of 2.8 mM D-glucose, the regression line concerning the release of insulin at increasing concentrations of tannic acid (logarithmic scale) suggested a threshold concentration for the inhibitory action of tannic acid close to 3.1 μM and an ED_{50} close to 65.6 μM. Two further series of experiments provided the following results. It was first observed that tannic acid (100 μM) indeed inhibits the secretory response to 16.7 mM, while failing to affect significantly insulin output at 8.3 mM D-glucose. The latter finding was confirmed, no significant difference in insulin output being observed when the islets were incubated in the presence of 8.3 mM D-glucose and increasing concentrations of tannic acid. Pooling together all available data, the output of insulin recorded in the presence of 8.3 mM D-glucose and 100 μM tannic acid averaged 92.7 ± 5.7 % ($n = 35$; $p > 0.35$) of the mean corresponding control values recorded within the same experiment in the absence of tannic acid (100.0 ± 5.4 %; $n = 36$). In the presence of 16.7 mM D-glucose and 100 μM tannic acid, however, the output of insulin represented no more than 57.2 ± 3.5 % ($n = 44$; $p < 0.001$) of the mean corresponding control values recorded in the absence of tannic acid. The output

of insulin remained significantly higher ($p < 0.001$) in the presence of 16.7 mM D-glucose and 100 μM tannic acid than in the sole presence of 8.3 mM D-glucose.

D-glucose Metabolism

The results of the experiments aiming at assessing the effects of tannic acid upon D-glucose metabolism in rat islets provided the following information. Relative to the mean value for D-[U-^{14}C]glucose oxidation recorded within each of four experiments in islets exposed to 16.7 mM in the absence of tannic acid (100.0 ± 8.7 %; $n = 27$), the measurements made at 2.8 mM D-glucose also in the absence of tannic acid averaged 14.6 ± 1.3 % ($n = 26$; $p < 0.001$), while those found in the concomitant presence of 16.7 mM D-glucose and 100 μM tannic acid amounted to 140.3 ± 11.8 % ($n = 28$; $p < 0.009$). Inversely, the generation of ^{3}HOH from D-[5-^{3}H]glucose by islets exposed to 16.7 mM D-glucose in the presence of tannic acid represented no more than 55.3 ± 7.0 % ($n = 21$; $p < 0.003$) of the mean corresponding values recorded within the same experiments at the same hexose concentration but in the absence of tannic acid. As a result of these opposite metabolic effects of tannic acid, the mean absolute value for the paired ratio between D-[U-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization, which, in the absence of tannic acid, was much lower at 2.8 mM D-glucose than at 16.7 mM D-glucose, was, at the high concentration of the hexose, significantly higher in the presence of tannic acid than in its absence.

As judged from these findings and assuming that the difference between D-[5-^{3}H]glucose conversion to ^{3}HOH and that of D-[U-^{14}C]glucose to $^{14}CO_2$ corresponds to the generation of lactic acid from D-glucose, the ATP generation rate attributable to the catabolism of the hexose, which did not exceed 171.8 ± 18.4 pmol/islet per 90 min at 2.8 mM D-glucose, amounted to 995.4 ± 98.4 and 998.4 ± 77.1 pmol/islet per 90 min at 16.7 mM D-glucose, respectively, in the absence and presence of tannic acid. The latter two values being virtually identical, they indicate that, in the presence of tannic acid, the increased oxidation of D-glucose compensated, in terms of energy yield, for the decreased rate of glycolysis.

Bioelectrical Activity

Tannic acid (100 μM) was found to impair the bioelectrical activity induced by D-glucose (16.7 mM) in mouse β-cells. From a detailed analysis of the changes induced by tannic acid in the bioelectrical response to 16.7 mM D-glucose, it was calculated that, over the same period of time, the influx of Ca^{2+} ions only represented in the presence of tannic acid about 41.4 % of that taking place in its absence.

Concluding Remarks

A salient finding in this study consists in the fact that at a D-glucose concentration of 8.3 mM as distinct from 16.7 mM, tannic acid failed over 90-min incubation to cause any sizeable decrease in insulin output. Hence, it would appear that inhibition by tannic acid of anoctamin 1 preferentially impairs the increase in insulin output provoked by a rise in D-glucose concentration from 8.3 to 16.7 mM, i.e., in the range of hexose

concentrations in which the gating of volume-regulated anion channels may play its major role in the stimulus-secretion coupling of glucose-induced insulin secretion.

Expression of TMEM16A in Human Pancreatic Islets

In the same report at that mentioned in section "Expression of SLC4A4 in Human Pancreatic Islets" of the present chapter, the expression of TMEM16A was also assessed in human pancreatic islets (Hanzu et al. 2012).

In mirror image to that found for SLC4A4, qRT-PCR yielded higher gene expression of TMEM16A in isolated islets than in the total pancreas with a mean paired pancreas/islet ratio of 0.50.

At the protein level, immunohistochemistry for TMEM16A documented its presence in both insulin-producing cells and exocrine cells. The immunostaining of TMEM16A appeared somewhat less pronounced in insulin-producing cells than in the exocrine cells.

Soluble Adenylyl Cyclase

Insulin-producing β-cells have long been known to be equipped with a family of G protein-responsive transmembrane adenylyl cyclases. Incretins released by the intestine in response to food intake, such as glucagon-like peptide 1 (GLP-1), increase adenosine 3′-5′-cyclic monophosphate (cAMP) in β-cells, at the intervention of specific G protein-coupled receptors, e.g., GLP-1 receptor, by activating transmembrane adenylyl cyclase.

A rise in extracellular D-glucose concentration also provokes a rapid and sustained increase in the cAMP content of rat pancreatic islets. Other nutrient secretagogues, such as L-leucine, also increase cAMP generation. In both cases, the latter increase is suppressed when the islets are incubated in the absence of extracellular Ca^{2+} (Valverde et al. 1983). As a matter of fact, the accumulation of cAMP evoked by D-glucose in islet cells was proposed to be attributable to a calcium-dependent stimulation of adenylate cyclase by endogenous calmodulin indeed present in pancreatic islets (Valverde et al. 1979).

More recently, a soluble adenylyl cyclase was identified in insulin-producing INS-1E cells by RT-PCR, Western blot, and immunocytochemistry. The activity of this soluble adenylyl cyclase can be modulated by Ca^{2+}, bicarbonate, and ATP. It was proposed that this soluble adenylyl cyclase is the predominant source of glucose-induced cAMP, at least in INS-1E cells (Ramos et al. 2008).

This information led to further experimental work conducted within the framework of the issues discussed in the present chapter. Thus recent investigations aimed at exploring the interaction between adenosine 3′5′-cyclic monophosphate (cAMP), volume-regulated anion channels (VRAC), and the Na^{+}-HCO_3^{-}-cotransporter NBCe1 in the regulation of nutrient- and hypotonicity-induced insulin

release from both rat pancreatic islets and tumoral insulin-producing BRIN-BD11 cells (Bulur et al. 2013). The major findings collected in this last series of investigations may be summarized as follows.

Experiments in Rat Pancreatic Islets

Tenidap (50 μM) and the inhibitor of VRAC 5-nitro-2-(3-phenylpropylamino)benzoate (NPPB, 0.1 mM) inhibited the secretory response evoked by D-glucose (8.3 mM) in rat pancreatic islets. Either 8-bromoadenosine-3′-5′-cyclic monophosphate (8-Br-cAMP, 1.0 mM) or dibutyryladenosine-3′-5′-cyclic monophosphate (db-cAMP, 1.0 mM) increased glucose-stimulated insulin release. The relative magnitude of such an increase was not significantly different with each of these two cAMP analogs with an overall mean enhancing action of 41.1 ± 11.4 %, when the measurements of insulin output were corrected for basal value. In the presence of tenidap, the two cAMP analogs augmented the mean value for insulin release. The overall mean relative magnitude of such an increase was virtually identical to that recorded in the absence of any potential inhibitor of insulin release. In the islets exposed to NPPB, however, the enhancing action of the cAMP analogs failed to achieve statistical significance, suggesting that, under the present experimental conditions, NPPB suppressed an essential component of the secretory response to D-glucose. It is indeed well established that agents increasing the cAMP content of non-tumoral insulin-producing cells fail to augment insulin output from islets incubated at low D-glucose concentrations (Malaisse et al. 1967). In contrast, the maintenance of a significant positive response to the cAMP analogs in the presence of tenidap suggests that the participation of NBCe1 in ionic fluxes does not represent an essential permissive process for the expression of D-glucose insulinotropic action.

Experiments in BRIN-BD11 Cells

Reference Data

The basal insulin release from BRIN-BD11 cells incubated in the isotonic medium containing 1.1 mM D-glucose averaged 61.5 ± 4.1 μU/ml per 30 min ($n = 39$). It was increased by 70.0 ± 5.8 μU/ml per 30 min (paired comparison; $n = 33$) in a hypotonic medium and by 30.5 ± 2.8 μU/ml per 30 min (paired comparison; $n = 8$) in the presence of 2-ketoisocaproate (KIC; 10 mM).

Effects of cAMP Analogs and Phosphodiesterase Inhibitors

When BRIN-BD11 cells were incubated in an isotonic medium, the association of adenosine-3′,5′-cyclic monophosphate acetoxymethyl ester (cAMP-AM; 0.1–0.2 mM) and 3-isobutyl-1-methylxanthine (IBMX; 0.5 mM) and even the sole presence of IBMX approximately doubled insulin output. The association of cAMP and IBXM also augmented insulin output when the BRIN-BD11 cells were incubated in a hypotonic medium. In this case, however, the relative magnitude for the

increase in insulin output evoked by the association of cAMP and IBMX was much lower than in the isotonic medium, this difference coinciding with the fact that the control values found in the absence of cAMP and IBXM averaged, in the hypotonic medium, close to 250 % of that recorded in the isotonic medium.

Effects of NPPB

The inhibitor of VRAC, NPPB (0.1 mM), abolished the secretory response to KIC, the cAMP analogs failing to fully restore the insulinotropic action of KIC.

When BRIN-BD11 cells are incubated in an isotonic medium, NPPB (0.1 mM) slightly enhances basal insulin output to 115.9 ± 7.6 % ($n = 8$; $p < 0.005$) of paired control values (Beauwens et al. 2006). However, at the same concentration NPPB abolished the secretory response to hypotonicity. Once again, the cAMP analogs failed to restore the secretory response evoked by the exposure of the BRIN-BD11 cells to the hypotonic medium. Nevertheless, the well-known enhancing action of the phosphodiesterase inhibitor IBMX upon insulin secretion remained operative in the cells exposed to both NPPB and a cAMP analog.

Effects of Tenidap

In the 50–100 μM range, tenidap decreases insulin output from BRIN-BD11 cells incubated in an isotonic medium containing 1.1 mM D-glucose (Bulur et al. 2009). Even in the concomitant presence of IBMX (0.5 mM) and cAMP-AM (0.1 mM), tenidap (50 μM) decreased significantly insulin output from BRIN-BD11 cells incubated in the isotonic medium below the mean control value recorded in the absence of tenidap.

Tenidap (50 μM) also inhibited KIC-stimulated insulin release. The cAMP analogs 8-Br-cAMP (1.0 mM) or db-cAMP (0.1 mM) failed to augment significantly insulin release recorded in the presence of both KIC and tenidap. Only dioctanoyl adenosine-3′,5′-cyclic monophosphate (dioctanoyl-cAMP; 1.0 mM) and 2′-O-monosuccinyladenosine 3′,5′-tyrosyl methyl ester (0.1 mM) augmented significantly insulin release evoked by KIC in the presence of tenidap.

When BRIN-BD11 cells were incubated in a hypotonic medium in the presence of tenidap, the release of insulin was significantly lower ($p < 0.01$) than the paired value recorded in an isotonic medium in the absence of tenidap. Among various cAMP analogs examined for such a purpose, and whether in the concomitant presence of IBMX (0.5 mM) or not, only dioctanoyl-cAMP (1.0 mM) increased, when tested in the absence of IBMX, insulin output to a sizeable extent from BRIN-BD11 cells exposed to the hypotonic medium in the presence of tenidap.

Effects of MAP-Kinase Inhibitors

The MAP-kinase inhibitors U0126 (1,4-diamino-2,3-dicyano-1,4bis(O-aminophenylmercapto)butadiene ethanolate; 10 μM) and PD98,059 (2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one; 50 μM) decreased modestly, and to the

same relative extent, the release of insulin from BRIN-BD11 cells incubated in the isotonic medium, whether in the presence or absence of KIC (10 mM) or in the hypotonic medium. These results are compatible with the participation of cAMP-responsive MAP-kinase in the secretory activity of BRIN-BD11 cells.

Effects of 2-Hydroxyestriol

The inhibitor of soluble adenylate cyclase 2-hydroxyesteriol, when tested at a 50 μM concentration, failed to affect significantly insulin release, whether from BRIN-BD11 cells incubated in an isotonic medium or exposed to the hypotonic medium. Even at a 100 μM concentration, 2-hydroxyesteriol only decreased insulin output to 84.2 ± 3.1 % ($n = 8$; $p < 0.002$) of the paired control value found under the same experimental conditions (isotonic or hypotonic medium) in the absence of 2-hydroxyestriol. Within the same experiments the release of insulin from BRIN-BD11 cells exposed to the hypotonic medium was decreased to a comparable extent by either 2-hydroxyestriol (100 μM) or the membrane permeant, metabolically stable inhibitor of cAMP-dependent protein kinase 8-bromoadenosine-3′,5′-cyclic monophosphorothioate (Rp-8-Br-cAMPS; also 100 μM) with an overall mean value of 82.8 ± 5.6 % ($n = 8$; $p < 0.025$) of the paired measurement made, also in the hypotonic medium, in the absence of the latter two agents. These findings could suggest a limited participation of soluble adenylate cyclase in the secretory activity of BRIN-BD11 cells. It should be underlined, however, that in this case, like in the experiments conducted with the MAP-kinase inhibitors, basal and stimulated insulin output from the BRIN-BD11 cells were affected to a comparable relative extent by the tested inhibitors.

Effects of HCO_3^- and/or Cl^- Omission

The omission of $NaHCO_3$ severely decreased the secretory response to either KIC (10 mM) or extracellular hypotonicity. In the absence of $NaHCO_3$, a modest further decrease in insulin output was noticed when tenidap (50 μM) was present in the incubation medium. Under the latter experimental conditions, neither 8-Br-cAMP (1.0 mM) nor db-cAMP (also 1.0 mM) affected significantly insulin output. Dioctanoyl-cAMP (1.0 mM), however, still dramatically increased insulin release from the BRIN-BD11 cells exposed, in the absence of $NaHCO_3$ and presence of tenidap, to either KIC or a hypotonic medium. The inhibition of insulin release from BRIN-BD11 cells attributable to the absence of $NaHCO_3$ is reminiscent of comparable results recorded in rat pancreatic islets (Malaisse et al. 1979b; Sener and Malaisse 2012).

In the absence of Cl^- or both Cl^- and HCO_3^-, the paired ratio between insulin output in the hypotonic/isotonic medium was also abnormally low.

Effects of Na^+ Omission

The release of insulin from BRIN-BD11 cells incubated in an isotonic medium deprived of Na^+, as achieved by the substitution of NaCl (115 mM) by an equimolar mixture of 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS), *N*-methyl-D-glucosamine, and sucrose and that of $NaHCO_3$ (24 mM) by an equimolar amount of

choline bicarbonate, was two to three times higher than that found, within the same experiment, in the usual isotonic medium. The hypotonic/isotonic ratio for insulin output, which was as expected above 200 % under the usual experimental conditions, did not exceed 103.6 ± 3.3 % in the Na^+-free hypotonic medium. When the BRIN-BD11 cells were exposed to the Na^+-free hypotonic medium, a sizeable increase in insulin output was provoked by either 8-Br-cAMP (1.0 mM) or both IBMX (0.5 mM) and cAMP-AM (0.1 mM). These findings may suggest a favorable effect of Na^+ omission, on basal insulin output at least. This could, conceivably, involve a lesser consumption of ATP by the Na^+, K^+-ATPase.

Facts and Hypotheses

In a physiologically relevant perspective and, hence, in the experiments conducted in rat pancreatic islets, a salient finding was that, under experimental conditions in which tenidap and NPPB inhibited to a comparable extent the insulinotropic action of 8.3 mM D-glucose, the enhancing action of cAMP analogs was suppressed in the islets exposed to NPPB, but not so in the islets exposed to tenidap. This finding is compatible with the view that NPPB, by opposing the gating of VRAC, suppressed an essential component of the secretory response to D-glucose. In contrast, the maintenance of a significant positive response to cAMP analogs in the presence of tenidap suggests that the participation of NBCe1 in ionic fluxes does not represent an essential permissive process for the expression of D-glucose insulinotropic action. A different situation prevailed in tumoral insulin-producing cells which, however, otherwise display a relatively poor secretory response to D-glucose and apparently express a lower level of TMEM16A mRNA than that found in rat pancreatic islets. The experiments conducted in these tumoral cells also failed to ascribe to activation or inactivation of soluble adenylyl cyclase a key role in their response to either a nutrient secretagogue or extracellular hypoosmolarity.

Possible Roles of Aquaporins

Several insulinotropic agents were recently reported to cause β-cell swelling. The possible participation of aquaporins to water transport in pancreatic islet cells was investigated, therefore, in several recent reports. Aquaporins are channel-forming membrane proteins which allow water movement through the plasma membrane (Agre 2004). Aquaglyceroproteins represent a subfamily of aquaporins permeable not only to water but also to small solutes like glycerol and urea (Agre 2004; Rojek et al. 2008). Aquaglyceroporin 7 (AQP7) is expressed in rat and mouse pancreatic islet β-cells and tumoral insulin-producing BRIN-BD11 cells (Best et al. 2009; Delporte et al. 2009; Matsumura et al. 2007). Five recent publications deal with the possible role of AQP7 and other aquaporins in β-cell function.

Matsumura et al. (2007) first found expression of AQP7, but not that of AQP3 or AQP9, in mouse pancreatic islets at both the mRNA and protein levels.

Immunohistochemistry revealed a complete overlap between insulin and AQP7 immunostaining in the pancreatic islet. Intraislet glycerol and triglyceride content was increased in AQP7$^{-/-}$ mice. Despite reduced pancreatic β-cell mass and islet insulin content, islets isolated from AQP7$^{-/-}$ mice secreted insulin at a higher rate both under basal low-glucose conditions and on exposure to a high concentration of D-glucose (25.0 mM). Incidentally and quite surprisingly, assuming an islet protein content close to 1.0 μg/islet, the secretion of insulin by islets from AQP7$^{+/+}$ mice recorded in the presence of 25.0 mM D-glucose was about two orders of magnitude lower (ca. 23.8 ± 1.5 pg/μg protein per hour; $n = 3$) in the study by Matsumura et al. (2007) than that found by Li et al. (2009) in islets from wild-type mice incubated in the presence of 20.0 mM D-glucose (about 2.0 ± 0.1 ng/μg protein per hour; $n = 9$–23) or by Bulur et al. (2010) in islets from NRMI mice incubated at 16.7 mM D-glucose (3.8 ± 0.5 ng/μg protein per hour). An even more pronounced difference (about 400-fold) prevails when comparing the insulin content from wild-type mice in the report by Matsumura et al. (113.4 ± 7.2 pg/μg protein; $n = 8$) and either Li et al. (47 ± 3 ng/μg protein; $n = 6$–8) or Bulur et al. (46 ± 1 ng/μg protein; $n = 88$).

Louchami et al. then documented by RT-PCR the expression, in addition to AQP7, of AQP5 and AQP8 mRNA in mice pancreatic islets, as well as the presence of AQP5 and AQP8 in insulin-producing β-cells by immunostaining (Louchami et al. 2012). In the same study, the secretion of insulin evoked by the omission of 50 mM NaCl, the substitution of 50 mM NaCl by 100 mM glycerol, or a rise in D-glucose concentration from 2.8 to 8.3 and 16.7 mM was severely impaired in the islets from AQP7$^{-/-}$ mice. Yet, exposure of β-cells to either the hypotonic medium or a rise in D-glucose concentration caused a similar degree of cell swelling and comparable pattern of electrical activity in cells from AQP7$^{+/+}$ and AQP7$^{-/-}$ mice. Both the cell swelling and change in membrane potential were only impaired in AQP7$^{-/-}$ cells when exposed to 50 mM glycerol. These findings are consistent with the previous suggestion that AQP7 mediates both the influx (Delporte et al. 2009) and efflux (Matsumura et al. 2007) of glycerol from insulin-producing cells. Second, they apparently imply the existence of at least one water transport pathway in mouse β-cells other than AQP7. Last, the impaired insulin secretory activity found in the islets from AQP7$^{-/-}$ mice, despite normal volume and electrical responses, to insulinotropic stimuli other than glycerol suggests that the glyceroaquaporin AQP7 could play a role at a distal site of the exocytotic pathway. For example, it might imply the perturbed participation of some cytosolic protein otherwise tightly coupled in functional terms to AQP7. Alternatively, the impaired secretory activity of AQP7$^{-/-}$ β-cells could be related to a secondary consequence of AQP7 absence, such as the accumulation of triglyceride previously reported in these cells (Matsumura et al. 2007).

In another study, the functional role of AQP7 expression in the tumoral pancreatic β-cell line BRIN-BD11 was investigated (Delporte et al. 2009). The BRIN-BD11 cell line is an insulin-secreting cell line established by electrofusion of normal rat pancreatic β-cell from New England Deaconess Hospital with immortalized RINm5F cells (McClenaghan et al. 1996). AQP7 mRNA and protein were

detected by RT-PCR and Western blot analysis, respectively, in these BRIN-BD11 cells. In an isoosmolar medium, the net uptake of [2-^{3}H]glycerol displayed an exponential time course reaching an equilibrium plateau value close to its extracellular concentration. Within 2 min of incubation in a hypotonic medium (caused by a 50 mM decrease in NaCl concentration), the [2-^{3}H]glycerol uptake averaged 143.2 ± 3.8 % ($n = 24$; $p < 0.001$) of its control value in isotonic medium, declining thereafter consistently with previously demonstrated volume regulatory decrease. When isoosmolarity was restored by the addition of 100 mM urea to the hypotonic medium, [2-^{3}H]glycerol uptake remained higher (112.1 ± 2.8 %, $n = 24$; $p < 0.001$) than its matched control under isotonic conditions, indicating rapid entry of urea and water. Insulin release by BRIN-BD11 cells was three times higher in hypotonic than in isotonic medium. When glycerol (100 mM) or urea (100 mM) was incorporated in the hypotonic medium, the insulin release remained significantly higher than that found in the control isotonic medium, averaging, respectively, 120.2 ± 4.2 and 107.0 ± 3.8 % of the paired value recorded in the hypotonic medium. These findings document the rapid entry of glycerol and urea in BRIN-BD11 cells, likely mediated by AQP7.

In the fourth report, rat pancreatic β-cells were investigated (Best et al. 2009). AQP7 mRNA was detected by RT-PCR in both rat pancreas and rat isolated pancreatic islets. The AQP7 protein was identified in rat pancreatic islets by Western blot analysis. Double fluorescent immunolabeling documented that AQP7 labeling overlaps with that of either insulin or somatostatin, but not with that of glucagon in rat pancreatic islets. The major functional results may be summarized as follows. The standard incubation medium used for islet cell preparation and incubation consisted of 130 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1 mM NaH_2PO_4, 1.2 mM $CaCl_2$, 25 mM Hepes-NaOH (pH 7.4), and 5 mM D-glucose. For isoosmotic substitution experiments, the basal medium contained 50 mM mannitol substituted for 25 mM NaCl. The addition of urea, glycerol, and 1,3 propanediol to the medium was then substituted for an equivalent amount of mannitol. The isoosmotic addition of urea (50 mM) increased relative cell volume in rat pancreatic β-cells. Such a cell swelling was followed by a gradual regulatory volume decrease (RVD). A similar degree of cell swelling was provoked by the isoosmotic addition of 50 mM glycerol. However, in this case no subsequent RVD was observed, possibly due to the intracellular accumulation of glycerol metabolites. Consistent with this suggestion, the isoosmotic addition of non-metabolizable 1,3 propanediol caused cell swelling followed by RVD. The isoosmotic addition of urea caused, as a rule, membrane depolarization and electrical activity in isolated rat β-cells. This effect of urea was transient, possibly reflecting the process of RVD. In contrast, the isoosmotic addition of glycerol (50 mM) caused a marked and sustained depolarization with a brief period of electrical activity. Last, 1,3 propanediol (50 mM) caused a modest and transient depolarization with resulting electrical activity in some cells. The volume-regulated anion channel (VRAC) inhibitor 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB, 50 μM) reversibly inhibited the depolarizing action of glycerol. The isoosmotic addition of urea, glycerol, or 1,3 propanediol evoked a noisy inward current at the whole-cell level

using the perforated patch configuration. The characteristics of this current resembled those of the VRAC current and were inhibited in the presence of NPPB. These findings are consistent with the uptake of urea, glycerol, or 1,3 propanediol, possibly via aquaporin, accompanied by water uptake leading to cell swelling, VRAC activation, depolarization, and electrical activity.

Last, in the most recent publications, the expression of several aquaporin isoforms was investigated in pancreatic islets from both wild-type and AQP7$^{-/-}$ knockout mice (Virreira et al. 2012). In the wild-type mice, RT-PCR detection revealed the presence of the mRNA of AQP1, AQP4, AQP5, AQP6, AQP8, AQP11, and AQP12, while that of AQP2, AQP3, and AQP9 was close to or below the limit of detection. With the exception of AQP7, comparable results were recorded in the AQP7$^{-/-}$ mice with, on occasion, an apparently somewhat more pronounced mRNA expression, e.g., in the case of AQP1, AQP4, and AQP11. This recent study thus draws attention to the high number of distinct aquaporin isoforms indeed expressed in mouse pancreatic cells. It was acknowledged, however, that further work is obviously required both to assess the possible physiological significance of these various aquaporin isoforms in mouse pancreatic islets and to conduct comparable investigations both in other species including humans and in distinct populations of endocrine cells (e.g., insulin- versus glucagon-producing cells).

Volume-Regulated Anion Channels and Glucagon Release

A possible role for volume-regulated anion channels in the process of glucose-induced inhibition of glucagon release was recently considered. Insulin-producing β-cells express a Na^+-K^+-2Cl^- cotransporter, which maintains a high chloride electrochemical potential gradient (Best 2005). Such is not the case in rat glucagon-producing cells (Majid et al. 2001). The latter cells, however, express K^+-Cl^- cotransporters (KCC) of the KCC1 and KCC4 isoforms, which are not present in either β-cells or δ-cells (Davies et al. 2004). Exposure of α-cells to hypotonic solutions caused cell swelling followed by a regulatory volume decrease (RVD). An inhibitor of KCC blocked such an RVD in α-cells, while having no effect on the RVD in β-cells. Inversely, an activator of KCC significantly decreased α-cell volume, but had no effect on β-cell volume (Davies et al. 2004). Under physiological conditions, the K^+-Cl^- cotransporter extrudes Cl^- from the cell interior and would therefore be expected to maintain the chloride electrochemical potential gradient at a low value. In such a case, the activation of volume-regulated anion channels, e.g., by D-glucose, would result in Cl^- entry into the cell, thus generating an outward, hyperpolarizing current, cell membrane hyperpolarization and, hence, inhibition of glucagon release. This proposed sequence of events was also considered in the light of a study documenting the expression and localization of the cystic fibrosis transmembrane conductance regulatory (CFTR) protein at much higher level in glucagon-secreting α-cells then in insulin-producing β-cells in the rat endocrine pancreas (Boom et al. 2007).

Concluding Remarks

The present chapter deals mainly with the expression and role of anionic transporters and channels in insulin-producing cells.

In this respect, the first issue concerns the cotransporter(s) Na^+-HCO_3^- of the NBCe1 family. The expression of both the mRNA and protein of distinct NBCe1 isoforms was documented in rat islets. The expression of NBCe1 mRNA and protein was also assessed in tumoral insulin-producing cells of the BRIN-BD11 line and human pancreatic islets. In this first set of experiments, tenidap was used as a potential inhibitor of NBCe1. Attention is drawn however on an apparent lack of specificity of tenidap toward the NBCe1 cotransporter, this drug also causing the gating of ATP-sensitive K^+ channels, as documented in a subsequent study. In addition to this reservation, a major so far unsolved question concerns the precise subcellular location of the NBCe1 cotransporters and the anionic flux mediated by these cotransporters. For instance, the question comes inevitably in mind whether such cotransporters mediate either the influx or efflux of Na^+ and HCO_3^- across the β-cell plasma membrane. In turn, changes in the Na^+ and HCO_3^- cytosolic concentration may participate in the stimulus-secretion coupling of insulin release. To cite only one example, soluble adenylyl cyclase may be activated by HCO_3^-. In considering the just-mentioned question, it could be argued that the insulin-producing β-cell acts mainly as a fuel-sensor cell in which the CO_2 generated by nutrient secretagogues, such as D-glucose, escapes from the cell mainly as HCO_3^- generated in a reaction catalyzed by a mitochondrial carbonic anhydrase (Sener et al. 2007). Hence, in situations of sustained stimulation of insulin release by D-glucose or other nutrient secretagogues, the efflux of the bicarbonate anion may appear as a more relevant movement across the plasma membrane rather than the opposite influx of the same anion. Admittedly, however, the efflux of HCO_3^- may occur mainly at the intervention of volume-regulated anion channels.

The latter remark leads to the second major issue considered in the present chapter. Based on a number of prior findings, the present work was indeed conceived in the framework of the so-called VRAC hypothesis. This hypothesis postulates that the gating of volume-regulated anion channels represents, in addition to the closing of ATP-sensitive K^+ channels, the second essential component of stimulus-secretion coupling in the process of glucose-stimulated insulin secretion and prevails in the range of concentration of the hexose well above the threshold value for the insulinotropic action of this nutrient. In the present study, evidence is provided to support the view that anoctamin 1 represents, to say the least, one of the volume-regulated anion channels in insulin-producing cells. The expression of the mRNA for anoctamin 1 and the presence of anoctamin 1 protein, as assessed by immunohistochemistry, were indeed documented in mouse, rat, and human pancreatic islet cells. Moreover, tannic acid, an inhibitor of anoctamin 1, impaired the secretory and bioelectrical response to a high concentration of D-glucose (16.7 mM) in rat pancreatic islets and mouse β-cells, respectively. Somewhat unexpectedly, tannic acid also affected both D-[5-^{3}H]glucose utilization and D-[U-^{14}C]glucose oxidation, the decrease in glycolytic flux provoked by tannic acid being compensated by an

increased oxidation of the hexose, with no change in the total energy yield resulting from the catabolism of the sugar in rat pancreatic islets. The underlying determinants of these metabolic effects of tannic acid remain to be identified. Likewise, the possible role of anoctamin 6 and anoctamin 10 in islet cells remains to be explored. It may also be safe to extend the present work by use of other inhibitors of TMEM16A such as the aminophenylthiazole (T16A(inh)AO1) (Namkung et al. 2011). Last, TMEM16A being referred to as a calcium-activated chloride channel, further work is obviously desirable to assess the effects of its potential inhibitors on cytosolic Ca^{2+} concentration in glucose-stimulated insulin-producing cells. In such a respect, it seems worthwhile to remind that, already in 1977, it was observed that the Ca^{2+} antagonist verapamil, which inhibits both ^{45}Ca uptake and glucose-stimulated insulin release by isolated islets and which does not affect the total production of lactate by islets exposed up to 90 min to 16.7 mM D-glucose, somewhat unexpectedly decreases the output of lactate into the incubation medium, this resulting in an increase of the lactate content of the islets (Malaisse et al. 1977). This finding may indeed be relevant to the participation of the Ca^{2+}-activated volume-regulated anion channel anoctamin 1 in the process of lactate anion efflux from pancreatic islets.

With these concluding remarks in mind, there is little risk to propose that the participation of anionic transporters and channels in pancreatic insulin-producing islet cells remains a field widely open to further investigations.

References

Agre P (2004) Aquaporin water channels. Angew Chem Int Ed Engl 43:4278–4290

Almaca J, Tian Y, Aldehni F, Ousingsawat J, Kongsuphol P, Rock JR, Harfe BD, Schreiber R, Kunzelmann K (2009) TMEM16A proteins produce volume regulated chloride currents that are reduced in mice lacking TMEM16A. J Biol Chem 284:28571–28578

Beauwens R, Best L, Markadieu N, Crutzen R, Louchami K, Brown P, Yates AP, Malaisse WJ, Sener A (2006) Stimulus-secretion coupling of hypotonicity-induced insulin release in BRIN-BD11 cells. Endocrine 30:353–363

Best L (1997) Glucose and a-ketoisocaproate induce transient inward currents in rat pancreatic β cells. Diabetologia 40:1–6

Best L (1999) Cell-attached recordings of the volume-sensitive anion channel in rat pancreatic B cells. Biochim Biophys Acta 1419:248–256

Best L (2000) Glucose-sensitive conductances in rat pancreatic β-cells: contribution to electrical activity. Biochim Biophys Acta 1468:311–319

Best L (2002) Study of a glucose-activated anion-selective channel in rat pancreatic β-cells. Pflugers Arch 445:97–104

Best L (2005) Glucose-induced electrical activity in rat pancreatic β-cells: dependence in intracellular chloride concentration. J Physiol (Lond) 568:137–144

Best L, Trebilcock R, Tomlinson S (1992) Lactate transport in insulin-secreting β-cells: contrast between rat islets and HIT-T15 insulinoma cells. Mol Cell Endocrinol 86:49–56

Best L, Sheader EA, Brown PD (1996) A volume-activated anion conductance in insulin-secreting cells. Pflugers Arch 431:363–370

Best L, Brown PD, Tomlinson S (1997) Anion fluxes, volume regulation and electrical activity in the mammalian β cell. Exp Physiol 82:957–966

Best L, Miley HE, Brown PD, Cook LJ (1999) Methylglyoxal causes swelling and activation of a volume-sensitive anion conductance in rat pancreatic β-cells. Membr Biol 167:65–71

Best L, Speake T, Brown PD (2001) Characterization of the volume-sensitive anion channel in rat pancreatic β-cells. Exp Physiol 86:145–150

Best L, Brown PD, Yates AP, Perret J, Virreira M, Beauwens R, Malaisse WJ, Sener A, Delporte C (2009) Contrasting effects of glycerol and urea transport on rat pancreatic β-cell function. Cell Physiol Biochem 23:255–264

Best L, Brown PD, Sener A, Malaisse WJ (2010a) Opposing effects of tenidap on the volume-regulated anion channel and K_{ATP} channel activity in rat pancreatic β-cells. Eur J Pharmacol 629:159–163

Best L, Brown PD, Sener A, Malaisse WJ (2010b) Electrical activity in pancreatic islet cells: the VRAC hypothesis. Islets 2:59–64

Blackard WG, Likuchi M, Rabinovitch A, Renold AE (1975) An effect of hypoosmolarity on insulin release in vitro. Am J Physiol 228:706–713

Boom A, Lybaert P, Pollet J-F, Jacobs P, Jijakli H, Golstein PE, Sener A, Malaisse WJ, Beauwens R (2007) Expression and localization of cystic fibrosis transmembrane conductance regulator in the endocrine pancreas. Endocrine 32:197–205

Bukowiecki L, Trus M, Matschinsky FM, Freinkel N (1979) Alteration in pancreatic islet phosphate content during secretory stimulation with glucose. Biochim Biophys Acta 583:370–377

Bulur N, Virreira M, Soyfoo MS, Louchami K, Delporte C, Perret J, Beauwens R, Malaisse WJ, Sener A (2009) Expression of the electrogenic Na^+ -HCO_3^- cotransporter NBCe1 in tumoral insulin-producing BRIN-BD11 cells. Cell Physiol Biochem 24:187–192

Bulur N, Zhang Y, Malaisse WJ, Sener A (2010) Insulin release from isolated pancreatic islets, dispersed islet cells and tumoral insulin producing cells: a re-examination. Metab Funct Res Diab 3:20–24

Bulur N, Crutzen R, Malaisse WJ, Sener A, Beauwens R, Golstein P (2013) Interaction between 3′,5′-cyclic monophosphate, volume-regulated anion channels and the Na^+-HCO_3^- cotransporter NBCe1 in the regulation of nutrient- and hypotonicity-induced insulin release from rat pancreatic islets and tumoral insulin-producing BRIN-BD11 cells. Mol Med Rep 7:1666–1672

Carpinelli AR, Malaisse WJ (1980) The stimulus-secretion coupling of glucose-induced insulin release. XLIV. A possible link between glucose metabolism and phosphate flush. Diabetologia 19:458–464

Carpinelli AR, Malaisse WJ (1981) Regulation of ^{86}Rb outflow from pancreatic islets. V. The dual effect of nutrient secretagogues. J Physiol (Lond) 315:143–156

Casas S, Gomis R, Gribble FM, Altirriba J, Knuutila S, Novials A (2007) Impairment of the ubiquitin-proteasome pathway is a downstream endoplasmic reticulum stress response induced by extracellular human islet amyloid polypeptide and contributes to pancreatic β-cell apoptosis. Diabetes 56:2284–2294

Cook DL, Hales CN (1984) Intracellular ATP directly blocks K^+ channels in pancreatic β-cells. Nature 311:271–273

Crutzen R, Shlyonsky V, Louchami K, Virreira M, Hupkens E, Boom A, Sener A, Malaisse WJ, Beauwens R (2012) Does NAD(P)H oxidase-derived H_2O_2 participate in hypotonicity-induced insulin release by activating VRAC in β-cells? Eur J Physiol 463:377–390

Davies SL, Roussa E, Le Rouzic P, Thevenod F, Alper SL, Best L, Brown PD (2004) Expression of K/Cl cotransporters in the a-cells of rat endocrine pancreas. Biochim Biophys Acta 1667:7–14

Delporte C, Virreira M, Crutzen R, Louchami K, Sener A, Malaisse WJ, Beauwens R (2009) Functional role of aquaglyceroporin 7 expression in the pancreatic β-cell line BRIN-BD11. J Cell Physiol 221:424–429

Freinkel N (1979) Phosphate translocation during secretory stimulation of pancreatic islets. In: Camerini-Davalos A, Hanover B (eds) Treatment of early diabetes. Plenum Press, New York, pp 71–77

Freinkel N, El Younsi C, Bonnar J, Dawson MC (1974) Rapid transient efflux of phosphate ions from pancreatic islets as an early action of insulin secretagogues. J Clin Invest 54:1179–1189

Freinkel N, El Younsi C, Dawson RMC (1976) Insulin release and phosphate ion efflux from rat pancreatic islets induced by L-leucine and its nonmetabolizable analogue, 2-amino-bicyclo [2-2-1]heptane-2-carboxylic acid. Proc Natl Acad Sci USA 73:3403–3407

Gawenis LR, Bradford EM, Prasad V, Lorenz JN, Simpson JE, Charke LL, Woo AL, Grisham C, Sanford LP, Doetschman T, Miller ML, Shull GE (2007) Colonic anion secretory defects and metabolic acidosis in mice lacking the NBC1 Na^+/HCO_3^- cotransporter. J Biol Chem 282:9042–9052

Hanzu FA, Gasa R, Bulur N, Lybaert P, Gomis R, Malaisse WJ, Beauwens R, Sener A (2012) Expression of TMEM16A and SLC4A4 in human pancreatic islets. Cell Physiol Biochem 29:61–64

Henquin JC, Detimary P, Gembal M, Jonas JC, Shepherd RM, Warnotte C, Gilon P (1994) Aspects biophysiques du contrôle de la sécrétion d'insuline. Journées de Diabétologie de l'Hôtel-Dieu:21–32

Huang F, Rock JR, Harfe BD, Cheng T, Huang X, Jan YN, Jan LY (2009) Studies on expression and function of the TMEM16A calcium-activated chloride channel. Proc Natl Acad Sci USA 106:21413–21418

Li D-Q, Jing X, Salehi A, Collins SC, Hoppa MB, Rosengren AH, Zhang E, Lundquist I, Oloffson CS, Mörgerlin M, Eliasson L, Rorsman P, Renström E (2009) Suppression of sulfonylurea- and glucose-induced insulin secretion in vitro and in vivo in mice lacking the chloride transport protein CIC-3. Cell Metab 10:309–315

Louchami K, Zhang Y, Beauwens R, Malaisse WJ, Sener A (2007) Is the glucose-induced phosphate flush in pancreatic islets attributable to gating of volume-sensitive anion channels? Endocrine 31:1–4

Louchami K, Best L, Brown P, Virreira M, Hupkens E, Perret J, Devuyst O, Uchida S, Delporte C, Malaisse WJ, Beauwens R, Sener A (2012) A new role for aquaporin 7 in insulin secretion. Cell Physiol Biochem 29:65–74

Majid A, Speake T, Best L, Brown PD (2001) Expression of the Na-K-2Cl cotransporter in and β cells isolated from the rat pancreas. Pflugers Arch 442:570–576

Malaisse WJ, Orci L (1979) The role of the cytoskeleton in pancreatic β-cell function. In: Gabbiani G (ed) Methods of achievements in experimental pathology, vol 9. S. Karger, Basel, pp 112–136

Malaisse WJ, Sener A, Koser M, Herchuelz A (1976) The stimulus-secretion coupling of glucose-induced insulin release. XXIV. The metabolism of α- and β-D-glucose in isolated islets. J Biol Chem 251:5936–5943

Malaisse WJ, Herchuelz A, Levy J, Sener A (1977) Calcium-antagonists and islet function. III. The possible site of action of verapamil. Biochem Pharmacol 26:735–740

Malaisse WJ, Sener A, Herchuelz A, Hutton JC (1979a) Insulin release: the fuel hypothesis. Metabolism 28:373–386

Malaisse WJ, Hutton JC, Kawazu S, Herchuelz A, Valverde I, Sener A (1979b) The stimulus-secretion coupling of glucose-induced insulin release. XXXV. The links between metabolic and cationic events. Diabetologia 16:331–341

Malaisse WJ, Malaisse-Lagae F, Mayhew D (1967) A possible role for the adenylcyclase system in insulin secretion. J Clin Invest 46:1724–1734

Malaisse WJ, Sener A, Welsh M, Malaisse-Lagae F, Hellerström C, Christophe J (1983) Mechanism of 3-phenylpyruvate-induced insulin release. Metabolic aspects. Biochem J 210:921–927

Malaisse WJ, Zhang Y, Louchami K, Jijakli H (2004) Stimulation by D-glucose of $^{36}Cl^-$ efflux from prelabeled rat pancreatic islets. Endocrine 25:23–25

Malaisse WJ, Best L, Beauwens R, Sener A (2008) Ionic determinants of the insulinotropic action of glucose: the anion channel hypothesis. Metab Funct Res Diab 1:2–6

Malaisse WJ, Virreira M, Zhang Y, Crutzen R, Bulur N, Lybaert P, Golstein PE, Sener A, Beauwens R (2012) Role of anoctamin 1 (TMEM16A) as a volume regulated anion channel in insulin-producing cells. Diabetologia 55(Suppl 1):S204

Malaisse WJ, Crutzen R, Bulur N, Virreira M, Rzajeva A, Golstein PE, Sener A, Beauwens R (2013) Effects of the inhibitor of anoctamin 1, tannic acid, on insulin-producing cells. Diabetologia 56(Suppl 1):S196

Martins JR, Faria D, Kongsuphol P, Schreiber R, Kunzelmann K (2011) Anoctamin 6 is an essential component of the outwardly rectifying chloride channel. Proc Natl Acad Sci USA 108:18168–18172

Matsumura K, Chang BH, Fujimiya M, Chen W, Kulkarni RN, Eguchi Y, Kimura H, Kojima H, Chan L (2007) Aquaporin 7 is a β-cell protein and regulator of intraislet glycerol content and glycerol kinase activity, β-cell mass, and insulin production and secretion. Mol Cell Biol 27:6026–6037

McClenaghan NH, Barnett CR, O'Harte FP, Flatt PR (1996) Mechanisms of amino acid-induced insulin secretion from the glucose-responsive BRIN-BD11 pancreatic β-cell line. J Endocrinol 151:349–357

McGlasson L, Best L, Brown PD (2011) The glucokinase activator GKA50 causes an increase in cell volume and activation of volume-regulated anion channels in rat pancreatic β-cells. Mol Cell Endocrinol 342:48–53

Miley HE, Sheader EA, Brown PD, Best L (1997) Glucose-induced swelling in rat pancreatic β-cells. J Physiol (Lond) 504:191–198

Muller-Berger S, Ducoudret O, Diakov A, Frömter E (2001) The renal Na-HCO_3^- cotransporter expressed in *Xenopus laevis* oocytes: change in stoichiometry in response to elevation of cytosolic Ca^2 concentrations. Pflugers Arch 442:718–728

Namkung W, Phuan PW, Verkman AS (2011) TMEM16A inhibitors reveal TMEM16A as a minor component of calcium-activated chloride channel conductance in airway and intestinal epithelial cells. J Biol Chem 286:2365–2374

Orci L, Malaisse WJ (1980) Hypothesis: single and chain release of insulin secretory granules is related to anionic transport at exocytotic sites. Diabetes 29:943–944

Parker MD, Boron WF (2008) Sodium-coupled bicarbonate transporters. In: Alpern RJ, Hebert SC (eds) Seldin and Giebisch's. The kidney physiology and pathophysiology. Elsevier Academic Press, Amsterdam, pp 1481–1497

Ramos LS, Zippin JH, Kamenetsky M, Buck J, Levin LR (2008) Glucose and GLP-1 stimulate camp production via distinct adenylyl cyclases in $INS\text{-}1^E$ insulinoma cells. J Gen Physiol 132:329–338

Rojek A, Praetorius J, Froklaer J, Nielsen S, Fenton RA (2008) A current view of the mammalian aquaglyceroporins. Annu Rev Physiol 70:301–327

Schreiber R, Uliyakina I, Kongsuphol P, Warth R, Mirza M, Martins JR, Kunzelmann K (2010) Expression and function of epithelial anoctamins. J Biol Chem 285:7838–7845

Sehlin J (1978) Interrelationship between chloride fluxes in pancreatic islets and insulin release. Am J Physiol 235:E501–E508

Sener A, Malaisse WJ (1976) Measurement of lactic acid in nanomolar amounts. Reliability of such a method as an index of glycolysis in pancreatic islets. Biochem Med 15:34–41

Sener A, Malaisse WJ (2012) Secretory, ionic and metabolic events in rat pancreatic islets deprived of extracellular $NaHCO_3$. Metab Funct Res Diab 5:1–3

Sener A, Malaisse-Lagae F, Malaisse WJ (1981) Stimulation of islet metabolism and insulin release by a nonmetabolizable amino acid. Proc Natl Acad Sci U S A 78:5460–5464

Sener A, Welsh M, Lebrun P, Garcia-Morales P, Saceda M, Malaisse-Lagae F, Herchuelz A, Valverde I, Hellerström C, Malaisse WJ (1983) Mechanism of 3-phenylpyruvate-induced insulin release. Secretory, ionic and oxidative aspects. Biochem J 210:913–919

Sener A, Jijakli H, Zahedi Asl S, Courtois P, Yates AP, Meuris S, Best LC, Malaisse WJ (2007) Possible role of carbonic anhydrase in rat pancreatic islets: enzymatic, secretory, metabolic, ionic, and electrical aspects. Am J Physiol 292:E1624–E1630

Somers G, Blondel B, Orci L, Malaisse WJ (1979) Motile events in pancreatic endocrine cells. Endocrinology 104:255–264

Somers G, Sener A, Devis G, Malaisse WJ (1980) The stimulus-secretion coupling of glucose-induced insulin release. XLV. The anion-osmotic hypothesis for exocytosis. Pflugers Arch 388:249–253

Soyfoo MS, Bulur N, Virreira M, Louchami K, Lybaert P, Crutzen R, Perret J, Delporte C, Roussa E, Thevenod F, Best L, Yates AP, Malaisse WJ, Sener A, Beauwens R (2009) Expression of the electrogenic Na^+ -HCO_3^- cotransporters NBCe1-A and NBCe1-B in rat pancreatic islet cells. Endocrine 35:449–458

Valverde I, Vandermeers A, Anjaneyulu R, Malaisse WJ (1979) Calmodulin activation of adenylate cyclase in pancreatic islets. Science 206:225–227

Valverde I, Garcia-Morales P, Ghiglione M, Malaisse WJ (1983) The stimulus-secretion coupling of glucose-induced insulin release. LIII. Calcium-dependency of the cyclic AMP response to nutrient secretagogues. Horm Metab Res 15:62–68

Virreira M, Malaisse WJ, Sener A, Beauwens R (2012) Expression of aquaporin isoforms in mouse pancreatic islets. Metab Funct Res Diab 5:27–28

Zhao C, Wilson MC, Schuit F, Halestrap AP, Rutter GA (2001) Expression and distribution of lactate/monocarboxylate transporter isoforms in pancreatic islets and the exocrine pancreas. Diabetes 50:361–366

Chloride Channels and Transporters in β-Cell Physiology

14

Mauricio Di Fulvio, Peter D. Brown, and Lydia Aguilar-Bryan

Contents

While this chapter was in press, two papers published simultaneously, one by Qiu et al. (Cell 157:447-458, 2014) and another by Voss et al. (Science, published online 10 April 2014, DOI:10.1126/science.1252826) identified the product of LRRC8A gene as an essential component of VRAC. In fact, multimerization of LRRC8A with the products of four homologous genes (LRRC8B-E) appears necessary to functionally reconstitute native VRAC properties, as we know them. Indeed, the reconstituted VRAC or the LRRC8A protein alone, named SWELL1 by Qiu et al., presented the typical biophysical properties and pharmacological profiles of VRAC in several cells when over-expressed. These included hypotonicity-stimulated anion fluxes, intermediate single-channel conductance, outwardly rectifying current-voltage (I-V) relationship, inhibition with [4-(2-butyl-6, 7-dichloro-2-cyclopentyl-indan-1-on-5-yl] oxobutyric acid (DCPIB), DIDS-sensitivity, high permeability to Cl^- ions and the ability to funnel out the osmoregulator taurine.

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_34,

Abstract

The ability of β-cells to depolarize, regulate $[Ca^{2+}]_i$, and secrete insulin even in the absence of functional K_{ATP} channels strongly suggests the presence of additional ionic cascades of events within the process of stimulus-secretion coupling. The purpose of this review is to introduce the reader to the role of the long-relegated and largely ignored subject of intracellular Cl^- concentration ($[Cl^-]_i$). The regulation of $[Cl^-]_i$ by transporters and channels, and their potential involvement in glucose-induced insulin secretion, is also discussed. It is important to keep in mind that, in the last decade, the molecular identification and functional characterization of many diverse regulators of $[Cl^-]_i$ in β-cells have added to the extraordinary complexity of the β-cell secretory response. We have therefore concentrated on key concepts, and on what we consider may be the most important players involved in the regulation of $[Cl^-]_i$ in β-cells, but time will tell.

Keywords

$[Cl^-]_i$ • Thermodynamic equilibrium • VRAC • Ca^{2+}-activated Cl^- channels • CFTR • NKCCs • Depolarization • Insulin secretion

Introduction: The Consensus Model of Glucose-Induced Insulin Secretion: Still an Incomplete View

> "*Stimulus-secretion coupling in β-cells is a complex process with multiple facets that cannot be simply incorporated in any single comprehensible model*." (Henquin et al. 2009)

Pancreatic β-cells secrete insulin in a very precise manner, by a process involving a remarkably wide variety of factors encompassing neurotransmitters (GABA, norepinephrine/epinephrine), hormones (glucagon, somatostatin, growth hormone), and incretins (GLP-1 and GIP). Perhaps more importantly, β-cells are also able to transduce changes in their metabolic status, i.e., plasma concentrations of nutrients in particular glucose and amino acids, into biophysical and biochemical secretory signals of exceptional complexity (Fig. 1a). β-Cells must therefore have the

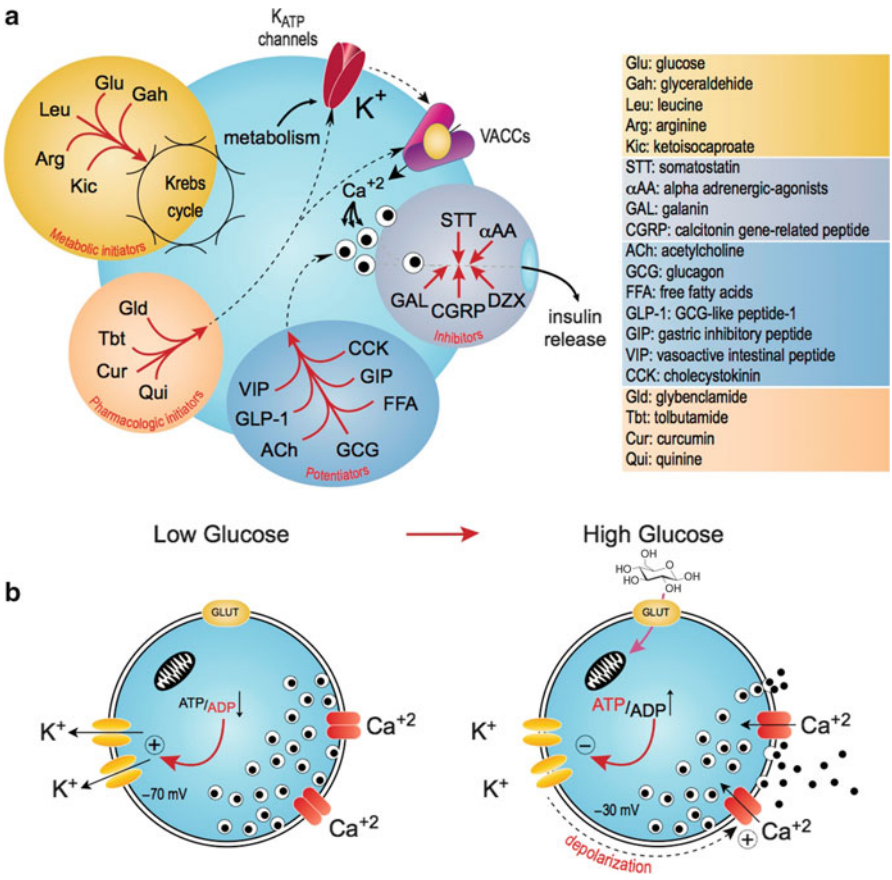

Fig. 1 Regulation of insulin secretion: nutrients, secretagogues, and other compounds. (**a**) Insulin secretion is exquisitely influenced by a wide variety of agents, which can be widely classified as follows: (i) metabolic initiators, i.e., agonists coupling the metabolic machinery of the β-cell to direct closure of K_{ATP} channels such as glucose, certain amino acids, and other substrates of the Krebs' cycle; (ii) pharmacologic initiators involved in either closing K_{ATP} channels or stimulating voltage-activated Ca^{2+} channels (*VACC*) such as sulfonylureas (tolbutamide, glibenclamide or BayK8644, respectively); (iii) potentiators implicated in insulin secretion by mechanisms independent of plasma membrane depolarization; and (iv) inhibitors, most of them involved in granule biology. (**b**) A recapitulation of the consensus model of insulin secretion depicted in textbooks. Under conditions of low or normal blood glucose, the low metabolic rate of the cell, as reflected by low ATP/ADP ratios, keeps K_{ATP} channels in the open state allowing the movement of K^+ according to the driving force of the cation established by the constant action of the Na^+/K^+-ATPase and the resting membrane potential of ~ −65 mV. When blood glucose rises, the sugar is moved into the β-cell via GLUT transporters stimulating metabolism and increasing the ATP/ADP ratio resulting in closure of K_{ATP} channels, plasma membrane depolarization, activation of VACC, influx of Ca^{2+}, and insulin secretion

capacity to integrate a variety of both stimulatory and inhibitory signals in order to promote the appropriate release of insulin (Henquin et al. 2003).

In spite of the complexity in signaling pathways, glucose-induced insulin secretion by pancreatic β-cells is commonly condensed into a very simple consensus model. This model is remarkably similar although not identical to the well-characterized depolarization-secretion coupling observed in neurons, chromaffin cells, or lactotrophs (reviewed in Misler (2012)). It involves the following sequence of events: glucose metabolism, closure of ATP-sensitive potassium channels (K_{ATP} channels) in the plasma membrane, depolarization, influx of Ca^{2+} through voltage-dependent calcium channels, and a rise in cytosolic-free Ca^{2+} concentration ($[Ca^{2+}]_i$) that induces exocytosis of insulin-containing granules (Fig. 1b and chapter "► ATP-Sensitive Potassium Channels in Health and Disease"). While this model adequately describes the control of insulin secretion, we contend that it may not completely explain the regulation of β-cell activity.

Oral hypoglycemic agents, like sulfonylureas, are used in the treatment of type 2 and neonatal diabetes mellitus and some forms of MODY (Aguilar-Bryan and Bryan 2008; Babenko et al. 2006; Klupa et al. 2012) because they stimulate insulin release from β-cells. They act by binding to the regulatory subunit of the K_{ATP} channel, SUR1 or sulfonylurea receptor, inhibiting K_{ATP} channels and depolarizing the plasma membrane (Panten et al. 1996). However, they may also exert K_{ATP} channel-independent effects on the β-cell, e.g., tolbutamide exerts paradoxical effects on $^{86}Rb^+$ efflux in islets (an index of K^+ permeability) (Best et al. 2004; Henquin 1980; Henquin and Meissner 1982a). While tolbutamide inhibits $^{86}Rb^+$ efflux in the absence of glucose, reflecting K_{ATP} channel inhibition, in the presence of glucose (5 mM or more), this compound increases the rate of $^{86}Rb^+$ efflux. This latter effect is clearly inconsistent solely with K_{ATP} channel inhibition and may reflect an increased driving force for K^+ efflux due to depolarization of the β-cell membrane potential due to other electrogenic events.

Furthermore in the absence of functional K_{ATP} channels, β-cells still depolarize, regulate $[Ca^{2+}]_i$, and secrete insulin in response to glucose (Henquin et al. 2009; Best et al. 2010; Dufer et al. 2004; Gembal et al. 1992; Rosario et al. 2008; Szollosi et al. 2007). This suggests the presence of additional membrane transport events associated with glucose stimulation. These additional mechanisms may include the activation of transient receptor potential (TRP) nonspecific cation channels or the activation of anion channels. It is the second of these possibilities, which is the focus of this chapter.

Thus, the aims of this chapter are to:

- Introduce the reader to the contribution of $[Cl^-]_i$ to plasma membrane potential and to discuss generic properties of Cl^--transporting proteins and channels.
- Discuss the evidence for the expression of these proteins in β-cells, and describe how they may modulate plasma membrane potential in response to glucose stimulation.

Intracellular Chloride Concentration and Cell Membrane Potential

> "*Anion channels have been relegated to the sidelines of ion channel research for more than 50 years...*" (Nilius and Droogmans 2003), however it has recently been recognized that: "*Some cells actively extrude Cl^-, others actively accumulate it, but few cells ignore it.*" (Alvarez-Leefmans 2012)

All cells, including electrically excitable ones such as neurons, myocytes, and pancreatic β-cells, exhibit a membrane resting potential (Em), defined by the difference between the electrical potential outside and inside of the cell. Although variable in magnitude, Em in electrically excitable cells is normally around −70 mV. It is generated and maintained by (i) the activity of the Na^+/K^+-ATPase, which actively loads into the cell $2K^+$ in exchange for $3Na^+$ ions resulting in a net loss of a positive charge per transport cycle, and (ii) the activity of a number of K^+ channels, which allow the "leaky" exit of K^+ ions from the cell (Sperelakis 2012). Thus, the Na^+/K^+-ATPase maintains a higher intracellular concentration of K^+ ($[K^+]_i$) in comparison with the outside resulting in a K^+ concentration gradient across the plasma membrane. The opening of some K^+ channels permits the exit of K^+ following its concentration gradient also known as chemical driving force, thus increasing the positive charges outside and the negative charges inside the cell. Therefore, the increased difference between the electrical potential outside and the inside of the cell, i.e., -Em, constitutes the electrical driving force that opposes to the K^+ chemical driving force, preventing additional exit of K^+ ions from the cell. When the net transmembrane flux of K^+ ions is zero, Em becomes stable at the particular negative Em value of that cell.

It has long been recognized that other ions notably Na^+ and Ca^{2+} are also asymmetrically distributed across the membrane. Therefore, changes in the permeability to these ions will also contribute to and modulate Em. The role of Cl^- in modulating Em is much less familiar. In fact the opening of Cl^- channels may either depolarize (efflux) or hyperpolarize Em (influx). The direction of Cl^- movement, and the resultant change in Em, is determined by (i) the difference between $[Cl^-]_i$ and extracellular chloride concentration ($[Cl^-]_o$) and (ii) the difference between Em and the electric potential for Cl^-, i.e., the Em where the net flux of Cl^- is zero (E_{Cl}). Hence, influx or efflux of Cl^- ions will result in the shift of Em towards more negative (hyperpolarizing) or positive (depolarizing) values, respectively. From this example, it is evident that at physiological $[Cl^-]_o$ of ~ 123 mM if $Em < E_{Cl}$, Cl^- will tend to enter the cell, whereas the reverse situation will be found when $Em > E_{Cl}$. When $Em = E_{Cl}$, then $[Cl^-]_i$ is passively distributed, i.e., the net flux of Cl^- is zero; the influx of Cl^- ions is identical in magnitude to its efflux, conditions under which $[Cl^-]_i$ reaches thermodynamic equilibrium.

Under conditions of thermodynamic equilibrium, $[Cl^-]_i$ can be easily calculated by the following expression derived from the Nernst equation:

$$[Cl^-]_i = [Cl^-]_o e^{EmF/RT}$$

where e is the Euler's number (~ 2.71), F the Faraday's constant (96.5 JmV^{-1}), R the gas constant (8.31 JK^{-1} mol^{-1}), and T the absolute temperature in the Kelvin scale (K = °C + 273.15). Therefore, $[Cl^-]_i$ in a resting excitable cell with Em of −70 mV, at 37 °C, and assuming $[Cl^-]_o$ = 123 mM, can be calculated to be ~ 10 mM. In other words, Cl^- in the cell would attain an intracellular concentration close to 10 mM, only if it was passively distributed across the plasma membrane according to the Nernst equation.

Until recently, the importance of $[Cl^-]_i$ as a physiological regulator was ignored, despite the fact that Cl^- is the most abundant anion in the body. This was because it was generally accepted that Cl^- distributes across plasma membranes strictly according to the Nernst equation, i.e., passively disseminated following its electrical and chemical gradients. This supposition is now known to be true for only very few specialized cells, and it is now clear that Cl^- is actively transported and tightly regulated in virtually all cells (as expertly documented by Alvarez-Leefmans (2012)). By virtue of its nonequilibrium distribution, Cl^- participates in the regulation of many cellular functions, including γ-amino butyric acid (GABA)-mediated synaptic signaling (Alvarez-Leefmans and Delpire 2009), cell volume and pH regulation (Hoffmann et al. 2009), cell growth and differentiation (Kunzelmann 2005; Iwamoto et al. 2004; Panet et al. 2006; Shiozaki et al. 2006), transepithelial salt and water transport (Hoffmann et al. 2007), and Em stabilization (Sperelakis 2012). Within the context of the pancreatic islet or in particular the pancreatic β-cells, $[Cl^-]_i$ may also play a role in growth and development or directly on the exocytotic machinery.

The direction that Cl^- follows in a given cell is determined at least by two factors: Em and the Cl^- concentration gradient. One of the most interesting aspects of the nonequilibrium distribution of Cl^-, i.e., $Em \neq E_{Cl}$, is that the same stimulus may have an opposite effect on Em. Accordingly, Cl^- plays a fundamental role in synaptic signaling involving ligand-gated Cl^- channels, e.g., the ionotropic GABA receptor type A ($GABA_A$). Indeed, GABA-signaling in neurons is depolarizing (excitatory) or hyperpolarizing (inhibitory) depending on $[Cl^-]_i$. In immature neurons and nociceptors, activation of $GABA_A$ allows Cl^- efflux because $[Cl^-]_i$ in these cells is kept above electrochemical equilibrium (Alvarez-Leefmans and Delpire 2009). Electrogenic in nature, Cl^- efflux depolarizes the plasma membrane, i.e., takes the resting Em to more positive values. Conversely, activation of $GABA_A$ in mature central neurons results in a hyperpolarizing inhibitory inward current of Cl^-. Therefore, when Em is close to E_{Cl}, activation of $GABA_A$ or any other anion channel allowing the passage of Cl^- may not further depolarize the plasma membrane, and it may in fact allow entrance of Cl^- following its concentration gradient (Alvarez-Leefmans and Delpire 2009; Wright et al. 2011).

An Overview of Cl^--Transporting Proteins

The ability of mammalian cells to regulate the entry and exit of Cl^-, and thus maintain a particular $[Cl^-]_i$ depends on the functional expression of Cl^--transporting proteins and channels (Alvarez-Leefmans 2012). Depending on the cell in

question, these include transport proteins that actively accumulate or extrude Cl^-, while Cl^- channels tend to dissipate the gradients established by the Cl^- accumulators and extruders (Fig. 2a). Chloride accumulators and extruders belong to several gene families all included within the group of solute carrier superfamily of genes (SLC), a very large group of genes organized in at least 46 families based on gene homology and sequence identity (Fredriksson et al. 2008; Hediger et al. 2004).

Three SLC families are known to have members directly involved in the regulation of $[Cl^-]_i$. These are: (i) SLC12A, also known as the cation (Na^+/K^+)-Cl^- cotransporter (CCC) superfamily, (ii) SLC4A also known as anion exchangers (AEs) or Cl^--bicarbonate exchangers (CBE), and (iii) SLC26A (also generally known as anion exchangers) (Table 1). In the following sections, we will describe the properties of the SLC12A family of genes, which include prototypical Cl^- loaders and extruders, and as we will see later in this chapter, these transporters may play significant roles in determining pancreatic β-cell excitability. However, it is important to keep in mind that many members of the SLC4A and SLC26A families (Table 1) are also involved in $[Cl^-]_i$ regulation in mammalian cells. For an in-depth insight into the molecular physiology, pharmacology, and regulation of these families of transporters, we refer the reader to specialized reviews by Alvarez-Leefmans (2012), Alper and Sharma (2013), Arroyo et al. (2013), Parker and Boron (2013), Romero et al. (2013), and Soleimani (2013)

Chloride Accumulators: SCL12A1, SLC12A2, and SLC12A3 Proteins

Three genes of the SLC12A family i.e., SLC12A1, SLC12A2, and SLC12A3, are considered Cl^- accumulators, whereas SLC12A4, SLC12A5, SLC12A6, and SLC12A7 are Cl^- extruders (see section "Chloride Extruders: SLC12A4, SLC12A5, SLC12A6, and SLC12A7 Proteins").

The SLC12A1 and SLC12A2 genes encode the $Na^+K^+2Cl^-$ cotransporter 2 (NKCC2) and 1 (NKCC1), respectively, whereas the SLC12A3 gene encodes the Na^+Cl^- cotransporter (NCC) (Table 1) (reviewed in Di Fulvio and Alvarez-Leefmans (2009)). These transporters exhibit distinctive expression patterns and have several splice variants. NKCC1, for instance, is considered a ubiquitously expressed and highly N-glycosylated protein of ~ 170 kDa (Alvarez-Leefmans 2012). In comparison, NKCC2 has been considered, until very recently, a transporter that is confined to cells of the kidney tubule (Arroyo et al. 2013). In the last 5 years, however, NKCC2 has been shown to express in several cell types of the gastrointestinal tract, the endolymphatic sac, retina (Xue et al. 2009; Zhu et al. 2011; Gavrikov et al. 2006; Akiyama et al. 2007, 2010; Kakigi et al. 2009; Nishimura et al. 2009; Nickell et al. 2007), and even pancreatic β-cells (Corless et al. 2006; Ghanaat-Pour and Sjoholm 2009; Bensellam et al. 2009; Alshahrani et al. 2012). Undoubtedly, NKCC2 shows the highest expression in the kidney where it is known to play a fundamental role in salt reabsorption (Carota et al. 2010).

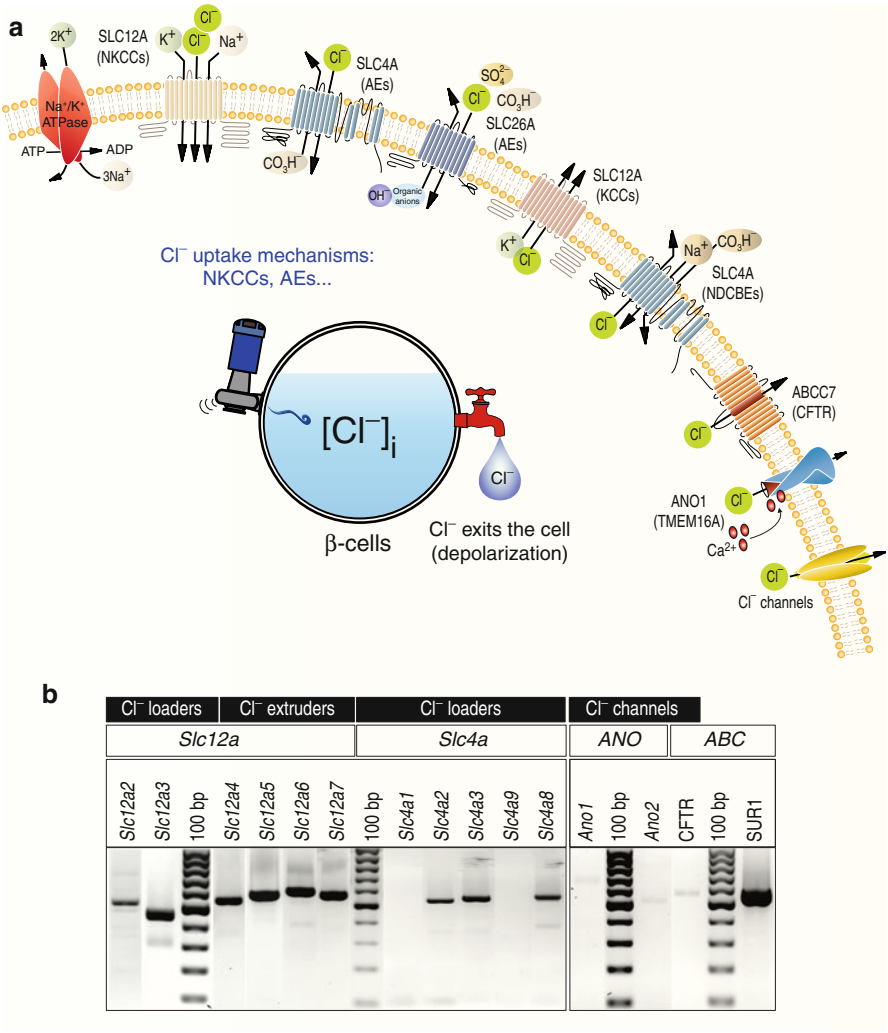

Fig. 2 Regulation of $[Cl^-]_i$ in cells. (**a**) The natural direction of K^+ and Na^+ ionic flows, as determined by the action of the Na^+/K^+-ATPase in all cells, i.e., Na^+ inward and K^+ outward provide the driving force to cotransport Cl^- in or out the cell via NKCCs or KCCs, respectively. Notably, other transporters may also contribute to net Cl^- transport. Indeed, also shown are representative members of the anion exchanger families (AEs: SLC4A, and SLC26A) involved in Cl^- uptake or extrusion. Some of these exchangers, e.g., AE1, AE2, or AE3, and some members of the SLC26A family, e.g., pendrin, use the outwardly directed driving force of HCO_3^- or other anion in exchange of Cl^- resulting in net uptake of Cl^- ions and reduced intracellular pH. Other members of the SLC4A family, e.g., NDCBEs (Na^+-driven Cl^-/bicarbonate exchangers), extrude Cl^- from the cell in exchange for Na^+ and HCO_3^- in an electrogenic manner. Cl^- channels, here represented by CFTR (ABCC7), TMEM16A (ANO1), and Cl^- channels in general, dissipate the electrochemical gradient of Cl^- which is determined by the functional balance of Cl^- loaders and extruders expressed in the cell. Some of these Cl^- channels are activated by cAMP (CFTR) or

NCC is abundantly, but not exclusively, expressed in epithelial cells of the distal convoluted tubule where it is responsible for the reabsorption of 5–10 % of filtered Na^+ and Cl^- (Reilly and Ellison 2000). NCC is commonly known and labeled as the "thiazide-sensitive" Na^+Cl^- cotransporter (Arroyo et al. 2013), however, it is important to note that ~ 50 % of thiazide-sensitive Na^+Cl^- reabsorption by the collecting duct occurs in the absence of NCC (Leviel et al. 2010).

Functional Properties

The function of the CCCs has been characterized extensively in heterologous expression systems such as the *Xenopus laevis* oocyte (Gamba 2005). NKCC1, NKCC2, and NCC are involved in the electroneutral accumulation of Cl^- in cells using the energy stored in the combined Na^+/K^+ and Cl^- chemical gradients. Normally, NKCCs generate and maintain an outwardly directed Cl^- gradient responsible for a wide variety of cellular functions including cell volume regulation, GABA-mediated synaptic signaling, and transepithelial ion/water transport (reviewed in Alvarez-Leefmans (2012)).

Selectivity

In the classic definition, NKCC1 and NKCC2 are considered Na^+-dependent K^+ and Cl^- cotransporters. However, depending on species or splice variant, they do show different affinities for these ions (Gamba 2005). In general, NKCCs exhibit high selectivity for Cl^- and Br^-, but not for I^- or F^- (Russell 2000). This does not mean that NKCCs cannot transport I^-, but that they "prefer" Cl^- as the anion to be transported. This preference may change depending on the cell type and the concentration of other halides relative to Cl^-. NKCCs also efficiently cotransport NH_4^+ in place of K^+ (Kinne et al. 1986; Amlal et al. 1994; Wall and Fischer 2002; Worrell et al. 2008), a property frequently exploited experimentally to determine NKCC1 and NKCC2 activity in vitro (Bachmann et al. 2003; Zaarour et al. 2012).

Fig. 2 (continued) Ca^{2+} ions (TMEM16A), others by changes in cell volume (VRAC) or after binding certain agonists such as GABA (not displayed). Note that VRAC is not displayed because of its unknown molecular identity(ies). In the *center* of the figure, shown is a hypothetical β-cell where the predominant action of Cl^- loaders, e.g., NKCCs or AEs (represented by a "pump" using the ionic driving force established by the Na^+/K^+-ATPase), determines $[Cl^-]_i$ above electrochemical equilibrium and therefore makes possible Cl^- exit from the cell upon activation of any channel with the ability to funnel Cl^- ions, e.g., CFTR, TMEM16A, $GABA_A$-receptors, VRAC, or any other Cl^- channel. This concept is represented in the figure as a faucet. Electrogenic in nature, Cl^- exiting from the cell causes plasma membrane depolarization. (**b**) Expression analysis of representative members of the SLC12A, SLC4A, ANO, and ABC family of genes (CFTR and SUR1, as control) performed by reverse transcription coupled to the polymerase chain reaction using total RNA purified from MIN6 β-cells (kindly provided by Dr. Jun-Ichi Miyazaki (1990)). It is important to note that these and other members of those families of genes are also expressed in human pancreatic islets, as demonstrated in expression arrays performed by Mahdi et al. and publicly available GEO-profiles database under accession number GSE41762 (Mahdi et al. 2012) or in recently published ChIP sequencing and RNA sequencing analysis performed in β, non β and exocrine cells of the human pancreas (Bramswig et al. 2013)

Table 1 Members of the SLC12A, SLC4A and SLC26A family of genes

Gene Family	Gene	Common name	Tissue expression[a]	Ion subtrates
SLC12A	**SLC12A1**	NKCC2	Kidney	$1Na^+$, $1\ K^+$, $2Cl^-$
	SLC12A2	NKCC1	Ubiquitous	$1Na^+$, $1\ K^+$, $2Cl^-$
	SLC12A3	NCC	Kidney, placenta	Na^+, Cl^-
	SLC12A4	KCC1	Ubiquitous	K^+, Cl^-
	SLC12A5	KCC2	Brain	K^+, Cl^-
	SLC12A6	KCC3	Widely expressed	K^+, Cl^-
	SLC12A7	KCC4	Widely expressed	K^+, Cl^-
SLC4A	**SLC4A1**	AE1	Erythrocytes, heart, colon, intercalated cells	Cl^-, HCO_3^-
	SLC4A2	AE2	Widely expressed	Cl^-, HCO_3^-
	SLC4A3	AE3	Brain, testicle, heart, kidney, gastrointestinal tract	Cl^-, HCO_3^-
	SLC4A4	NBCe1	Widely expressed	$1Na^+$, $2HCO_3^-$, Na^+, CO_3^{-2}
	SLC4A5	NBCe2	Testes, liver, spleen	$1Na^+$, $3HCO_3^-$, Na^+, CO_3^{-2}
	SLC4A7	NBCn1	Skeletal muscle, brain, heart, kidney, liver, lung	Na^+, HCO_3^-
	SLC4A8	NDCBE	Brain, testis, amygdala, heart, caudate nucleus, frontal lobe, kidney, ovaries	Na^+, HCO_3^-, Cl^-, CO_3^{-2}
	SLC4A9	AE4	Kidney, testis, lung, placenta	Na^+, HCO_3^- (unresolved)
	SLC4A10	NBCn2	Cerebellum, lung, brain, hippocampus	Na^+, HCO_3^-
	SLC4A11	BTR1	Thalamus, kidney, salivary glands, thyroid	Not defined yet
SLC26A	**SLC26A1**	SAT1	Liver, kidney, intestine	SO_4^{2-}, Cl^-, oxalate, glyoxylate
	SLC26A2	DTDST	Brain, condrocytes, kidney, intestine, pancreas	SO_4^{2-}, Cl^-, Ox^-, HO^-, I^-, Br^-, NO_3^-
	SLC26A3	DRA	Colon, red cells, sperm, Epididymis	Cl^-, oxalate, HCO_3^-
	SLC26A4	Pendrin	Cochlea, thyroid, amygdala, mesangial, endothelial and type-B intercalated cells	Cl^-, I^-, HCO_3^-
	SLC26A5	Prestin	Cochlea, testis, brain	Cl^-, SO_4^{2-}, formate, oxalate
	SLC26A6	PAT1	Placenta, duodenum, kidney, pancreas, heart, sperm	Cl^-, SO_4^{2-}, formate, oxalate, HCO_3^-, HO^-, NO_3^-, SCN^-
	SLC26A7	SLC26A7	Testis, lung, endothelial gastric parietal and type-A intercalated cells	Cl^-, $HCO3^-$, SO_4^{2-}, oxalate, (NO_3^-, Br^-, Cl^-)-channel
	SLC26A8	TAT1	Kidney, male germ cells, lung	Cl^-, SO_4^{2-}, oxalate
	SLC26A9	SLC26A9	Kidney, male germ cells, lung, brain	Cl^-, HCO_3, Cl^--channel Na^+Cl^--transport
	SLC26A10	SLC26A10	Widely expressed	Unknown
	SLC26A11	SLC26A11	Endothelial and renal intercalated cells, pancreas, placenta, brain, thyroid, cervix	Cl^--channel?

[a]Tissue distribution is compiled here according to reported abundance and sources of primary cDNA clones which can be found in www.ncbi.nlm.nih.gov/gene/. Therefore, it should not be taken as definitive (see text for particular details related to gene expression/distribution).

An important functional difference between NKCC1 and NKCC2 is their capability to cotransport water. Indeed, NKCC1 is a robust water transporter (Hamann et al. 2010), whereas NKCC2 is considered a "dry" transporter due to its lack of water transport capacity (Zeuthen and Macaulay 2012). It is not clear whether NCCs are able to cotransport ions other than Na^+ and Cl^- (Monroy et al. 2000), or if they transport water.

Regulation

The regulatory mechanism involved in activation/inactivation of NKCCs and NCCs has been the subject of intense research (reviewed in Kahle et al. (2010)). In general, NKCCs and NCCs are directly and acutely regulated by phosphorylation cascades directly or indirectly initiated by several serine-threonine kinases of the WNK family (with no lysine = K) or Ste20-type kinases SPAK/OSR1, respectively. These kinases are activated by cell shrinkage brought about hypertonic stress and/or a decrease in $[Cl^-]_i$. Activation of these kinases via phosphorylation modulates the quality and quantity of specific phosphosites located mainly in the N-terminus of NKCCs and NCCs. In addition to phosphorylation cascades, the availability of NKCCs and NCCs in the plasma membrane appears to be regulated by incompletely defined post-translational mechanisms where complex N-glycosylation may play a role (Arroyo et al. 2013). When compared to the wealth of information related to the acute post-translational regulatory mechanisms involved in activation/deactivation of plasma membrane-located NKCCs or plasma membrane insertion of NKCCs and NCCs, the mechanisms involved in long-term genetic regulation of NKCCs and NCCs remain virtually undefined (Di Fulvio and Alvarez-Leefmans 2009).

Pharmacology

NKCCs and NCCs are the targets of different kinds of clinically relevant diuretics. NKCCs are potently inhibited by loop diuretics of the sulfamoyl family such as bumetanide and furosemide. On the other hand, the thiazide group of diuretics targets NCCs. These include chlorothiazide and hydrochlorothiazide. It is important to mention that these diuretics may be selective but not specific of a particular transporter. Indeed, in addition to being an effective inhibitor of NKCC1 and NKCC2 activities, bumetanide also targets other transporters of the SLC12A family, such as SLC12A4-7 (Reid et al. 2000), as well as non-transporter proteins (Yang et al. 2012). Thus, experimental data involving such pharmacological agents should be looked at with some caution. This may be particularly the case when considering bumetanide as a "diabetogenic drug" (Sandstrom 1988) (see section "Chloride Channels and Transporters in Diabetes").

Molecular Diversity

The molecular identities of $Na^+K^+2Cl^-$ and Na^+Cl^- cotransporters are not simple, and many different alternatively spliced variants of SLC12A1, SLC12A2, and SLC12A3 are known (reviewed in Di Fulvio and Alvarez-Leefmans (2009)).

Some have been characterized at the functional level, whereas others have unknown functional or pharmacological properties. In addition, the expression of more than one splice variant in a single cell and the inability to distinguish between them with inhibitors add an extra layer of complexity to the interpretation and molecular identification of particular transport systems.

Associated Human Diseases

Homozygous or compound heterozygous mutations of the human SLC12A1 and SLC12A3 genes cause Bartter's syndrome (antenatal type 1, omim.org/entry/601678) and Gitelman's syndrome (omim.org/entry/263800), respectively (Simon et al. 1996; Simon and Lifton 1998). Antenatal Bartter's syndrome type 2 is a rare and severe life-threatening condition characterized by hypokalemic alkalosis, hypercalciuria, hyperprostaglandinemia, and severe volume depletion. Gitelman's, on the other hand, is a relatively common and much less severe renal tubular disorder characterized by hypomagnesemia and hypocalciuria (Glaudemans et al. 2012). In relation to the SLC12A2, there are no human diseases associated with mutations in this gene.

Animal Models

Targeted truncation of the first 3.5 kb of the Slc12a1 gene in mice eliminates expression of all NKCC2 variants and results in severe volume depletion and phenotypic manifestations resembling Bartter's syndrome in humans. Mice lacking NKCC2 do not survive beyond the first 2 weeks of life (Takahashi et al. 2000). Interestingly, elimination of individual spliced variants of NKCC2, e.g., NKCC2A or NKCC2B, did not result in obvious phenotypic manifestations (Oppermann et al. 2006, 2007). Several animal models deficient in NKCC1 (NKCC1KO) have been generated by different strategies. A key phenotypic feature of these mice is deafness and imbalance due to inner-ear dysfunction, which occurs irrespective of the genetic strategy used to knockout Slc12a2 expression (Pace et al. 2000; Delpire et al. 1999; Flagella et al. 1999). Apart from additional common or unique manifestations observed in NKCC1KO mice (reviewed in Gagnon and Delpire (2013) and summarized in Table 2), recent evidence suggests that NKCC1KO mice have increased glucose tolerance and improved insulin secretory capacity when compared to wild type (Alshahrani and Di Fulvio 2012). Disruption of the Slc12a3 gene in mice only partially mimics the phenotypic features of Gitelman's syndrome (Schultheis et al. 1998; Yang et al. 2010), and this is probably due to activation of transporters which compensate for the lack of NCC (Soleimani 2013).

Chloride Extruders: SLC12A4, SLC12A5, SLC12A6, and SLC12A7 Proteins

Nomenclature

The branch of SLC12A gene family including SLC12A4, SLC12A5, SLC12A6, and SLC12A7 encodes the typical Cl^- extruders of the CCC family also commonly

Table 2 Genetically engineered animal models developed to study the physiological impact of Cftr, Ano1 or Slc12a transporters in vivo

Genetic alteration	Phenotypes[a]	Advantages/Disadvantages
Cftr^{tm1Unc} (FABP-hCFTR)	Partially recapitulates human CF, viable, no spontaneous diabetes	Transgene prolongs lifespan (carries hCFTR transgene)
Cftr^{tm1Kth} (global for ΔF508)	Partially recapitulates human CF, poor survival, no spontaneous diabetes	Models misfolding mutations
Cftr^{tm1Uth} (global for R117H)	Partially recapitulates human CF, viable, no spontaneous diabetes	Models partial activity mutants, high survival without FABP transgene
Cftr (pig global for Cftr)	Severe spontaneous lung infections, meconium ileus, exocrine pancreatic insufficiency, focal biliary cirrhosis	Recapitulates newborn human CF
Cftr (ferret global for Cftr)	Absence of vas deferens, lung infection, exocrine pancreas destruction, abnormal endocrine pancreas function	Recapitulates many of the human CF, complete post-natal morbidity/lethality
Ano1^{tm1Bdh} (global KO TMEM16A)	Aerophagia, impaired weight gain, cyanosis, tracheomalacia	Early post-natal lethal
Ano1$^{tm1.1Jwo}$ (floxed/frt TMEM16A)	Increased thermal nociceptive threshold, abnormal nociceptor morphology	Viable and fertile
Slc12a1^{tm1Tkh} (global KO NKCC2)	Growth retardation, severe dehydration, hypercalciuria, hydronephrosis, polyuria, nephrocalcinosis and kidney failure	Complete post-natal lethality
Slc12a1^{tm2Haca} (global KO NKCC2A)	Minimal kidney dysfunction	Viable, fertile, no gross abnormalities
Slc12a1^{tm1Haca} (global KO NKCC2B)	Urine hypoosmolarity, altered tubulo-glomerular feedback	Viable, fertile, no gross abnormalities
Slc12a2^{tm1Ges} ***Slc12a2^{tm1Bhk}*** ***Slc12a2^{tm2Bhk}*** ***Slc12a2^{tm1Dlp}*** (global KO NKCC1)	Decreased fat tissue, abnormal balance, deafness, circling, spinning, hypotension, coiled cecum, hyposalivation, post-natal growth retardation, high thermal nociceptive threshold, increased glucose tolerance and insulin secretion	Partial post-natal lethality, male infertility and reduced female fertility,
Slc12a3^{tm1Ges} (global KO NCC)	Hypotension, hypomagnesemia, reduced urinary calcium, chloride and sodium, abnormal morphology of the distal convoluted tubules	Viable, fertile, no gross abnormalities
Slc12a4^{tm1Cah} (global KO KCC1)	No phenotypic manifestations	Normal mice, viable and fertile
Slc12a5^{tm1Dlp} ***Slc12a5^{tm1Tjj}*** (global KO KCC2)	Severe motor deficits, prone to seizures, growth retardation, abnormal interneuron morphology, akinesia, abnormal nociception, atelectasis	Complete post-natal lethality
Slc12a6^{tm1Dlp} (global KO KCC3)	Impaired coordination, paraparesis, demyelination, axon degeneration	Infertility
Slc12a7^{tm1Tjj} (global KO KCC4)	Deafness, renal tubular acidosis	No obvious defects in vision or motor function, grossly normal and fertile

[a]The phenotypic manifestations compiled here are neither exhaustive nor complete.

known as K^+Cl^- cotransporters (KCCs) KCC1, KCC2, KCC3, and KCC4 (reviewed in Adragna et al. (2004a)).

Functional Properties

KCCs play important roles in cell volume regulation and in the maintenance of $[Cl^-]_i$ below electrochemical equilibrium. They actively extrude Cl^- from cells driven by the product of the K^+ and Cl^- gradients (Adragna et al. 2004a). Although KCCs are typical efflux transporters under most physiological conditions, KCCs can also operate in the "wrong" direction (i.e., mediate K^+ and Cl^- influx) if the chemical gradients for these ions dictate (Payne 1997).

Selectivity

In general, K^+ and Cl^- ions transported by KCCs can be replaced by other ions of similar size and charge, e.g., NH_4^+ or Br^-, SCN^-, I^-, NO_3^-, and $MeSO_4^-$, respectively (reviewed in Gibson et al. (2009)).

Regulation

Except for KCC2, and possibly KCC3, the functionality of KCC1 and KCC4 requires an increase in cell volume using a hypotonic challenge, in order to detect transport activity. This property, in particular, for KCC1 when coupled to its wide distribution in tissues and cells makes this CCC an excellent candidate for the regulation of cell volume and $[Cl^-]_i$. The product of the Slc12a5, KCC2, has long been considered a neuron-specific cotransporter. However, KCC2 is not only minimally expressed or absent in nociceptive neurons (Mao et al. 2012), but it is expressed at the mRNA or protein levels in vascular smooth muscle cells (Di Fulvio et al. 2001), testis (Uvarov et al. 2007), osteoblasts (Brauer et al. 2003), endometrial cells (Wei et al. 2011), cardiac myocytes (Antrobus et al. 2012), lens cells (Lauf et al. 2012), and pancreatic islets (Taneera et al. 2012). At the functional level, KCC2 is considered the prototypical neuronal Cl^- extruder, which makes possible the hyperpolarizing (inhibitory) effect of GABA in mature neurons of the central nervous system (Kahle et al. 2008; Blaesse et al. 2009). In fact, when compared to other KCCs, only KCC2 is clearly functional under basal isotonic conditions. The fifth CCC, i.e., KCC3, plays a key role in K^+Cl^- homeostasis, cell volume regulation, and electrical responses to GABA and glycine. In fact, KCC2 and KCC3 are both considered part of the regulatory machinery involved in $[Cl^-]_i$ regulation in neurons (Blaesse et al. 2009). However, unlike KCC2, KCC3 has a clear impact in cell volume regulation when over-expressed in cell lines or oocytes in spite of the fact that both cotransporters appear constitutively active under normotonic physiological conditions (Uvarov et al. 2007; Antrobus et al. 2012; Race et al. 1999). Therefore, in the case of neurons or any cell type where KCC2 and KCC3 were co-expressed, these cotransporters may play coordinated but distinctive regulatory roles in $[Cl^-]_i$ homeostasis or cell volume regulation under physiological conditions. When compared to the other KCC members, much less is known regarding KCC4, the product of the SLC4A7 gene. Interestingly, KCC4 is not a constitutively active transporter under normotonic conditions, when it is heterologously expressed. KCC4 could be ubiquitously expressed, but a systematic

search has not been done (see Gene Expression Omnibus Database, www.ncbi.nlm.nih.gov/geoprofiles/4697431). A key functional characteristic of KCC4 is its strong activation when exposed to hypotonic solutions (Mercado et al. 2000).

Pharmacology

Loop diuretics inhibit all KCCs, but at higher concentrations than those required to inhibit NKCCs (Reid et al. 2000; Jean-Xavier et al. 2006). At least two non-diuretic drugs inhibit KCCs at low doses, i.e., low μM range in some cells. These are 5-isothiocyanate-2-[2-(4-isothiocyanato-2-sulfophenyl) ethenyl] benzene-1-sulfonic acid (DIDS) (Delpire and Lauf 1992) and dihydroindenyl-oxyacetic acid (DIOA) (Fujii et al. 2007). Recently, new highly selective and specific inhibitors of KCC2 and KCC3 have been developed (Delpire et al. 2009). Most notably, a new highly selective agonist of KCC2 has been developed (Gagnon et al. 2013).

Molecular Diversity

Multiple splice variants of KCC1, KCC2, KCC3, and KCC4 are found in many tissues, and all of them are considered part of the general machinery responsible for cell volume regulation (Adragna et al. 2004a). However, our knowledge of the functional properties of most of their splice variants, as well as their relative contribution to the total functional KCC pool in cells, is very limited (reviewed in Gagnon and Di Fulvio (2013)).

Associated Diseases in Humans

Although there are no human diseases associated with mutations in the SLC12A4 (KCC1), SLC12A5 (KCC2), or SLC12A7 (KCC4), mutations in the SLC12A6 (KCC3) gene are associated with Andermann's syndrome also known as Charlevoix disease or sensorimotor polyneuropathy with or without agenesis of corpus callosum (Dupre et al. 2003).

Animal Models

Mice lacking functional Slc12a4, Slc12a5, Slc12a6, and Slc12a7 have been generated and characterized (reviewed in Gagnon and Delpire (2013) and summarized in Table 2). Interestingly, ablation of KCC1, a ubiquitous transporter involved in cell volume regulation in all cells, does not result in obvious phenotypic manifestations. This suggests that KCC1 is dispensable for cell volume regulation (Boettger et al. 2003; Byun and Delpire 2007; Rust et al. 2007). Nevertheless, caution should be taken before drawing the conclusion that KCC1 is not involved in cell volume regulation, as its dispensability does not exclude the role. In fact, it actually tells us that the function of this transporter may be replaceable by other KCCs once KCC1 is absent. In this respect, mice deficient in KCC3 result in functional impairment of multiple organs and systems (Gagnon and Delpire 2013) and show phenotypic manifestations reflecting volume depletion such as hypertension and increased water consumption coupled to increased $[Cl^-]_i$ and shrinkage in neurons isolated from these mice (Boettger et al. 2003; Adragna et al. 2004b). Mice with targeted

disruption of the Slc12a6 gene exhibit several characteristics observed in Andermann's syndrome (Howard et al. 2002).

Absence of KCC2 is fatal for mice; they die after birth due to severe motor deficits and respiratory failure (Hubner et al. 2001). Elimination of one KCC2 variant, i.e., KCC2b, bypasses early lethality likely due to expression of KCC2a, a variant commanded by an alternative distal promoter in the Slc12a5 gene (Uvarov et al. 2007). However, absence of KCC2b results in pups prone to tonic or clonic seizures leading to their deaths before weaning (Woo et al. 2002). Further studies using neurons lacking KCC2b confirmed that this variant mediates the developmental decrease in $[Cl^-]_i$ observed in mature neurons, a key component in inhibitory GABA-ergic synaptic signaling (Zhu et al. 2005). Disruption of KCC4 in mice results in hearing loss and renal tubular acidosis (Boettger et al. 2002).

Chloride Channels: A Synopsis of Some of Them

> "*Not long ago, Cl^- channels were the Rodney Dangerfield of the ion channel field. Rodney Dangerfield (1921–2004) was a comedian who became famous for his joke*": "*I get no respect. I played hide-and-seek, and they wouldn't even look for me*." (Duran et al. 2010)

Anion channels are widely distributed and ubiquitously expressed. In general, anion channels form a structurally heterogeneous group of proteins with a common functional characteristic: the formation of a transmembrane-conductive pathway for anions. These channels have been classified as follows: (i) ligand-gated channels, such as GABA and glycine receptors that open after binding of an extracellular ligand, i.e., GABA and glycine, respectively; (ii) voltage-gated Cl^- channels (CLC); (iii) the volume-regulated anion channels (VRACs); (iv) Ca^{2+}-activated Cl^- channels (CaCCs); and (v) the phosphorylation-regulated cystic fibrosis (CF) transmembrane conductance regulator (CFTR) channel. The next part of this chapter will focus on three classes of these channels: VRAC, CaCCs, and CFTR.

Volume-Regulated Anion Channels (VRAC)

The original concept of cell-swelling-activated Cl^- channels came in the early 1960s as a result of electrophysiological studies performed on intact frog skin (Macrobbie and Ussing 1961). The hypothesis was further elaborated in the 1980s by volume regulation experiments on Ehrlich ascites tumor cells (Hoffmann and Simonsen 1989) and human lymphocytes (Grinstein et al. 1984). These experiments demonstrated that Cl^- channels play an important role in anion efflux during the process of regulatory volume decrease (RVD), whereby the cell regulates its volume in response to cell swelling (Hoffmann et al. 2007). A variety of putative volume-regulated anion channels with different electrophysiological properties were subsequently identified using single-channel patch-clamp methods (Hudson and Schultz 1988; Kunzelmann et al. 1992). These somewhat inconsistent observations, however, were soon superseded by whole-cell experiments, which identified currents carried by channels, now widely recognized as VRAC (Solc and Wine 1991; Worrell et al. 1989). It is slightly unwise to state that a protein is ubiquitously

expressed unless very many tissues have been screened, but these channels have now been identified in a vast variety of mammalian cells. They are even expressed in cells in which they are not the principal pathways for Cl^- efflux during RVD, e.g., lacrimal gland acinar cells (Majid et al. 2001).

Nomenclature

Many names have been assigned, e.g., "volume-activated," "volume-regulated," and "volume-expansion-sensing," each name representing the fundamental property that their activation depends on an increase in cell volume (Nilius and Droogmans 2003; Okada 1997). They are also frequently referred to as anion channels rather than Cl^- channels, because a well-documented property of these channels is that they are permeable to a range of anions rather than just Cl^- (Strange et al. 1996). A lack of discrimination between anions is, however, thought to be a biophysical limitation of all anion channels and transporters (Wright and Diamond 1977). A final term frequently employed to describe these channels is "outward rectifier" (Okada 1997), as this refers to the ability of the channel to permit the passage of more positive current than negative current. The term, however, causes confusion, particularly among students, as it is misleading in two ways: (i) a true rectifier permits current flow in only one direction, while these channels do pass current in both, and (ii) "outward" refers to a positive current caused by the efflux of cations from the cell. For anions, however, their influx causes a positive current.

Functional Properties

While several reports showing minor variations between channels in different cell types have been published, the major functional properties of VRAC are fairly well defined. However, precise details of many of the channel attributes have been difficult to establish not only because the molecular identity of the channel remains elusive but also because VRAC might be the result of multiple molecular identities working in concert. What follows is a brief overview of the most widely accepted properties of VRAC. For more detailed information, please consult previous reviews (Nilius and Droogmans 2003; Okada 1997; Strange et al. 1996; Eggermont et al. 2001).

Selectivity

As stated above outward-rectifying current–voltage relationship permits more anion influx than efflux. However, it is important to note that VRAC still permits significant and measureable anion efflux, particularly for Cl^-. Indeed, the outwardly directed electrochemical gradient of Cl^- in most cells favors its efflux, not its influx. The permeability (P) sequence of VRAC to halides is $I^- > Br^- > Cl^- > F^-$ (Arreola et al. 1995; Rasola et al. 1992). This is referred to as Eisenman's sequence I (Wright and Diamond 1977). VRAC is also permeable to a range of larger anions, e.g., HCO_3^- ($P_{Bicarbonate}$: $P_{Cl} = 0.48$) (Rasola et al. 1992) and acetate ($P_{Acetate}$: $P_{Cl} = 0.47$) (Arreola et al. 1995), and in some cells (although not all), VRAC permits the efflux of larger organic osmolytes, e.g., taurine, glycine, or myoinositol (Roy and Banderali 1994; Kirk et al. 1992).

Regulation

The precise mechanism by which VRAC is activated by changes in cell volume is not understood. There are significant bodies of evidence, however, to suggest the involvement of tyrosine kinases and rho kinases in VRAC activation (these data are summarized in the excellent review of Eggermont and collaborators (2001)). Swelling-induced VRAC activation depends on the availability of intracellular ATP, but in most cells ATP hydrolysis is not required (Nilius and Droogmans 2003; Okada 1997; Strange et al. 1996; Eggermont et al. 2001). In chromaffin cells, VRAC is activated by GTP-γ-S, probably due to G protein activation, in absence of cell swelling (Doroshenko and Neher 1992).

Pharmacology

VRAC is blocked by classic inhibitors of anion channels such as DIDS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonate (SITS), 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), or 9-anthracenecarboxylic acid (9AC). All of these compounds are nonspecific inhibitors, as they also block other Cl^- channels and many anion transporters (Nilius and Droogmans 2003; Macrobbie and Ussing 1961). In addition to these drugs, substrates of p-glycoprotein, e.g., 1,9-dideoxyforskolin and tamoxifen, also block VRAC (Macrobbie and Ussing 1961). It is important to mention a paper by Hélix et al. where a new group of acidic diaryl ureas (not currently available) was synthesized and tested on Cl^- conductance in human erythrocytes. Of those, NS3728 was the most potent VRAC blocker, with an $IC_{50} = 0.40$ μM (Helix et al. 2003).

Molecular Identity

The identification of VRAC is based on the anion conductance under hypoosmotic challenge, outwardly directed current rectification and sensitivity to classic anion channel inhibitors. However, the molecular identity of the VRAC has remained elusive for over 20 years. Several candidate proteins have been proposed over this period, and these include p-glycoprotein, pICln, ClC-2, and ClC-3. However, as has been reviewed extensively elsewhere (Nilius and Droogmans 2003; Okada 1997; Strange et al. 1996; Doroshenko and Neher 1992), the claim for each of these "pretenders" has proved ill founded. Work that is more recent has suggested that proteins from the bestrophin family of Cl^- channels may contribute to VRAC (Fischmeister and Hartzell 2005), but there is little conclusive evidence in support of this hypothesis (Chien and Hartzell 2008). Similarly, TMEM16 proteins may also contribute to VRAC channels (Almaca et al. 2009). This viewpoint, however, is also not widely supported (Shimizu et al. 2013).

Ca^{2+}-Activated Cl^- Channels

Calcium-activated chloride channels (CaCCs) were initially described in the early 1980s by Miledi (1982) and Barish (1983) using *Xenopus* oocytes. It is now clear that they are broadly expressed proteins, which play multiple functions by mediating Ca^{2+}-dependent Cl^- secretion in glands and flat epithelia and by modifying cellular responses to appropriate stimuli (Duran et al. 2010; Kunzelmann

et al. 2011). They play important roles in cell physiology, including epithelial secretion of electrolytes and water, sensory transduction, regulation of neuronal and cardiac excitability, regulation of vascular tone, and maintaining $[Cl^-]_i$ by dissipating the intracellular Cl^- gradient generated by Cl^- transporters (Alvarez-Leefmans 2012).

Nomenclature

While several proteins have been proposed to be responsible for classical CaCC currents, as described in oocytes and acinar cells, the recently identified anoctamin family, also known as ANO or TMEM16, displays characteristics most similar to those expected for the classical CaCCs. "Anoctamin" was the term coined, because of their ANion selectivity and the existence of eight (OCT) transmembrane domains (Yang et al. 2008). Ten members of this family have been identified so far (ANO1-10 or TMEM16A–K), which are thought to play a role during tissue development because of their differential temporal and spatial expression. As reported by Schreiber et al (2010) TMEM16A, F, G, I, J, and K are expressed in a variety of epithelial tissues, while TMEM16B–E are more constrained to neuronal and musculoskeletal tissues. The only two channels in this family that have been shown conclusively to be CaCCs are TMEM16A and B. Some of the different names given to TMEM16A are related to its overexpression in different cancers, and they include TAOS2, ORAOV2, and DOG-1. In this review, we will concentrate on TMEM16A and TMEM16B.

Functional Properties

At the electrophysiological level, CaCCs have been studied for more than 30 years (Hartzell et al. 2009). CaCC currents recorded in whole-cell configuration have very similar properties in many different cell types, including *Xenopus* oocytes, various secretory epithelial cells, hepatocytes, gut smooth muscle cells, and pulmonary artery endothelial cells, among others. In general, these currents exhibit (i) Ca^{2+} and voltage sensitivity, (ii) slow activation by depolarization, (iii) linear instantaneous current–voltage relationship, (iv) outwardly rectifying steady-state current–voltage relationship, (v) higher permeability to I^- than Cl^-, and (vi) incomplete sensitivity to DIDS (100–500 μM), NPPB (100 μM), and NFA (100 μM) (Hartzell et al. 2005). Although whole-cell $I_{Cl.Ca}$ seem quite similar in different tissues, there is considerable diversity in the properties of single CaCCs. There appear to be at least four types of CaCCs by conductance in different cell types (Hartzell et al. 2009). Whether this diversity of single-channel conductance truly reflects the variety of single channels that underlie the typical macroscopic $I_{Cl.Ca}$ remains debatable, because rarely have investigators carefully linked single-channel measurements with macroscopic currents.

Selectivity

Selectivity for various ions, which is a key feature of all channels, differs enormously between ion channels. For instance, voltage-gated cation channels are highly selective for one ion. Therefore, voltage-gated K^+ channels select for K^+

over Na^+ by a factor of > 100 to 1 (Hille 2001). This high selectivity for K^+ ions is due to the presence of a binding site in the channel pore for ions the size of K^+ (Doyle et al. 1998). With these channels, the geometry of the protein and the binding site for ions is crucial for selectivity. On the other hand, most Cl^- channels including CaCCs are relatively nonselective (Jentsch 2002) which in the case of CaCC translates to selecting only ~ 10-fold between ions that differ in radius by ~ 1.5 °A versus 0.5 °A.

Regulation

The Ca^{2+} that activates CaCCs can come from either Ca^{2+} influx or Ca^{2+} release from intracellular stores. In certain tissues, it has been documented that specific types of Ca^{2+} channels are coupled to CaCCs, including the following: (i) rat dorsal root ganglion (DRG) neurons, where CaCCs are activated by both Ca^{2+} influx and Ca^{2+}-induced Ca^{2+} release from internal stores (Ivanenko et al. 1993; Kenyon and Goff 1998; Ayar et al. 1999). (ii) In mouse sympathetic neurons, there appears to be a selective coupling of different kinds of voltage-activated Ca^{2+} channels (VACCs), to Ca^{2+}-activated Cl^- and K^+ channels: Ca^{2+} entering through L- and P-type channels activates CaCCs, whereas Ca^{2+} entering through N-type channels activates Ca^{2+}-activated K^+ channels (Martinez-Pinna et al. 2000). Ca^{2+} can activate CaCCs by direct binding to the channel protein or indirectly, via Ca^{2+}-binding proteins. The distinction between these two mechanisms results from the observation that many CaCCs can be stably activated in excised patches by Ca^{2+} in the absence of ATP (Koumi et al. 1994; Kuruma and Hartzell 2000; Gomez-Hernandez et al. 1997), suggesting that in some preparations, activation does not require phosphorylation. In other tissues, however, channel activity runs down quickly after excision, suggesting the possibility that intracellular components, in addition to Ca^{2+}, are required to open the channel (Nilius et al. 1997; Reisert et al. 2003; Klockner 1993).

Precise details on the mechanism(s) of direct Ca^{2+} gating remain the subject of speculation, because the molecular architecture of the TMEM16 proteins has still to be fully determined. Evidence supporting direct gating of CaCCs by Ca^{2+} has been obtained using inside-out patches isolated from hepatocytes and from *Xenopus* oocytes exposed to increasing Ca^{2+} on the cytosolic side of the excised patch. Application of Ca^{2+} to an excised patch activates both single channels and macroscopic currents even in the absence of any ATP required for phosphorylation. The quick activation of CaCCs by rapid application of Ca^{2+} to excised patches (Kuruma and Hartzell 2000), or by photo-releasing Ca^{2+} in acinar cells isolated from pancreas and parotid glands (Park et al. 2001) is also consistent with the hypothesis that CaCCs are directly gated by Ca^{2+} ions.

Pharmacology

Specific blockers are indispensable for identifying ion channels physiologically and for isolating specific currents from a mixture of currents. Blockers are also valuable tools for resolving the structure of the pore, analyzing tissue distribution, or for the affinity purification of channel proteins. Unfortunately, few specific potent anion

channel blockers are available, and even fewer exist for CaCCs. Most of them require high concentrations to completely block Cl^- currents and may have undesirable side effects. The features of the available Cl^- channel blockers have been discussed in detail in several reviews (Hartzell et al. 2005; Jentsch 2002; Eggermont 2004). The most common blockers for native CaCCs are niflumic acid (NFA) and flufenamic acid (White and Aylwin 1990). These drugs block CaCCs overexpressed in *Xenopus* oocytes at concentrations in the 10 μM range (Hartzell et al. 2005). NFA is often considered a specific blocker and has been used to identify anion currents as CaCCs in different tissues. However, NFA is far from being a perfect tool to isolate CaCCs, because in addition to its blocking effect, NFA also enhances $I_{Cl.Ca}$ in smooth muscle at negative voltages. Other commonly used Cl^- channel blockers include tamoxifen, DIDS, SITS, NPPB, A9C, and DPC. However, these drugs are even less effective than the flufenamates on CaCCs (Frings et al. 2000). Larger blocking molecules are less voltage-dependent, suggesting that they lodge at sites less deep in the channel. DPC and DIDS block at a site about 30 % into the voltage field, whereas NFA appears to block at the external mouth of the channel.

Molecular Identity

The molecular identity of CaCCs was elusive for more than 30 years (Huang et al. 2012). A flurry of activity and excitement in the field of CaCCs was generated in 2008 with the almost simultaneous publication of three papers reporting that the "transmembrane protein with unknown function 16A," i.e., TMEM16A, is a bona fide CaCC (Yang et al. 2008; Schroeder et al. 2008; Caputo et al. 2008). These publications have elicited much interest in the membrane biology field; as it turns out the functional expression of TMEM16A in heterologous systems yielded a conductance that for the first time showed the classical characteristics of the CaCCs, e.g., anion-selective channels activated by increases in $[Ca^{2+}]_i$ within the range of 0.25 μM (Galietta 2009). The accepted in silico-predicted structure of TMEM16A consists of eight transmembrane domains, with cytosolic N- and C-termini. TMEM16A exists as different protein variants generated by alternative splicing, all of them with associated CaCC activity, although with different functional properties. When compared to TMEM16A, higher $[Ca^{2+}]_i$ are required to activate TMEM16B (anoctamin-2) although the latter has faster activation and deactivation kinetics than the former (Scudieri et al. 2012).

Associated Human Diseases

Although to date, no mutations in ANO1 or ANO2 genes have been identified as causing human disease, it is important to keep in mind that several cancers show overexpression of TMEM16A and that it may be a useful and sensitive diagnostic biomarker and prognostic tool (Duran and Hartzell 2011).

Animal Models

TMEM16A and TMEM16B are expressed in many tissues. The only available mouse model (Table 2) suggests or proposes different roles for these channels, including the following: (i) a secondary Cl^- channel role in airway epithelia

because of the presence of CFTR, (ii) a role in gut motility and tracheal development, (iii) as a mediator of nociceptive signals triggered by bradykinin, and (iv) as a contributor in photoreceptor function (Duran and Hartzell 2011). We have a long way to go to clearly understand the role of these two components of the TMEM16 family in human and rodent tissues. Interestingly, the tracheas of both null mice, the Tmem16a and the cftr, revealed similar congenital defects in cartilage that may reflect a common Cl^- secretory defect mediated by the expression of these two different Cl^- channels (Rock et al. 2009).

CFTR

We have already invoked CFTR here and there, as if it were a silent spectator or a modulator of other channels or transporters. However, CFTR per se functions as a transepithelial anion channel providing a pathway for Cl^-, gluconate, and HCO_3^- transport (Lubamba et al. 2012). Cystic fibrosis (CF) is a disease of deficient epithelial anion transport, resulting from genetic mutations that cause a loss of function of the cystic fibrosis transmembrane conductance regulator (CFTR). Recently, it has been reported that CFTR is not only expressed in secretory epithelia but also in rat pancreatic β-cells (Fig. 2b) (Boom et al. 2007) (Di Fulvio and Aguilar-Bryan, unpublished rodent and human data).

Functional Properties

Identification of the CFTR gene as a member of the ATP-binding cassette superfamily of proteins (ABCC7) and subsequent functional studies confirmed CFTR as the affected gene in CF disease and its protein product as an epithelial Cl^- channel. Transfection of functional wild-type CFTR into cultured CF respiratory and digestive epithelial cells corrected the chloride transport defect. Conclusive evidence was brought by the demonstration that insertion of wild-type CFTR into artificial bilayer membranes generates chloride channels with individual properties of CFTR-associated conductance. These properties are: (i) increase in channel open probability by cAMP-dependent phosphorylation and intracellular nucleotides, (ii) the anion permeability selectivity which is $Br^- > Cl^- > I^- > F^-$, (iii) the current–voltage relationship which is linear, and (iv) single-channel conductance in the 6–11 pS range (Riordan 2008; Quinton 2007; Davis 2006). Anion flow through this channel is needed for normal function of epithelia such as those that line airways and the intestinal tract and ducts in the pancreas, testes, and sweat glands. Without anion flow, water movement slows and dehydrated mucus clogs the ducts, explaining the multiorgan pathology of CF.

As suggested by its name, cystic fibrosis transmembrane regulator, CFTR, interacts with different proteins including a variety of ion channels such as the epithelial Na^+ channel (ENaC), VRACs, CaCCs, and transporters of the SLC26A family (Alper and Sharma 2013; Kunzelmann 2001). Although highly expressed in lung, gut, and exocrine pancreas, CFTR has been reported in many other tissues. Expression of CFTR reduces $I_{Cl.Ca}$. Airway epithelial cells from CF patients and CF mouse models have an increased $I_{Cl.Ca}$ (Hartzell et al. 2005; Perez-Cornejo and Arreola 2004).

Regulation

CFTR is atypical, both as an ion channel and as an ABC protein, having adopted the basic ABC transporter structural architecture to generate a ligand-gated channel whose level of activity is quantitatively controlled by the phosphorylation state of its unique regulatory (R) domain. Although CFTR has been known to function as an apical epithelial Cl^- channel, dysregulated transport of Na^+ is an additional, well-described phenomenon proposed to play a major role in CF lung disease. Accordingly, stimulation of CFTR by cAMP agonists inhibits the amiloride-sensitive epithelial Na^+ channel, ENaC (Zhou et al. 2008), and this activity is increased in CF respiratory epithelia (Knowles et al. 1981). An additional role for CFTR has been assigned in the regulation of the outwardly rectifying Cl^- channel (ORCC) that can only be activated by PKA and ATP when CFTR is functionally intact (Jovov et al. 1995). As mentioned, CFTR may functionally control many other ion channels, including CaCCs, which involves the interaction of the C-terminal part of the CFTRs R-domain with CaCCs (Kunzelmann 2001), renal outer medullar K^+ (ROMK) channels, the Na^+/H^+ exchanger-3 (NHE3), and aquaporins (Kunzelmann 2001; Stutts et al. 1995). The best-known modulator of the CFTR activity is intracellular cAMP. In addition, it has been shown that cGMP-dependent protein kinases might be involved in phosphorylation and activation of CFTR in the intestine. CFTR can regulate other transporters including Cl^-/HCO_3^- exchange in pancreatic tissue (Lee et al. 1999). In other respiratory epithelia, CFTR and ENaC are inversely regulated (Donaldson and Boucher 2007). Evidence from molecular, functional, and pharmacological experiments also indicates that CFTR inhibits TMEM16A functionality by mechanisms likely involving direct interaction between both channels (Kunzelmann et al. 2011).

Pharmacology

Two main inhibitors of CFTR Cl^- conductance have been widely used, both of which are nonspecific and have low efficacy. The original class of CFTR inhibitors includes the thiazolidinone CFTRinh-172 and the hydrazide GlyH-101. The former has been widely used in CF research to investigate the involvement of CFTR in cellular processes. Patch-clamp and site-directed mutagenesis studies indicate that CFTRinh-172 stabilizes the CFTR channel closed state by binding at or near arginine-347 on the CFTR cytoplasmic surface. The IC_{50} for inhibition of CFTR Cl^- current by CFTRinh-172 is ~ 300 nM. By using the patch-clamp technique, the use of glycine hydrazide GlyH-101 showed an altered CFTR current–voltage relationship that changed from linear to inwardly-rectifying. These findings, together with additional biophysical data, suggested an external pore-blocking inhibition mechanism. The IC_{50} for channel blockage is of ~ 8 μM. More recently the use of new chemical chaperons or corrector/potentiator has extended the "therapeutic" pharmacology by addressing the underlying defects in the cellular processing and Cl^- channel function. Correctors are principally targeted at correcting cellular misprocessing of the most common human mutant of CFTR, i.e., ΔF508, whereas potentiators are intended to restore

cAMP-dependent chloride channel activity of all mutants of CFTR at the cell surface (Rowe and Verkman 2013; Verkman et al. 2013).

Molecular Identity

As mentioned, CFTR is a member of the ATP-binding cassette (ABC) superfamily of membrane proteins involved in the transport of a wide variety of substrates across membranes. In the case of CFTR, however, open/close gating allows transmembrane flow of anions down their electrochemical gradient. The canonical model contains two sets of transmembrane domains, with typically six membrane-spanning α-helices each and two cytoplasmic nucleotide-binding folds (NBDs) and, in the case of CFTR, an important regulatory (R) domain with several consensus sites for PKA-mediated phosphorylation needed for successful transmission of NBD events to the channel gate. The complete family comprises 48 ABC proteins, and CFTR is the only one that functions as an ion channel. It is also thought that each CFTR channel appears to be built from one CFTR polypeptide (Zhang et al. 2005; Dean and Allikmets 2001; Bear et al. 1992; Riordan et al. 1989).

Associated Human Diseases

Cystic fibrosis is caused by loss-of-function mutations in the CF transmembrane conductance regulator protein, expressed mainly at the apical plasma membrane of secretory epithelia. CF is the most common fatal recessive disease among Caucasians and is characterized by substantial clinical heterogeneity. Nearly 2,000 mutations in the CFTR gene have been identified as cause of the disease by impairing CFTR translation, cellular processing, and/or Cl^- channel gating. Although present in almost every ethnic group, the incidence among them is thought to vary significantly (Quinton 2007). The resultant disease affects all exocrine epithelia, consistent with the idea that CFTR acts as a node within a network of signaling proteins. CFTR not only is a regulator of multiple transport proteins and controlled by numerous kinases but also participates in many signaling pathways that are disrupted after expression of its commonest mutation in Caucasians, ΔF508 (Drumm et al. 2012).

Animal Models

Because numerous conditional and knockout animal models (mouse, piglet, and ferret) have been generated in the past two decades (Keiser and Engelhardt 2011; Wilke et al. 2011), in this report, we briefly mention the most representative ones in Table 2.

The Link Between Cl^- Transport and Insulin Secretion

In the previous sections of this chapter, we have discussed Cl^- transport in a generic way. In the remainder of this article, we will review the possible role that Cl^- has in modulating the activity of pancreatic β-cells.

The earliest evidence for the non-equilibrium distribution of Cl^- ions in pancreatic islet cells and its potential modulation of Em was published almost exactly 35 years ago. In 1978 Sehlin demonstrated that $^{36}Cl^-$ is actively accumulated against its electrochemical gradient in isolated rat β-cells and that Cl^- exiting from these cells contributes to the depolarizing effect of glucose (Sehlin 1978). The precise mechanisms involved were not understood at the time, mainly because there was still much confusion in the literature about the diversity of the CCC family. This confusion was only fully resolved after the molecular identification of the members of the SLC12A family of proteins in the 1990s.

Further evidence for Cl^- modulation of insulin secretion in response to glucose was soon provided by experiments performed by Orci, Malaisse, and others (Orci and Malaisse 1980; Somers et al. 1980). In these experiments, incubation of rat islets with isethionate, a commonly used Cl^- ion substitute, resulted in a fast, reversible, and dose-dependent inhibition of insulin release in response to insulinotropic glucose (16.7 mM) or α-ketoisocaproate, but not at very low or non-stimulatory concentrations of glucose (5.5 mM). Similar results were obtained when $[Cl^-]_o$ was decreased from the physiological 123 mM to less than 38 mM (Somers et al. 1980), or when β-cells were treated with the loop-diuretic furosemide a known inhibitor of Cl^- transporters of the SLC12A family (Sehlin 1981). These initial observations uncovered an intriguing parallelism between Cl^- fluxes and insulin secretion.

Next, to gain insights into new possible mechanism regulating insulin secretion, were Tamagawa and Henquin (1983) who tested the ability of Cl^- to modulate the action of secretagogues or potentiators in the secretory response, including theophylline, tolbutamide, glyceraldehyde, and the amino acids leucine, lysine, and arginine. In agreement with previous work (Somers et al. 1980), replacement of Cl^- with increasing concentrations of isethionate or methylsulfate inhibited insulin secretion in response to high glucose by a mechanism dependent on Ca^{2+} influx. Manipulation of Cl^- concentrations did not, however, affect insulin secretion in response to all secretagogues or potentiators, suggesting that $[Cl^-]_i$ may impact stimulus-secretion coupling differentially according to the agonist used (Tamagawa and Henquin 1983). Although the underlying anionic mechanisms involved in insulin secretion were not pin-pointed at that time, the common observation that changes in $[Cl^-]_i$ regulate cell volume and pH led to the proposition that insulin secretion could be influenced by changes in β-cell osmolarity or pH. This was subsequently supported by several studies from Sehlin's laboratory using pancreatic islets from *ob*/*ob* mice. A few years later, Lindstrom et al. (1986) demonstrated that K^+ and Cl^- fluxes regulate β-cell volume, whereas Sandstrom and Sehlin (1987) established for the first time that β-cells contain a loop diuretic (bumetanide)-sensitive mechanism for $^{36}Cl^-$ uptake (Lindstrom et al. 1986; Sandstrom and Sehlin 1987).

These previous findings elicited a series of experiments to test the hypothesis that loop diuretics, the classic inhibitors of NKCCs, may impair insulin secretion. In a series of three papers, Sandstrom and Sehlin reported that (i) a single dose of

furosemide results in transient hyperglycemia, (ii) furosemide produces short- and long-term glucose intolerance, and (iii) the inhibitory effect of furosemide on insulin secretion may be secondary to the inhibition of Cl^- and Ca^{2+} fluxes (Sandstrom and Sehlin 1988a, b, c). These results coupled with the finding that β-cells express a furosemide-sensitive cotransport system for Na^+, K^+, and Cl^- (Lindstrom et al. 1988) supported the idea that active accumulation of Cl^- in β-cells does impact on insulin secretion. To close the loop, in 1988, Sehlin and Meissner tested this hypothesis using mouse pancreatic β-cells and demonstrated that manipulation of $[Cl^-]_i$ impacts voltage-gated Ca^{2+} channels (Sehlin and Meissner 1988), establishing for the first time a link between glucose, electrogenic Cl^- fluxes, and insulin secretion.

Electrophysiology of Cl^- Transport in Pancreatic β-Cells

The recognition that Cl^- contributes to the electrophysiology of β-cells dates from the late 1970s when Sehlin (1978) stated: "*Because it appears from the data of this study that β-cells have an inwardly-directed, active transport of* Cl^-, *an increase of anion permeability by sugars may, by analogy with GABA-action in nerve cells, participate in depolarization of β-cells through a net efflux of* Cl^- *toward its equilibrium potential*" (Sehlin 1978). The excitement surrounding the discovery of the K_{ATP} channel soon after this was written, however, meant that this "Cl^- hypothesis" was not tested directly using electrophysiological methods for another 15 years.

VRAC in β-Cells

In 1992, Len Best somewhat serendipitously developed a keen interest in the potential roles of anion channels in β-cell regulation. Best and his colleagues were examining the importance of glycolysis on β-cell function, when they observed changes in membrane potential, intracellular $[Ca^{2+}]$, and insulin secretion upon the addition of lactate to experimental solutions (Best et al. 1992; Lynch et al. 1991). These effects were rapid and observed with both D-lactate and L-lactate, suggesting that metabolism was not required. One potential explanation for these data was that lactate modulates the β-cell membrane potential by electrogenic transport via an ion channel.

The first electrophysiological evidence for the presence of a Cl^- channel in β-cells, a VRAC, was published in two papers which appeared only a few weeks apart in December 1995 (Kinard and Satin 1995) and January 1996 (Best et al. 1996a). Both papers were based on experiments performed using two different β-cell lines, i.e., HIT-T15 and RINm5F cells, but they were soon complemented by data obtained from β-cells isolated from rat pancreas (Best et al. 1996b). The β-cell VRAC exhibited many characteristics in common with VRAC expressed in other

tissues, i.e., activation by cell-swelling, outward-rectifying current–voltage relationships, and inhibition by DIDS or NPPB (Kinard and Satin 1995; Best et al. 1996a, b). Further studies showed that the channel was also blocked by extracellular ATP, 1, 9-dideoxyforskolin, and 4-OH tamoxifen (Best et al. 2001; Best 2002a).

Channel activity was dependent on the presence of intracellular ATP and supported by other nucleotides such as GTP (Kinard and Satin 1995; Best et al. 1996a; Miley et al. 1999). Activity was also supported by ATP-γ-S (Miley et al. 1999), indicating that nucleotide hydrolysis was not required for channel activity, as it is also the case for VRAC in other cell types (Okada 1997). However, the β-cell channel appeared to be different from that in most other cell types, at least based on halide selectivity because the channel was more permeable to Cl^- in comparison to I^- (Kinard and Satin 1995; Best et al. 1996a). This is in marked contrast to all other cells in which the channel is most permeable to I^- (reviewed in Okada (1997)). Further characterization demonstrated a finite permeability to lactate ($P_{Lactate}$: $P_{Cl} = 0.38$) and to other organic anions (Best et al. 2001). In the initial characterization, Best also reported a significant Na^+ permeability (P_{Na}: $P_{Cl} = 0.32$; (Best et al. 1996a)). This value, however, may reflect a small contribution to the whole-cell current from cation-selective TRP channels, some of which we now know are also expressed in β-cells (Colsoul et al. 2011; Islam 2011; Uchida and Tominaga 2011; Takii et al. 2006; Cao et al. 2012). In summary, the VRAC of pancreatic β-cells appears to have at least one unique biophysical property which distinguishes it from VRACs in other cells (see section "Volume-Regulated Anion Channels (VRAC)"). The lack of the molecular identity for VRAC in any cell, however, means that it still is not known whether the VRAC in β-cells has a unique molecular identity.

Hypotonic Solutions Stimulate Insulin Secretion by Activating VRAC: An "Exciting" Phenomenon!

In 1975 Blackard and collaborators had demonstrated that insulin secretion is stimulated when β-cells are exposed to hypotonic media, but the mechanism underlying this phenomenon was not investigated (Blackard et al. 1975). A decade later similar observations were made in chromaffin cells of the adrenal medulla, where hypotonic solutions caused epinephrine secretion (Doroshenko and Neher 1992; Wakade et al. 1986). The authors of this study fully investigated the mechanisms involved. They found that the response was independent of metabolism or receptor antagonism, but it was blocked by reducing $[Ca^{2+}]_o$ or by using DIDS (Doroshenko and Neher 1992). As a result it was hypothesized that the activation of VRAC in the chromaffin cells evoked a depolarization of Em, which was sufficient to open voltage-gated Ca^{2+} channels. The resultant increase in $[Ca^{2+}]_i$ could then trigger exocytosis of vesicles containing epinephrine.

In subsequent studies in pancreatic islet cells, it was observed that exposure of mouse β-cells to hypotonic media caused a transient depolarization of Em resulting in enhanced electrical activity (Best et al. 1996a; Britsch et al. 1994). Best et al. described a similar phenomenon in rat β-cells and also showed that the depolarization of Em was inhibited by DIDS. Furthermore, insulin secretion from the rat cells in response to hypotonic solutions was also inhibited by DIDS (Best et al. 1996a). These data suggested that, as in chromaffin cells, the activation of VRAC is responsible for the depolarization of Em. This leads to an increase in $[Ca^{2+}]_i$ via the activation of voltage-gated Ca^{2+} channels and the consequent triggering of insulin secretion.

The role of VRAC in these events was further supported by precisely parallel time courses of plasma membrane depolarization and the changes in insulin secretion. These changes were both biphasic, so that an initial peak is then followed by a decline towards control values over the next 5–10 min. The dynamic time courses probably occur because the β-cells have the capacity to regulate their volume. Using video-imaging methods (Best et al. 1996b) it was demonstrated that β-cells exhibit an RVD in response to hypotonically induced cell swelling; thus after rapid swelling to a maximum volume due to osmotic water influx, β-cells recover their volume towards control over the next 5–10 min. This RVD is similar to that described in most other cells, where it is due to ion efflux either via K^+ and Cl^- channels and/or K^+Cl^- cotransporters (Hoffmann and Simonsen 1989). The RVD in β-cells was inhibited by either VRAC inhibitors or by Ca^{2+}-activated K^+ channel blockers (Best et al. 1996a; Sheader et al. 2001), suggesting a contribution of these channels to ion loss during RVD. This conclusion was also supported by earlier $^{86}Rb^+$ flux studies, which showed that K^+ ($^{86}Rb^+$) efflux was transiently increased by hypotonic swelling (Sandstrom and Sehlin 1988c; Engstrom et al. 1991). Thus, VRAC activity (and the resultant depolarization of Em) can be expected to be maximal at the peak of cell swelling and then decline as cell volume recovers towards the basal level.

Further evidence for a central role of VRAC in hypotonicity-induced insulin secretion was provided by experiments in which $[Ca^{2+}]_i$ in cells of the RINm5F line was measured with Fura-2. Figure 3a shows that exposing the cells to a hypotonic solution caused a biphasic increase in $[Ca^{2+}]_i$, with a time course similar to that for RVD, the hypotonicity-induced depolarization and hypotonicity-induced insulin secretion. The hypotonicity-induced increase in $[Ca^{2+}]_i$ was greatly attenuated by the addition of the Cl^- channel inhibitor DIDS (Fig. 3b). The increase in $[Ca^{2+}]_i$ was also inhibited by the removal of extracellular Ca^{2+} or by the addition of the L-type Ca^{2+} channel inhibitor D600 (Sheader et al. 2001). These data therefore support the hypothesis that the increase in $[Ca^{2+}]_i$ is caused by Ca^{2+} entry via L-type channels which open in response to VRAC-mediated depolarization of Em. This idea is further reinforced by the data presented in Fig. 3c, which shows that the L-type channels (activated using increased KCl to depolarize Em experimentally) are not blocked by the addition of DIDS.

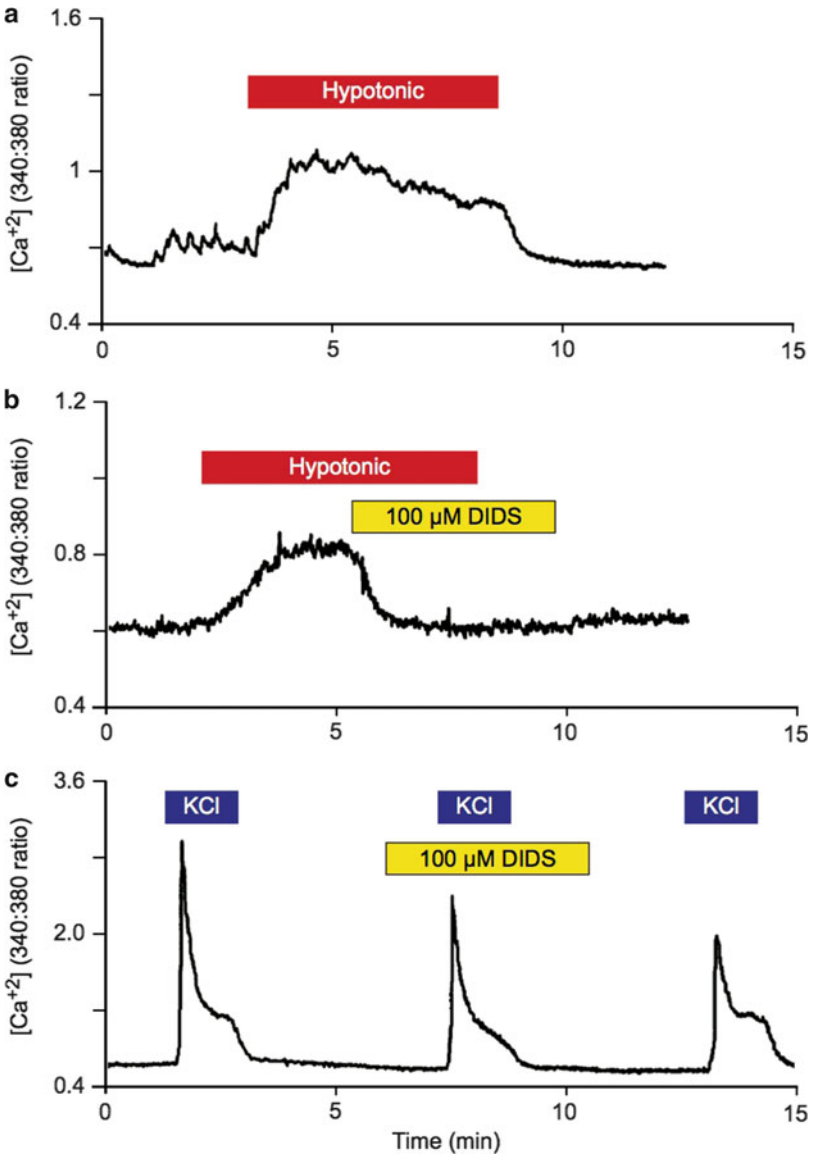

Fig. 3 Elevation of $[Ca^{2+}]_i$ in pancreatic β-cells by osmotically induced swelling. $[Ca^{2+}]_i$ was measured using the fluorescent indicator Fura-2. Results are presented as the ratio of light emitted at 500 nm in response to excitation at 340 and 380 nm (340:380 ratio). (**a**) Superfusion of cells with hypotonic solution causes a reversible increase in $[Ca^{2+}]_i$. (**b**) The hypotonic-induced increase in $[Ca^{2+}]_i$ is inhibited by DIDS (the classic Cl^- channel/VRAC inhibitor). (**c**) DIDS does not inhibit Ca^{2+} entry promoted by voltage (KCl)-gated Ca^{2+} channels (Figure adapted from Sheader et al. (2001))

Nutrient-Induced VRAC Activation: A Physiological Mechanism?

The effects of extracellular hypotonic solutions on β-cell activity can only be regarded as experimental phenomenon, because β-cells in vivo almost certainly never encounter large changes in extracellular osmolality. These data are very significant in that they clearly demonstrate that the opening of Cl^- channels results in depolarization of Em. Further experiments in which cells were exposed to hypertonic solutions which inhibited insulin secretion in the presence of stimulatory concentrations of glucose, however, went on to indicate that VRAC may indeed have a physiological role. In experiments examining the effects of Cl^- substitution, it had been observed that increasing extracellular osmolarity, by sucrose addition, inhibited glucose-stimulated insulin secretion (Orci and Malaisse 1980; Somers et al. 1980). In microelectrode studies, Britsch and collaborators (1994) demonstrated that glucose-induced electrical activity was also inhibited transiently by hyperosmotic solutions, so that maximum inhibition was observed almost immediately (60 s) on exposure with a gradual recovery of activity over the subsequent 10 min (Britsch et al. 1994).

These biphasic changes in electrical activity precisely mirror the changes observed in β-cell volume when exposed to hypertonic solutions (Miley et al. 1998), i.e., an initial and rapid cell shrinkage is followed by a more gradual recovery of cell volume (regulatory volume increase; RVI) over the following 10 min. This RVI occurs because of the activation of ion transporters, which mediate the influx of Na^+ and Cl^- (NKCCs, AEs, and NHEs) creating an osmotic gradient so water reenters the cell (Miley et al. 1998). The similarity between the time courses of volume change and electrical activity suggest that glucose-induced electrical activity is maximally inhibited when cell volume is at a minimum, and then as volume recovers so does electrical and secretory activity. These data therefore suggest that VRAC may make significant contributions to Em at stimulatory glucose concentrations.

In 1997, Helen Miley (from Len Best's group) published a key electrophysiological study of nutrient-induced electrical activity (Miley et al. 1997). Using the amphotericin-perforated patch method to gain electrical access to isolated β-cells, she showed that membrane depolarization caused by glucose was associated with a simultaneous increase in cell volume. Furthermore the glucose-induced depolarization of Em was associated with the generation of small negative (or inward) currents. The same negative currents were also observed in cells swollen by exposure to hypotonic solutions, and the glucose-induced or swelling-induced currents were inhibited by the Cl^- channel blocker DIDS (Best 1997). Subsequent experiments have demonstrated that both the glucose- and hypotonicity-induced currents were also blocked by 4OH-tamoxifen, a more specific VRAC inhibitor (Best 2002b). These data suggest that an anion channel, probably VRAC, was activated by increasing glucose concentrations.

In support of this hypothesis, glucose increased β-cell Cl^- permeability as assessed using fluorimetric methods (Eberhardson et al. 2000). Measuring the rates of efflux of labeled taurine and D-aspartate, which permeate VRAC, also indicated channel activation by glucose (Chan et al. 2002), although others were unable to confirm these findings (Jijakli et al. 2006). A more recent report has provided evidence that the long-established phenomenon of the "phosphate flush," i.e., an increase in phosphate efflux observed during the stimulation of islets with glucose, may also be attributable to VRAC activation (Louchami et al. 2007).

Chloride Transporter Expression in β-Cells

The Intracellular Concentration of Cl^- Determines β-Cell Excitability

As discussed in sections "Intracellular Chloride Concentration and Cell Membrane Potential" and "An Overview of Cl^--Transporting Proteins," virtually all cells tightly regulate $[Cl^-]_i$ (Alvarez-Leefmans 2012), and β-cells are certainly not the exception. VRAC activation in β-cells creates a depolarizing current. This requires $[Cl^-]_i$ above electrochemical equilibrium, i.e., E_{Cl} must be positive with respect to the membrane potential. The seminal study of Sehlin (1978), based on distribution of $^{36}Cl^-$ in *ob*/*ob* mouse islets, suggested that this is indeed the case providing estimates for E_{Cl} of between, −18 and +2.5 mV (Sehlin 1978). Fluorimetric measurements and electrophysiological approaches established that $[Cl^-]_i$ is at least three times above thermodynamic equilibrium in β-cells, a finding consistent with the active accumulation of Cl^- in these cells (Eberhardson et al. 2000; Kozak and Logothetis 1997). This section discusses the evidence that Cl^- accumulators are expressed and have essential roles in β-cells.

Best (2005) determined the influence of $[Cl^-]_i$ on the electrical activity of rat β-cells in an elegant series of electrophysiological experiments. In these experiments, the current-clamp recordings of Em, interspersed with whole-cell currents, were measured at voltage clamp potential of −65 mV, and recorded at $[Cl^-]_i$ of 6 mM (Fig. 4a) or 80 mM (Fig. 4b). In the presence of 6 mM Cl^-, increasing the extracellular glucose concentration from 4 to 16 mM produced a slight hyperpolarization of Em and a positive shift in the whole-cell current at Em = −65 mV. At 80 mM Cl^-, by contrast, glucose depolarized Em which was associated with a significant enhancement of the negative whole-cell current. The relationship between whole-cell currents (ΔI_G: pA) measured on glucose stimulation and $[Cl^-]_i$ over a range from 6 to 120 mM is shown in Fig. 4c. The glucose-induced currents were negative (depolarizing) at all $[Cl^-]_i$ except at 6 mM, because E_{Cl} is positive of Em = −65 mV except at 6 mM. Thus, the activation of Cl^- channels will induce depolarizing negative currents at all $[Cl^-]_i$ except 6 mM. These data showed clearly that $[Cl^-]_i$ has a key role in determining the electrical excitability of the β-cell.

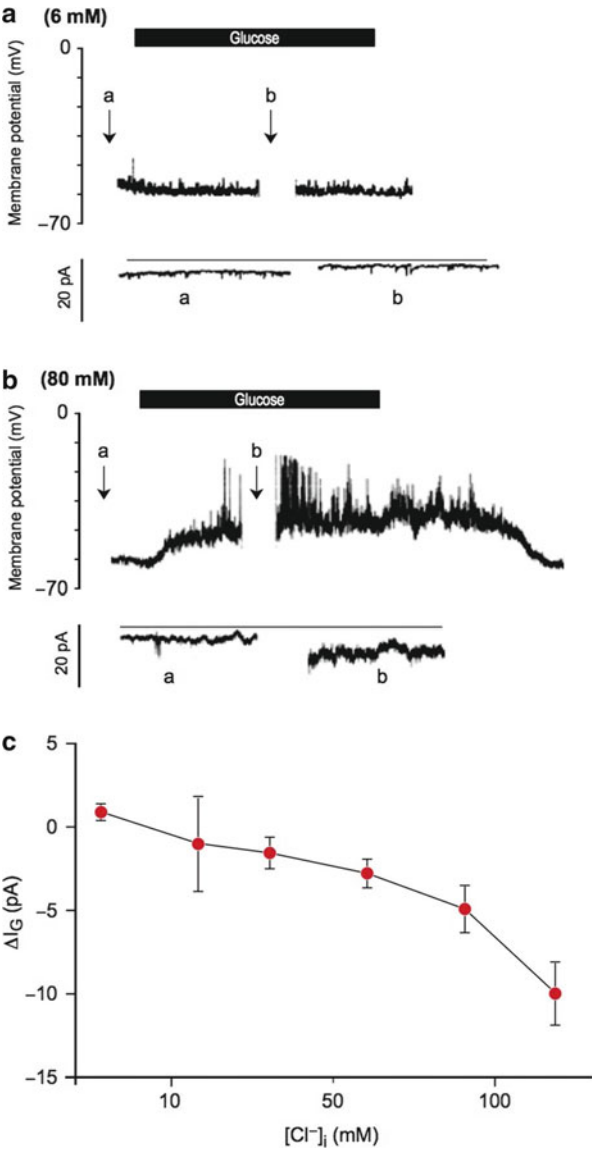

Fig. 4 Glucose-induced electrical activity in rat pancreatic β-cells as a function of $[Cl^-]_i$. Shown are representative experiments performed using amphotericin to gain electrical access to the cell. Amphotericin forms membrane pores, which are permeable to both monovalent cations and anions; thus, the final $[Cl^-]_i$ is "clamped" by that in the pipette solution. (**a**, **b**) Em measured in at 4 and 20 mM glucose (bars) by current clamp with a concentration of Cl^- in the pipette of 6 mM (**a**) or 80 mM (**b**). Each trace is interrupted by short periods of voltage clamp (*a*, *b*) to measure changes in glucose-induced current (ΔI_G). (**c**) ΔI_G plotted in log scale as a function of $[Cl^-]_i$ (Figure adapted from Best (2005))

SLC12A Expression in Pancreatic Islet Cells

The quest to correlate functional data supporting the existence of anion-dependent mechanisms involved in insulin secretion to specific transporters and channels began in 2001 when Majid and collaborators (2001) correlated bumetanide-sensitive β-cell volume regulation and NKCC1 expression in β- but not in α-cells, where no evidence of NKCC1 transcripts could be found Majid et al. (2001).

These results were consistent with the notion that β-cells actively accumulate Cl^- through NKCCs (Sandstrom 1990) and also supported Rorsman's observation that glucagon-secreting α-cells exhibit $[Cl^-]_i$ below electrochemical equilibrium as calculated from the reversal potential for GABA-mediated currents (Rorsman et al. 1989).

The link between cell volume regulation, NKCC1 expression, and insulin secretion was further supported by results obtained in gene expression experiments performed in rat or human pancreatic islets, establishing the expression of all KCC isoforms at the mRNA, protein, or functional levels (Taneera et al. 2012; Davies et al. 2004). However, in spite of the fact that KCCs are expressed in β-cells, their contribution to volume homeostasis was minimal (Davies et al. 2004), probably reflecting low expression levels. The finding that β-cells co-express NKCC1 and NKCC2 (Alshahrani et al. 2012), both involved in $[Cl^-]_i$ upload (Gamba 2005), further supported the notion that these cotransporters may impact $[Cl^-]_i$ in these cells. Clearly, if $[Cl^-]_i$ is kept above thermodynamic equilibrium in β-cells (Eberhardson et al. 2000; Kozak and Logothetis 1997), Cl^- uptake mechanisms prevail over KCC-mediated Cl^- extrusion, a concept that may also apply to human islets. Indeed, as it is the case of rodent β-cells and islets (see Alshahrani et al. (2012) and Fig. 2b), NKCC1, NKCC2, and KCCs are also co-expressed in human islets (Di Fulvio and Aguilar-Bryan, unpublished data).

Functional Evidence of the Importance of NKCC Activity in Pancreatic β-Cell Function

The experiments illustrated in Fig. 5 explored the importance of NKCC activity in determining $[Cl^-]_i$ in β-cells, by determining the effect of bumetanide (the inhibitor of NKCC1 and NKCC2) on glucose-induced β-cell activity. These experiments exploited important differences in the properties of the pore-forming antibiotics gramicidin and amphotericin. Gramicidin produces pores which are predominantly permeable to cations; thus in recordings made using this method, $[Cl^-]_i$ is still determined by the endogenous physiological Cl^- regulatory mechanisms expressed in the β-cell (Kyrozis and Reichling 1995; Rhee et al. 1994). In the gramicidin experiments, bumetanide (10 μM) almost completely abolished the normal glucose-induced depolarization. These data strongly suggest that NKCCs significantly contribute to $[Cl^-]_i$ in β-cells. Note that bumetanide had no effect on glucose-induced electrical activity when amphotericin was used to gain electrical access (Fig. 5b). In these experiments the Cl^- gradient imposed from the pipette solution and the lack of inhibition by bumetanide argues against the possibility of any nonspecific actions of the drug, such as a direct inhibition of VRAC. The involvement of NKCCs in insulin secretion is further supported by recent data showing that bumetanide at concentrations known to inhibit NKCC1 and NKCC2 inhibits insulin secretion from INS-1E β-cells at all concentrations of glucose tested (Alshahrani and Di Fulvio 2012).

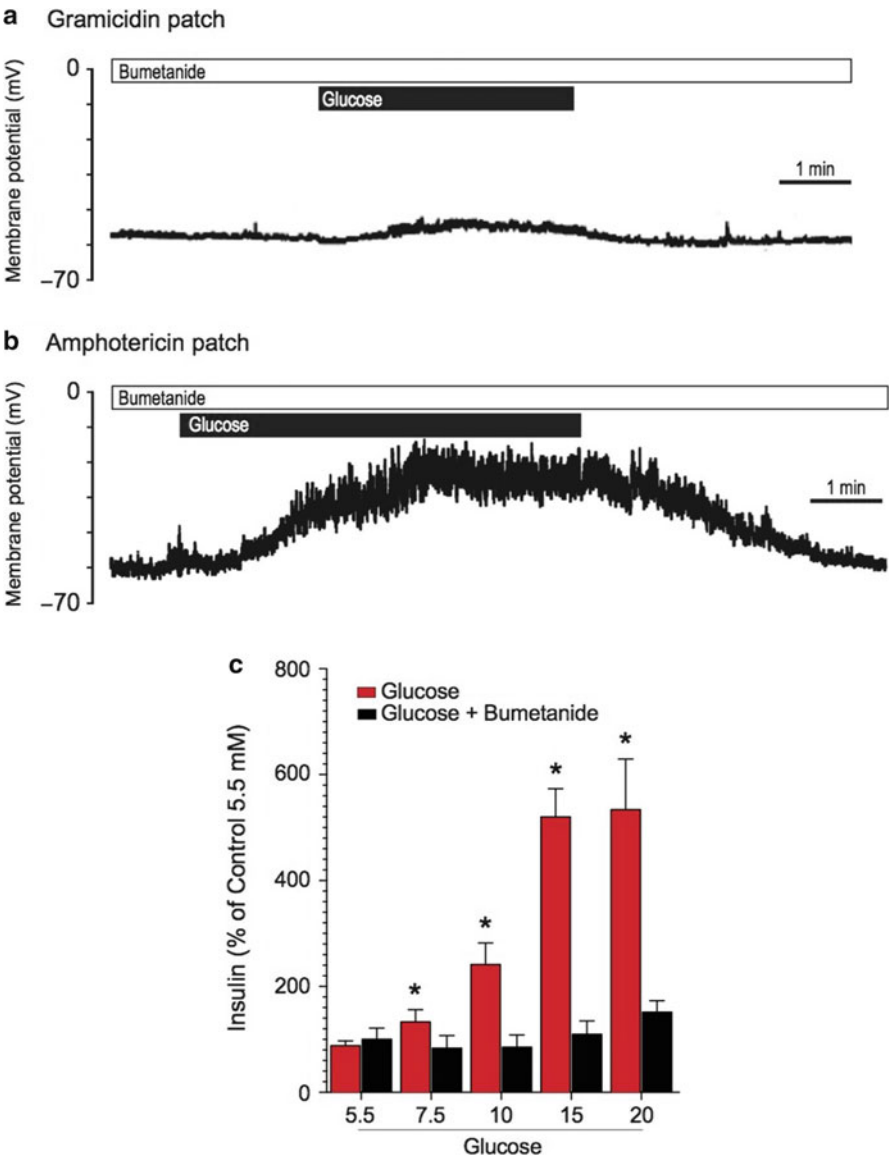

Fig. 5 Glucose-induced electrical activity and insulin secretion are attenuated by bumetanide. (**a**, **b**) Glucose-induced (16 mM; bar) electrical activity measured in the presence of 10 μM bumetanide. Experiments are perforated-patch, current-clamp recordings in which electrical access was gained using gramicidin (**a**) or amphotericin (**b**). (**c**) Insulin secretion from INS-1E β-cells in response to the indicated range of glucose expressed as percentage increase from basal secretion (5.5 mM glucose) in the absence or presence of bumetanide (10 μM) (Panels **a** and **b** adapted from Best (2005); panel **c** from Alshahrani and Di Fulvio (2012))

The VRAC Hypothesis and the *"Popular"* Consensus Model

> "*. . .This popularity has two drawbacks: the [consensus] model tends to become dogmatic or exclusive, and its limitations are no longer lucidly perceived.*" (Henquin et al. 2009)

In spite of the wealth of evidence accumulated in support of the role of Cl^- in β-cell physiology over the last four decades, the consensus K_{ATP} model of insulin secretion has gained and retained immense popularity over the same period. In this chapter, we have highlighted the VRAC-mediated Cl^- current induced by glucose stimulation. This current, which despite of its small magnitude, does appear to play a significant role in insulin secretion. The existence of this current should not be seen as challenging the consensus K_{ATP} model; instead, as a supplementary mechanism which complements the role of the K_{ATP} channels.

This additional mechanism requires glucose metabolism and is directly linked to volume-sensitive anion channels (Best et al. 2010; Miley et al. 1997; Jakab et al. 2006) of unknown molecular identity. Mechanistically, it may be schematized as follows: glucose metabolism induces osmotic water entry, transient β-cell swelling, and activation of VRAC, which are responsible for the efflux of Cl^- and probably other anions (Best et al. 2010; McGlasson et al. 2011). Naturally, electrogenic Cl^- efflux modulates membrane depolarization in response to glucose and therefore stimulates insulin secretion (Best et al. 2010; Drews et al. 2010). The efflux of Cl^- in response to glucose in β-cells is possible because $[Cl^-]_i$ is higher than that expected for a passive distribution of Cl^- across the cell membrane (Sehlin 1978; Britsch et al. 1994; Eberhardson et al. 2000; Kozak and Logothetis 1997; Henquin and Meissner 1982b; Meissner and Preissler 1980; Beauwens et al. 2006). Bumetanide-sensitive Cl^- cotransporters, i.e., NKCCs, appear to be largely responsible for maintaining this nonpassive distribution of Cl^- in β-cells (Alshahrani et al. 2012; Alshahrani and Di Fulvio 2012; Majid et al. 2001; Sandstrom and Sehlin 1988c; Lindstrom et al. 1988; Miley et al. 1998; Eberhardson et al. 2000; Sandstrom 1990; Sandstrom and Sehlin 1993; Best 2005). Figure 6 embodies a unified concept of an integrated, yet still incomplete, consensus model of insulin secretion.

It is important to remember that the mechanism whereby glucose activates VRAC or the molecular identity(ies) of the channels involved in Cl^- efflux remains unresolved. There is, however, abundant evidence of a role for VRAC in insulin secretion. Glucose has been shown to cause β-cell swelling in a concentration-dependent manner both during acute exposure (Miley et al. 1997; Semino et al. 1990) and in longer-term culture conditions (Chan et al. 2002). Swelling and VRAC activation also have an almost identical time course when measured simultaneously in a single cell (Miley et al. 1997). Both are also dependent on the metabolism of the sugar (Miley et al. 1997; Best 2002b). It is therefore conceivable that VRAC activation is the result of the intracellular accumulation of glucose metabolites, which leads to β-cell swelling. It remains to be established which metabolite(s) might be involved in this mechanism. However, an interesting feature of β-cells is that while

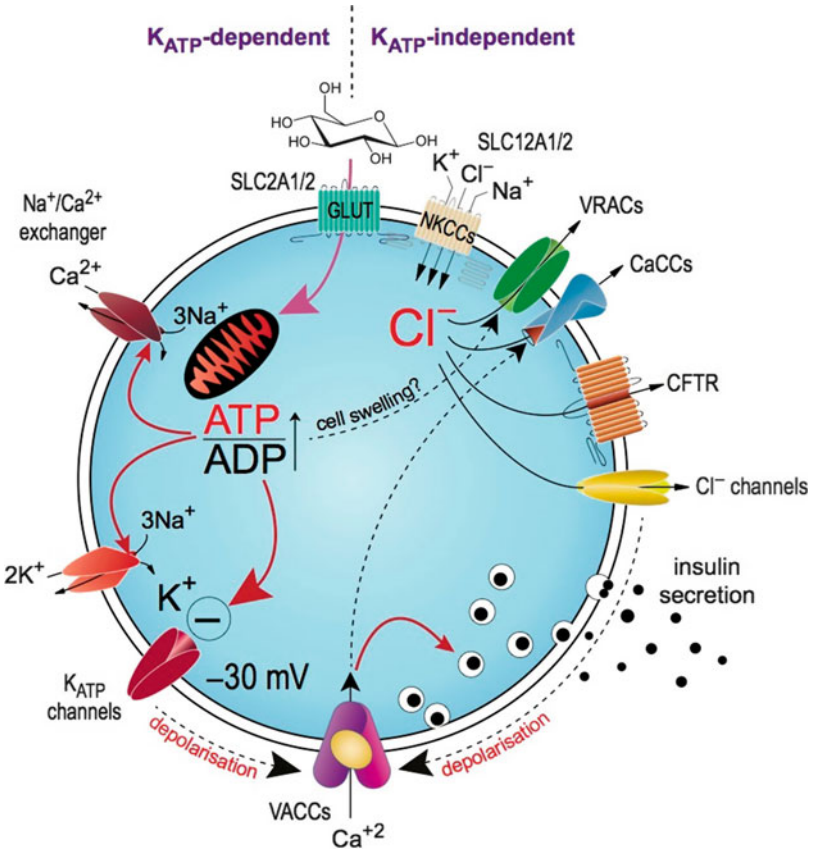

Fig. 6 An integrated view of the consensus model of insulin secretion. Shown are two main routes whereby plasma membrane depolarization and insulin secretion may occur. One is the well-known and widely accepted K_{ATP}-dependent pathway where glucose increases the ATP/ADP ratio, closing K_{ATP} channels, provoking rapid plasma membrane depolarization and increased Ca^{2+} influx via voltage-activated Ca^{2+} channels (*VACC*), and then ensuing insulin secretion. The other route, which by no means excludes the former, depicts an alternate K_{ATP}-independent pathway. In this road, Cl^- is maintained in β-cells at higher concentrations than the ones predicted by the Nernst equation due to the predominant functional presence of NKCCs and potentially other Cl^- loaders (see Fig. 2). Upon glucose entry, glucose is metabolized, not only increasing the ATP/ADP ratio but also producing osmotically active metabolites which in turn provoke cell swelling and activation of volume-regulated anion channels (*VRAC*) with subsequent exit of Cl^- from the cell. Further, increase in Ca^{2+} influx may stimulate Ca^{2+}-dependent Cl^- channels, also favoring additional Cl^- exiting from the cell. Hence, the electrogenic nature of Cl^- outflow contributes to plasma membrane depolarization and insulin secretion in response to glucose and other nutrients

glycolysis generates considerable amounts of lactate from glucose (Sener and Malaisse 1976; Best et al. 1989), activity and expression of the lactate (monocarboxylate) transporter MCT are low or absent (Zhao et al. 2001). This could conceivably lead to intracellular accumulation of lactate during glucose stimulation.

Another possible explanation is that the metabolism of glucose stimulates ion influx in the β-cells. AEs and NHEs are both activated by glucose metabolism in response to changes in intracellular pH (Lynch et al. 1989; Shepherd et al. 1996; Shepherd and Henquin 1995). Both of these transporter systems are also known to contribute to volume regulation in β-cells (Miley et al. 1998). Future research is necessary to determine which specific isoforms of these transporters are expressed in β-cells and to what extent they contribute to glucose-induced swelling.

Clearly, as additional Cl^- transporters and channels are identified and characterized in β-cells, confirmation of their role in insulin secretion will enrich our understanding of this very complex and fundamental signaling pathway. Braun et al. (2010) reported for the first time that $GABA_A$ activation by its ligand depolarizes isolated human β-cells firing action potentials and stimulating insulin secretion (Braun et al. 2010), pointing towards the idea that Cl^- may have different ways to leave the cell. These latter findings are in striking analogy with those results relating GABA-mediated synaptic depolarization and $[Cl^-]_i$ above equilibrium in immature or specific sensory neurons, a very well-defined subject (Alvarez-Leefmans and Delpire 2009) from which we could learn.

Chloride Channels and Transporters in Diabetes

Remarkably, diabetes mellitus has emerged as the most common comorbidity in cystic fibrosis and is considered a clinical entity (cystic fibrosis-related diabetes, CFRD) distinct from that of type 1 diabetes (T1DM) and type 2 diabetes (T2DM). The relevance of this diagnosis extends from not only its imposition of additional medical burden but also its association with worse health outcomes in individuals with CF. CFRD occurs most commonly in the setting of severe CF mutations associated with exocrine pancreatic insufficiency and has been considered as an insulin-insufficient state, although ketoacidosis is uncommon. Delayed and blunted insulin and C-peptide secretion typify the oral glucose tolerance tests in CF patients, even in the absence of CFRD. Abnormalities are more pronounced with worsening glycemic status (Kelly and Moran 2013). Intravenous challenges to glucose and other stimulatory agents reveal impaired first-phase insulin and C-peptide secretion, as observed in T2DM. Basal insulin secretion is generally at least partially preserved. Unlike T1DM, β-cell damage in CF does not appear to be of autoimmune origin (Gottlieb et al. 2012). Instead, according to the traditional "collateral damage" model of CFRD, abnormal Cl^- channel function results in thick viscous secretions that give rise to obstructive damage to the exocrine pancreas. Progressive fibrosis and fatty infiltration ensue and destroy islet architecture. Immunohistochemical studies of islets from patients with CFRD identified significantly reduced percentage of insulin-producing cells within islets when compared to islets of non-CFRD patients and controls (Abdul-Karim et al. 1986; Soejima and Landing 1986; Iannucci et al. 1984). This β-cell-specific destruction characterizes T1DM. In contrast, decreased glucagon secretion has been found in response to OGTT and various other stimuli in subjects with CF and worsening

glucose tolerance (Lang et al. 1997), suggesting islet cell destruction is not cell selective and is linked to exocrine pancreas fibrosis. Perhaps even more importantly, despite their limitations as retrospective studies, these postmortem findings highlighted the variability in β-cell mass and its lack of correlation with the diagnosis of CFRD. As the CF population ages, the normal decline in β-cell function that occurs with aging may allow underlying β-cell abnormalities to become more prominent. This normal decline coupled with compromised insulin secretory capacity may then give rise to diabetes and in some cases may obscure the distinction between T2DM and CFRD.

On the other hand, bumetanide, the prototypical inhibitor of NKCCs, has been considered a diabetogenic drug for many years, a concept partially supported by experimental data but not reproduced in all settings, particularly in humans. Indeed, low doses of bumetanide chronically administered to humans enhance insulin secretion and glucose tolerance (Robinson et al. 1981), an effect not observed after acute administration of the drug (Giugliano et al. 1980; Flamenbaum and Friedman 1982; Halstenson and Matzke 1983). These findings are not in contrast to the notion that loop diuretics impair insulin secretion. In fact, the inhibitory effect of bumetanide on insulin secretion from mice islets was observed in vitro only after acute administration of the drug (Sandstrom 1988, 1990), an effect that was recently confirmed in the rat β-cell line INS-1E (Alshahrani and Di Fulvio 2012). Although the impact of bumetanide on insulin secretion when chronically administered remains to be seen, NKCC1KO mice exhibit improved glucose tolerance and enhanced insulin secretion in vivo and in vitro when compared to wild-type mice, a phenomenon that could be potentially linked to NKCC2 expression in islets or to any other Cl^- transporter present there (Alshahrani et al. 2012). Nevertheless, acute bumetanide impairs glucose homeostasis in NKCC1KO an insulin secretion from islets lacking NKCC1 suggesting the presence of a bumetanide-sensitive mechanism involved in insulin secretion in the absence of NKCC1 (Alshahrani and Di Fulvio 2012).

Taken together, genetic, molecular, functional, and pharmacological evidence supports the notion that $[Cl^-]_i$ in β-cells is a key component of the insulin secretion machinery and, hence, it is included in the unified model shown in Figure 6.

Concluding Remarks

The information and thoughts presented in this chapter support the following long-standing, usually forgotten, and new remarks:

- $[Cl^-]_i$ is tightly regulated in β-cells, and its exit from these cells depolarizes the plasma membrane.
- NKCCs and Cl^- channels play a key role in β-cell physiology.
- Electrogenic Cl^- efflux depends on the metabolic state of the β-cell.
- The molecular identity of these Cl^- routes is unknown.
- Glucose may promote insulin secretion in the absence of K_{ATP} channels via anionic mechanisms.

- Cationic and anionic mechanisms are involved in insulin secretion, neither of which are exclusive.
- The consensus model of insulin secretion is incomplete and should be allowed to evolve with the incorporation of novel data.

Acknowledgments This work has been partially supported by funds awarded to MDiF from the American Diabetes Association, the Diabetes Action Research and Education Foundation, and from Wright State University (WSU), Boonshoft School of Medicine (BSoM), through the Seed Grant Program. LA-B is supported by NIH grant 5R01DK97829 and the Pacific Northwest Diabetes Research Institute (PNDRI). We are grateful to Dr. Len Best for useful discussion at the conception of this chapter. We are thankful to Shams Kursan, student of the Master's Program in Pharmacology and Toxicology at WSU-BSoM, for her excellent work in performing the RT-PCR experiments shown in Fig. 2b.

Cross-References

► (Dys)Regulation of Insulin Secretion by Macronutrients
► Anionic Transporters and Channels in Pancreatic Islet Cells
► ATP-Sensitive Potassium Channels in Health and Disease
► Electrophysiology of Islet Cells

References

Abdul-Karim FW, Dahms BB, Velasco ME, Rodman HM (1986) Islets of Langerhans in adolescents and adults with cystic fibrosis. A quantitative study. Arch Pathol Lab Med 110(7):602–606

Adragna NC, Di Fulvio M, Lauf PK (2004a) Regulation of K-Cl cotransport: from function to genes. J Membr Biol 201(3):109–137

Adragna NC, Chen Y, Delpire E, Lauf PK, Morris M (2004b) Hypertension in K-Cl cotransporter-3 knockout mice. Adv Exp Med Biol 559:379–385

Aguilar-Bryan L, Bryan J (2008) Neonatal diabetes mellitus. Endocr Rev 29(3):265–291

Akiyama K, Miyashita T, Mori T, Mori N (2007) Expression of the Na^+-K^+-$2Cl^-$ cotransporter in the rat endolymphatic sac. Biochem Biophys Res Commun 364(4):913–917

Akiyama K, Miyashita T, Matsubara A, Mori N (2010) The detailed localization pattern of Na^+/K^+/$2Cl^-$ cotransporter type 2 and its related ion transport system in the rat endolymphatic sac. J Histochem Cytochem 58(8):759–763

Almaca J, Tian Y, Aldehni F, Ousingsawat J, Kongsuphol P, Rock JR, Harfe BD, Schreiber R, Kunzelmann K (2009) TMEM16 proteins produce volume-regulated chloride currents that are reduced in mice lacking TMEM16A. J Biol Chem 284(42):28571–28578

Alper SL, Sharma AK (2013) The SLC26 gene family of anion transporters and channels. Mol Aspects Med 34(2–3):494–515

Alshahrani S, Di Fulvio M (2012) Enhanced insulin secretion and improved glucose tolerance in mice with homozygous inactivation of the $Na^+K^+2Cl^-$ co-transporter 1. J Endocrinol 215(1):59–70

Alshahrani S, Alvarez-Leefmans FJ, Di Fulvio M (2012) Expression of the Slc12a1 gene in pancreatic β-cells: molecular characterization and in silico analysis. Cell Physiol Biochem 30(1):95–112

Alvarez-Leefmans F (2012) Intracellular chloride regulation. In: Sperelakis N (ed) Cell physiology sourcebook: essentials of membrane biophysics, 4th edn. Elsevier/Academic, Amsterdam/Boston

Alvarez-Leefmans FJ, Delpire E (2009) Thermodynamic and kinetics of chloride transport in neurons: an outline. In: Alvarez-Leefmans FJ, Delpire E (eds) Physiology and pathology of chloride transporters and channels in the nervous system: from molecules to diseases. Elsevier/Academic, Amsterdam/Boston

Amlal H, Paillard M, Bichara M (1994) Cl^--dependent NH_4^+ transport mechanisms in medullary thick ascending limb cells. Am J Physiol 267(6 Pt 1):C1607–C1615

Antrobus SP, Lytle C, Payne JA (2012) K^+-Cl^- cotransporter-2 KCC2 in chicken cardiomyocytes. Am J Physiol Cell Physiol 303(11):C1180–C1191

Arreola J, Melvin JE, Begenisich T (1995) Volume-activated chloride channels in rat parotid acinar cells. J Physiol 484(Pt 3):677–687

Arroyo JP, Kahle KT, Gamba G (2013) The SLC12 family of electroneutral cation-coupled chloride cotransporters. Mol Aspects Med 34(2–3):288–298

Ayar A, Storer C, Tatham EL, Scott RH (1999) The effects of changing intracellular Ca^{2+} buffering on the excitability of cultured dorsal root ganglion neurones. Neurosci Lett 271(3):171–174

Bachmann O, Wuchner K, Rossmann H, Leipziger J, Osikowska B, Colledge WH, Ratcliff R, Evans MJ, Gregor M, Seidler U (2003) Expression and regulation of the Na^+-K^+-$2Cl^-$ cotransporter NKCC1 in the normal and CFTR-deficient murine colon. J Physiol 549(Pt 2):525–536

Babenko AP, Polak M, Cavé H, Busiah K, Czernichow P, Scharfmann R, Bryan J, Aguilar-Bryan L, Vaxillaire M, Froguel P (2006) Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. N Engl J Med 355(5):456–466

Barish ME (1983) A transient calcium-dependent chloride current in the immature Xenopus oocyte.J Physiol 342:309–325

Bear CE, Li CH, Kartner N, Bridges RJ, Jensen TJ, Ramjeesingh M, Riordan JR (1992) Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). Cell 68(4):809–818

Beauwens R, Best L, Markadieu N, Crutzen R, Louchami K, Brown P, Yates AP, Malaisse WJ, Sener A (2006) Stimulus-secretion coupling of hypotonicity-induced insulin release in BRIN-BD11 cells. Endocrine 30(3):353–363

Bensellam M, Van Lommel L, Overbergh L, Schuit FC, Jonas JC (2009) Cluster analysis of rat pancreatic islet gene mRNA levels after culture in low-, intermediate- and high-glucose concentrations. Diabetologia 52(3):463–476

Best L (1997) Glucose and α-ketoisocaproate induce transient inward currents in rat pancreatic β cells. Diabetologia 40(1):1–6

Best L (2002a) Inhibition of glucose-induced electrical activity by 4-hydroxytamoxifen in rat pancreatic β-cells. Cell Signal 14(1):69–73

Best L (2002b) Evidence that glucose-induced electrical activity in rat pancreatic β-cells does not require K_{ATP} channel inhibition. J Membr Biol 185(3):193–200

Best L (2005) Glucose-induced electrical activity in rat pancreatic β-cells: dependence on intracellular chloride concentration. J Physiol 568(Pt 1):137–144

Best L, Yates AP, Meats JE, Tomlinson S (1989) Effects of lactate on pancreatic islets. Lactate efflux as a possible determinant of islet-cell depolarization by glucose. Biochem J 259(2):507–511

Best L, Yates AP, Tomlinson S (1992) Stimulation of insulin secretion by glucose in the absence of diminished potassium $^{86}Rb^+$ permeability. Biochem Pharmacol 43(11):2483–2485

Best L, Sheader EA, Brown PD (1996a) A volume-activated anion conductance in insulin-secreting cells. Pflugers Arch 431(3):363–370

Best L, Miley HE, Yates AP (1996b) Activation of an anion conductance and β-cell depolarization during hypotonically induced insulin release. Exp Physiol 81(6):927–933

Best L, Speake T, Brown P (2001) Functional characterisation of the volume-sensitive anion channel in rat pancreatic β-cells. Exp Physiol 86(2):145–150

Best L, Davies S, Brown PD (2004) Tolbutamide potentiates the volume-regulated anion channel current in rat pancreatic β cells. Diabetologia 47(11):1990–1997

Best L, Brown PD, Sener A, Malaisse WJ (2010) Electrical activity in pancreatic islet cells: the VRAC hypothesis. Islets 2(2):59–64

Blackard WG, Kikuchi M, Rabinovitch A, Renold AE (1975) An effect of hyposmolarity on insulin release in vitro. Am J Physiol 228(3):706–713

Blaesse P, Airaksinen MS, Rivera C, Kaila K (2009) Cation-chloride cotransporters and neuronal function. Neuron 61(6):820–838

Boettger T, Hubner CA, Maier H, Rust MB, Beck FX, Jentsch TJ (2002) Deafness and renal tubular acidosis in mice lacking the K-Cl co-transporter Kcc4. Nature 416(6883):874–878

Boettger T, Rust MB, Maier H, Seidenbecher T, Schweizer M, Keating DJ, Faulhaber J, Ehmke H, Pfeffer C, Scheel O, Lemcke B, Horst J, Leuwer R, Pape HC, Volkl H, Hubner CA, Jentsch TJ (2003) Loss of K-Cl co-transporter KCC3 causes deafness, neurodegeneration and reduced seizure threshold. EMBO J 22(20):5422–5434

Boom A, Lybaert P, Pollet JF, Jacobs P, Jijakli H, Golstein PE, Sener A, Malaisse WJ, Beauwens R (2007) Expression and localization of cystic fibrosis transmembrane conductance regulator in the rat endocrine pancreas. Endocrine 32(2):197–205

Bramswig NC, Everett LJ, Schug J, Dorrell C, Liu C, Luo Y, Streeter PR, Naji A, Grompe M, Kaestner KH (2013) Epigenomic plasticity enables human pancreatic α to β cell reprogramming. J Clin Invest 123(3):1275–1284

Brauer M, Frei E, Claes L, Grissmer S, Jager H (2003) Influence of K-Cl cotransporter activity on activation of volume-sensitive Cl^- channels in human osteoblasts. Am J Physiol Cell Physiol 285(1):C22–C30

Braun M, Ramracheya R, Bengtsson M, Clark A, Walker JN, Johnson PR, Rorsman P (2010) Gamma-aminobutyric acid (GABA) is an autocrine excitatory transmitter in human pancreatic β-cells. Diabetes 59(7):1694–1701

Britsch S, Krippeit-Drews P, Gregor M, Lang F, Drews G (1994) Effects of osmotic changes in extracellular solution on electrical activity of mouse pancreatic β-cells. Biochem Biophys Res Commun 204(2):641–645

Byun N, Delpire E (2007) Axonal and periaxonal swelling precede peripheral neurodegeneration in KCC3 knockout mice. Neurobiol Dis 28(1):39–51

Cao DS, Zhong L, Hsieh TH, Abooj M, Bishnoi M, Hughes L, Premkumar LS (2012) Expression of transient receptor potential ankyrin 1 (TRPA1) and its role in insulin release from rat pancreatic β cells. PLoS One 7(5):e38005

Caputo A, Caci E, Ferrera L, Pedemonte N, Barsanti C, Sondo E, Pfeffer U, Ravazzolo R, Zegarra-Moran O, Galietta LJ (2008) TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity. Science 322(5901):590–594

Carota I, Theilig F, Oppermann M, Kongsuphol P, Rosenauer A, Schreiber R, Jensen BL, Walter S, Kunzelmann K, Castrop H (2010) Localization and functional characterization of the human NKCC2 isoforms. Acta Physiol (Oxf) 199(3):327–338

Chan CB, Saleh MC, Purje A, MacPhail RM (2002) Glucose-inducible hypertrophy and suppression of anion efflux in rat β cells. J Endocrinol 173(1):45–52

Chien LT, Hartzell HC (2008) Rescue of volume-regulated anion current by bestrophin mutants with altered charge selectivity. J Gen Physiol 132(5):537–546

Colsoul B, Vennekens R, Nilius B (2011) Transient receptor potential cation channels in pancreatic β cells. Rev Physiol Biochem Pharmacol 161:87–110

Corless M, Kiely A, McClenaghan NH, Flatt PR, Newsholme P (2006) Glutamine regulates expression of key transcription factor, signal transduction, metabolic gene, and protein expression in a clonal pancreatic β-cell line. J Endocrinol 190(3):719–727

Davies SL, Roussa E, Le Rouzic P, Thevenod F, Alper SL, Best L, Brown PD (2004) Expression of K^+-Cl^- cotransporters in the α-cells of rat endocrine pancreas. Biochim Biophys Acta 1667(1):7–14

Davis PB (2006) Cystic fibrosis since 1938. Am J Respir Crit Care Med 173(5):475–482

Dean M, Allikmets R (2001) Complete characterization of the human ABC gene family. J Bioenerg Biomembr 33(6):475–479

Delpire E, Lauf PK (1992) Kinetics of DIDS inhibition of swelling-activated K-Cl cotransport in low K sheep erythrocytes. J Membr Biol 126(1):89–96

Delpire E, Lu J, England R, Dull C, Thorne T (1999) Deafness and imbalance associated with inactivation of the secretory Na- K-2Cl co-transporter. Nat Genet 22(2):192–195

Delpire E, Days E, Lewis LM, Mi D, Kim K, Lindsley CW, Weaver CD (2009) Small-molecule screen identifies inhibitors of the neuronal K-Cl cotransporter KCC2. Proc Natl Acad Sci USA 106(13):5383–5388

Di Fulvio M, Alvarez-Leefmans FJ (2009) The NKCC and NCC genes: an in silico view. In: Alvarez-Leefmans FJ, Delpire E (eds) Physiology and pathology of chloride transporters and channels in the nervous system: from molecules to diseases. Academic, Amsterdam/Boston, pp 169–208

Di Fulvio M, Lincoln TM, Lauf PK, Adragna NC (2001) Protein kinase G regulates potassium chloride cotransporter-3 expression in primary cultures of rat vascular smooth muscle cells. J Biol Chem 276(24):21046–21052

Donaldson SH, Boucher RC (2007) Sodium channels and cystic fibrosis. Chest 132(5):1631–1636

Doroshenko P, Neher E (1992) Volume-sensitive chloride conductance in bovine chromaffin cell membrane. J Physiol 449:197–218

Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science 280(5360):69–77

Drews G, Krippeit-Drews P, Dufer M (2010) Electrophysiology of islet cells. Adv Exp Med Biol 654:115–163

Drumm ML, Ziady AG, Davis PB (2012) Genetic variation and clinical heterogeneity in cystic fibrosis. Annu Rev Pathol 7:267–282

Dufer M, Haspel D, Krippeit-Drews P, Aguilar-Bryan L, Bryan J, Drews G (2004) Oscillations of membrane potential and cytosolic Ca^{2+} concentration in SUR1$^{-/-}$ β cells. Diabetologia 47(3):488–498

Dupre N, Howard HC, Mathieu J, Karpati G, Vanasse M, Bouchard JP, Carpenter S, Rouleau GA (2003) Hereditary motor and sensory neuropathy with agenesis of the corpus callosum. Ann Neurol 54(1):9–18

Duran C, Hartzell HC (2011) Physiological roles and diseases of Tmem16/Anoctamin proteins: are they all chloride channels? Acta Pharmacol Sin 32(6):685–692

Duran C, Thompson CH, Xiao Q, Hartzell HC (2010) Chloride channels: often enigmatic, rarely predictable. Annu Rev Physiol 72:95–121

Eberhardson M, Patterson S, Grapengiesser E (2000) Microfluorometric analysis of Cl^- permeability and its relation to oscillatory Ca^{2+} signaling in glucose-stimulated pancreatic β-cells. Cell Signal 12(11–12):781–786

Eggermont J (2004) Calcium-activated chloride channels: (un)known, (un)loved? Proc Am Thorac Soc 1(1):22–27

Eggermont J, Trouet D, Carton I, Nilius B (2001) Cellular function and control of volume-regulated anion channels. Cell Biochem Biophys 35(3):263–274

Engstrom KG, Sandstrom PE, Sehlin J (1991) Volume regulation in mouse pancreatic β-cells is mediated by a furosemide-sensitive mechanism. Biochim Biophys Acta 1091(2):145–150

Fischmeister R, Hartzell HC (2005) Volume sensitivity of the bestrophin family of chloride channels. J Physiol 562(Pt 2):477–491

Flagella M, Clarke LL, Miller ML, Erway LC, Giannella RA, Andringa A, Gawenis LR, Kramer J, Duffy JJ, Doetschman T, Lorenz JN, Yamoah EN, Cardell EL, Shull GE (1999) Mice lacking the basolateral Na-K-2Cl cotransporter have impaired epithelial chloride secretion and are profoundly deaf. J Biol Chem 274(38):26946–26955

Flamenbaum W, Friedman R (1982) Pharmacology, therapeutic efficacy, and adverse effects of bumetanide, a new "loop" diuretic. Pharmacotherapy 2(4):213–222

Fredriksson R, Nordstrom KJ, Stephansson O, Hagglund MG, Schioth HB (2008) The solute carrier (SLC) complement of the human genome: phylogenetic classification reveals four major families. FEBS Lett 582(27):3811–3816

Frings S, Hackos DH, Dzeja C, Ohyama T, Hagen V, Kaupp UB, Korenbrot JI (2000) Determination of fractional calcium ion current in cyclic nucleotide-gated channels. Methods Enzymol 315:797–817

Fujii T, Ohira Y, Itomi Y, Takahashi Y, Asano S, Morii M, Takeguchi N, Sakai H (2007) Inhibition of P-type ATPases by [(dihydroindenyl)oxy]acetic acid (DIOA), a K^+ -Cl^- cotransporter inhibitor. Eur J Pharmacol 560(2–3):123–126

Gagnon KB, Delpire E (2013) Physiology of SLC12 transporters: lessons from inherited human genetic mutations and genetically engineered mouse knockouts. Am J Physiol Cell Physiol 304(8):C693–C714

Gagnon KB, Di Fulvio M (2013) A molecular analysis of Na^+-independent cation-chloride cotransporters. Cell Phys Biochem 32(7):14–31

Gagnon M, Bergeron MJ, Lavertu G, Castonguay A, Tripathy S, Bonin RP, Perez-Sanchez J, Boudreau D, Wang B, Dumas L, Valade I, Bachand K, Jacob-Wagner M, Tardif C, Kianicka I, Isenring P, Attardo G, Coull JA, De Koninck Y (2013) Chloride extrusion enhancers as novel therapeutics for neurological diseases. Nat Med. 19(11):1524-8

Galietta LJ (2009) The TMEM16 protein family: a new class of chloride channels? Biophys J 97(12):3047–3053

Gamba G (2005) Molecular physiology and pathophysiology of electroneutral cation-chloride cotransporters. Physiol Rev 85(2):423–493

Gavrikov KE, Nilson JE, Dmitriev AV, Zucker CL, Mangel SC (2006) Dendritic compartmentalization of chloride cotransporters underlies directional responses of starburst amacrine cells in retina. Proc Natl Acad Sci USA 103(49):18793–18798

Gembal M, Gilon P, Henquin JC (1992) Evidence that glucose can control insulin release independently from its action on ATP-sensitive K^+ channels in mouse B cells. J Clin Invest 89(4):1288–1295

Ghanaat-Pour H, Sjoholm A (2009) Gene expression regulated by pioglitazone and exenatide in normal and diabetic rat islets exposed to lipotoxicity. Diabetes Metab Res Rev 25(2):163–184

Gibson JS, Ellory JC, Adragna NC, Lauf PK (2009) Pathophysiology of the K^+-Cl^- cotransporters: paths to discovery and overview. In: Alvarez-Leefmans FJ, Delpire E (eds) Physiology and pathology of chloride transporters and channels in the nervous system: from molecules to diseases. Academic, Amsterdam/Boston, pp 27–42

Giugliano D, Varricchio M, Cerciello T, Varano R, Saccomanno F, Giannetti G (1980) Bumetanide and glucose tolerance in man. Farmaco Prat 35(8):403–408

Glaudemans B, Yntema HG, San-Cristobal P, Schoots J, Pfundt R, Kamsteeg EJ, Bindels RJ, Knoers NV, Hoenderop JG, Hoefsloot LH (2012) Novel NCC mutants and functional analysis in a new cohort of patients with Gitelman syndrome. Eur J Hum Genet 20(3):263–270

Gomez-Hernandez JM, Stuhmer W, Parekh AB (1997) Calcium dependence and distribution of calcium-activated chloride channels in Xenopus oocytes. J Physiol 502(Pt 3):569–574

Gottlieb PA, Yu L, Babu S, Wenzlau J, Bellin M, Frohnert BI, Moran A (2012) No relation between cystic fibrosis-related diabetes and type 1 diabetes autoimmunity. Diabetes Care 35(8):e57

Grinstein S, Rothstein A, Sarkadi B, Gelfand EW (1984) Responses of lymphocytes to anisotonic media: volume-regulating behavior. Am J Physiol 246(3 Pt 1):C204–C215

Halstenson CE, Matzke GR (1983) Bumetanide: a new loop diuretic (Bumex, Roche Laboratories). Drug Intell Clin Pharm 17(11):786–797

Hamann S, Herrera-Perez JJ, Zeuthen T, Alvarez-Leefmans FJ (2010) Cotransport of water by the Na^+-K^+-$2Cl^-$ cotransporter NKCC1 in mammalian epithelial cells. J Physiol 588(Pt 21):4089–4101

Hartzell C, Putzier I, Arreola J (2005) Calcium-activated chloride channels. Annu Rev Physiol 67:719–758

Hartzell HC, Yu K, Xiao Q, Chien LT, Qu Z (2009) Anoctamin/TMEM16 family members are Ca^{2+}-activated Cl^- channels. J Physiol 587(Pt 10):2127–2139

Hediger MA, Romero MF, Peng JB, Rolfs A, Takanaga H, Bruford EA (2004) The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins Introduction. Pflugers Arch 447(5):465–468

Helix N, Strobaek D, Dahl BH, Christophersen P (2003) Inhibition of the endogenous volume-regulated anion channel (VRAC) in HEK293 cells by acidic di-aryl-ureas. J Membr Biol 196(2):83–94

Henquin JC (1980) Tolbutamide stimulation and inhibition of insulin release: studies of the underlying ionic mechanisms in isolated rat islets. Diabetologia 18(2):151–160

Henquin JC, Meissner HP (1982a) Opposite effects of tolbutamide and diazoxide on $^{86}Rb^+$ fluxes and membrane potential in pancreatic B cells. Biochem Pharmacol 31(7):1407–1415

Henquin JC, Meissner HP (1982b) The electrogenic sodium-potassium pump of mouse pancreatic β-cells. J Physiol 332:529–552

Henquin JC, Ravier MA, Nenquin M, Jonas JC, Gilon P (2003) Hierarchy of the β-cell signals controlling insulin secretion. Eur J Clin Invest 33(9):742–750

Henquin JC, Nenquin M, Ravier MA, Szollosi A (2009) Shortcomings of current models of glucose-induced insulin secretion. Diabetes Obes Metab 11(Suppl 4):168–179

Hille B (2001) Ion channels of excitable membranes, 3rd edn. Sinauer, Sunderland

Hoffmann EK, Simonsen LO (1989) Membrane mechanisms in volume and pH regulation in vertebrate cells. Physiol Rev 69(2):315–382

Hoffmann EK, Schettino T, Marshall WS (2007) The role of volume-sensitive ion transport systems in regulation of epithelial transport. Comp Biochem Physiol A Mol Integr Physiol 148(1):29–43

Hoffmann EK, Lambert IH, Pedersen SF (2009) Physiology of cell volume regulation in vertebrates. Physiol Rev 89(1):193–277

Howard HC, Mount DB, Rochefort D, Byun N, Dupre N, Lu J, Fan X, Song L, Riviere JB, Prevost C, Horst J, Simonati A, Lemcke B, Welch R, England R, Zhan FQ, Mercado A, Siesser WB, George AL Jr, McDonald MP, Bouchard JP, Mathieu J, Delpire E, Rouleau GA (2002) The K-Cl cotransporter KCC3 is mutant in a severe peripheral neuropathy associated with agenesis of the corpus callosum. Nat Genet 32(3):384–392

Huang J, Shan J, Kim D, Liao J, Evagelidis A, Alper SL, Hanrahan JW (2012) Basolateral chloride loading by the anion exchanger type 2: role in fluid secretion by the human airway epithelial cell line Calu-3. J Physiol 590(Pt 21):5299–5316

Hubner CA, Stein V, Hermans-Borgmeyer I, Meyer T, Ballanyi K, Jentsch TJ (2001) Disruption of KCC2 reveals an essential role of K-Cl cotransport already in early synaptic inhibition. Neuron 30(2):515–524

Hudson RL, Schultz SG (1988) Sodium-coupled glycine uptake by Ehrlich ascites tumor cells results in an increase in cell volume and plasma membrane channel activities. Proc Natl Acad Sci USA 85(1):279–283

Iannucci A, Mukai K, Johnson D, Burke B (1984) Endocrine pancreas in cystic fibrosis: an immunohistochemical study. Hum Pathol 15(3):278–284

Islam MS (2011) TRP channels of islets. Adv Exp Med Biol 704:811–830

Ivanenko A, Baring MD, Airey JA, Sutko JL, Kenyon JL (1993) A caffeine- and ryanodine-sensitive Ca^{2+} store in avian sensory neurons. J Neurophysiol 70(2):710–722

Iwamoto LM, Fujiwara N, Nakamura KT, Wada RK (2004) Na-K-2Cl cotransporter inhibition impairs human lung cellular proliferation. Am J Physiol Lung Cell Mol Physiol 287(3):L510–L514

Jakab M, Grundbichler M, Benicky J, Ravasio A, Chwatal S, Schmidt S, Strbak V, Furst J, Paulmichl M, Ritter M (2006) Glucose induces anion conductance and cytosol-to-membrane transposition of ICln in INS-1E rat insulinoma cells. Cell Physiol Biochem 18(1–3):21–34

Jean-Xavier C, Pflieger JF, Liabeuf S, Vinay L (2006) Inhibitory postsynaptic potentials in lumbar motoneurons remain depolarizing after neonatal spinal cord transection in the rat. J Neurophysiol 96(5):2274–2281

Jentsch TJ (2002) Chloride channels are different. Nature 415(6869):276–277

Jijakli H, Zhang Y, Sener A, Malaisse WJ (2006) Tritiated taurine handling by isolated rat pancreatic islets. Endocrine 29(2):331–339

Jovov B, Ismailov II, Benos DJ (1995) Cystic fibrosis transmembrane conductance regulator is required for protein kinase A activation of an outwardly rectified anion channel purified from bovine tracheal epithelia. J Biol Chem 270(4):1521–1528

Kahle KT, Staley KJ, Nahed BV, Gamba G, Hebert SC, Lifton RP, Mount DB (2008) Roles of the cation-chloride cotransporters in neurological disease. Nat Clin Pract Neurol 4(9):490–503

Kahle KT, Rinehart J, Lifton RP (2010) Phosphoregulation of the Na-K-2Cl and K-Cl cotransporters by the WNK kinases. Biochim Biophys Acta 1802(12):1150–1158

Kakigi A, Nishimura M, Takeda T, Taguchi D, Nishioka R (2009) Expression of aquaporin1, 3, and 4, NKCC1, and NKCC2 in the human endolymphatic sac. Auris Nasus Larynx 36(2):135–139

Keiser NW, Engelhardt JF (2011) New animal models of cystic fibrosis: what are they teaching us? Curr Opin Pulm Med 17(6):478–483

Kelly A, Moran A (2013) Update on cystic fibrosis-related diabetes. J Cyst Fibros 12(4):318–331

Kenyon JL, Goff HR (1998) Temperature dependencies of Ca^{2+} current, Ca^{2+}-activated Cl^- current and Ca^{2+} transients in sensory neurones. Cell Calcium 24(1):35–48

Kinard TA, Satin LS (1995) An ATP-sensitive Cl^- channel current that is activated by cell swelling, cAMP, and glyburide in insulin-secreting cells. Diabetes 44(12):1461–1466

Kinne R, Kinne-Saffran E, Schutz H, Scholermann B (1986) Ammonium transport in medullary thick ascending limb of rabbit kidney: involvement of the Na^+, K^+, Cl^--cotransporter. J Membr Biol 94(3):279–284

Kirk K, Ellory JC, Young JD (1992) Transport of organic substrates via a volume-activated channel. J Biol Chem 267(33):23475–23478

Klockner U (1993) Intracellular calcium ions activate a low-conductance chloride channel in smooth-muscle cells isolated from human mesenteric artery. Pflugers Arch 424(3–4):231–237

Klupa T, Skupien J, Malecki MT (2012) Monogenic models: what have the single gene disorders taught us? Curr Diab Rep 12(6):659–666

Knowles M, Gatzy J, Boucher R (1981) Increased bioelectric potential difference across respiratory epithelia in cystic fibrosis. N Engl J Med 305(25):1489–1495

Koumi S, Sato R, Aramaki T (1994) Characterization of the calcium-activated chloride channel in isolated guinea-pig hepatocytes. J Gen Physiol 104(2):357–373

Kozak JA, Logothetis DE (1997) A calcium-dependent chloride current in insulin-secreting β TC-3 cells. Pflugers Arch 433(6):679–690

Kunzelmann K (2001) CFTR: interacting with everything? News Physiol Sci 16:167–170

Kunzelmann K (2005) Ion channels and cancer. J Membr Biol 205(3):159–173

Kunzelmann K, Kubitz R, Grolik M, Warth R, Greger R (1992) Small-conductance Cl^- channels in HT29 cells: activation by Ca^{2+}, hypotonic cell swelling and 8-Br-cGMP. Pflugers Arch 421(2–3):238–246

Kunzelmann K, Tian Y, Martins JR, Faria D, Kongsuphol P, Ousingsawat J, Thevenod F, Roussa E, Rock J, Schreiber R (2011) Anoctamins. Pflugers Arch 462(2):195–208

Kuruma A, Hartzell HC (2000) Bimodal control of a Ca^{2+}-activated Cl^- channel by different Ca^{2+} signals. J Gen Physiol 115(1):59–80

Kyrozis A, Reichling DB (1995) Perforated-patch recording with gramicidin avoids artifactual changes in intracellular chloride concentration. J Neurosci Methods 57(1):27–35

Lang I, Daneman A, Cutz E, Hagen P, Shandling B (1997) Abdominal calcification in cystic fibrosis with meconium ileus: radiologic-pathologic correlation. Pediatr Radiol 27(6):523–527

Lauf PK, Di Fulvio M, Srivastava V, Sharma N, Adragna NC (2012) KCC2a expression in a human fetal lens epithelial cell line. Cell Physiol Biochem 29(1–2):303–312

Lee MG, Wigley WC, Zeng W, Noel LE, Marino CR, Thomas PJ, Muallem S (1999) Regulation of Cl^-/HCO_3^- exchange by cystic fibrosis transmembrane conductance regulator expressed in NIH 3T3 and HEK 293 cells. J Biol Chem 274(6):3414–3421

Leviel F, Hubner CA, Houillier P, Morla L, El Moghrabi S, Brideau G, Hassan H, Parker MD, Kurth I, Kougioumtzes A, Sinning A, Pech V, Riemondy KA, Miller RL, Hummler E, Shull GE, Aronson PS, Doucet A, Wall SM, Chambrey R, Eladari D (2010) The Na^+-dependent chloride-bicarbonate exchanger SLC4A8 mediates an electroneutral Na^+ reabsorption process in the renal cortical collecting ducts of mice. J Clin Invest 120(5):1627–1635

Lindstrom P, Norlund L, Sehlin J (1986) Potassium and chloride fluxes are involved in volume regulation in mouse pancreatic islet cells. Acta Physiol Scand 128(4):541–546

Lindstrom P, Norlund L, Sandstom PE, Sehlin J (1988) Evidence for co-transport of sodium, potassium and chloride in mouse pancreatic islets. J Physiol 400:223–236

Louchami K, Zhang Y, Beauwens R, Malaisse WJ, Sener A (2007) Is the glucose-induced phosphate flush in pancreatic islets attributable to gating of volume-sensitive anion channels? Endocrine 31(1):1–4

Lubamba B, Dhooghe B, Noel S, Leal T (2012) Cystic fibrosis: insight into CFTR pathophysiology and pharmacotherapy. Clin Biochem 45(15):1132–1144

Lynch AM, Meats JE, Best L, Tomlinson S (1989) Effects of nutrient and non-nutrient stimuli on cytosolic pH in cultured insulinoma (HIT-T15) cells. Biochim Biophys Acta 1012(2):166–170

Lynch AM, Trebilcock R, Tomlinson S, Best L (1991) Studies of the mechanism of activation of HIT-T15 cells by lactate. Biochim Biophys Acta 1091(2):141–144

Macrobbie EA, Ussing HH (1961) Osmotic behaviour of the epithelial cells of frog skin. Acta Physiol Scand 53:348–365

Mahdi T, Hanzelmann S, Salehi A, Muhammed SJ, Reinbothe TM, Tang Y, Axelsson AS, Zhou Y, Jing X, Almgren P, Krus U, Taneera J, Blom AM, Lyssenko V, Esguerra JL, Hansson O, Eliasson L, Derry J, Zhang E, Wollheim CB, Groop L, Renstrom E, Rosengren AH (2012) Secreted frizzled-related protein 4 reduces insulin secretion and is overexpressed in type 2 diabetes. Cell Metab 16(5):625–633

Majid A, Speake T, Best L, Brown PD (2001) Expression of the Na^+K^+-2CI- cotransporter in α and β cells isolated from the rat pancreas. Pflugers Arch 442(4):570–576

Mao S, Garzon-Muvdi T, Di Fulvio M, Chen Y, Delpire E, Alvarez FJ, Alvarez-Leefmans FJ (2012) Molecular and functional expression of cation-chloride cotransporters in dorsal root ganglion neurons during postnatal maturation. J Neurophysiol 108(3):834–852

Martinez-Pinna J, McLachlan EM, Gallego R (2000) Distinct mechanisms for activation of Cl^- and K^+ currents by Ca^{2+} from different sources in mouse sympathetic neurones. J Physiol 527 (Pt 2):249–264

McGlasson L, Best L, Brown PD (2011) The glucokinase activator GKA50 causes an increase in cell volume and activation of volume-regulated anion channels in rat pancreatic β-cells. Mol Cell Endocrinol 342(1–2):48–53

Meissner HP, Preissler M (1980) Ionic mechanisms of the glucose-induced membrane potential changes in β-cells. Horm Metab Res Suppl Suppl 10:91–99

Mercado A, Song L, Vazquez N, Mount DB, Gamba G (2000) Functional comparison of the K^+-Cl^- cotransporters KCC1 and KCC4. J Biol Chem 275(39):30326–30334

Miledi R (1982) A calcium-dependent transient outward current in Xenopus laevis oocytes.Proc R Soc Lond B 215:491–497

Miley HE, Sheader EA, Brown PD, Best L (1997) Glucose-induced swelling in rat pancreatic β-cells. J Physiol 504(Pt 1):191–198

Miley HE, Holden D, Grint R, Best L, Brown PD (1998) Regulatory volume increase in rat pancreatic β-cells. Pflugers Arch 435(2):227–230

Miley HE, Brown PD, Best L (1999) Regulation of a volume-sensitive anion channel in rat pancreatic β-cells by intracellular adenine nucleotides. J Physiol 515(Pt 2):413–417

Misler S (2012) Stimulus–response coupling in metabolic sensor cells. In: Sperelakis N (ed) Cell physiology sourcebook: essentials of membrane biophysics, 4th edn. Elsevier/Academic, Amsterdam/Boston

Miyazaki J, Araki K, Yamato E, Ikegami H, Asano T, Shibasaki Y, Oka Y, Yamamura K (1990) Establishment of a pancreatic β cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. Endocrinology 127(1):126–132

Monroy A, Plata C, Hebert SC, Gamba G (2000) Characterization of the thiazide-sensitive Na^+-Cl^- cotransporter: a new model for ions and diuretics interaction. Am J Physiol Renal Physiol 279(1):F161–F169

Nickell WT, Kleene NK, Kleene SJ (2007) Mechanisms of neuronal chloride accumulation in intact mouse olfactory epithelium. J Physiol 583(Pt 3):1005–1020

Nilius B, Droogmans G (2003) Amazing chloride channels: an overview. Acta Physiol Scand 177(2):119–147

Nilius B, Prenen J, Szucs G, Wei L, Tanzi F, Voets T, Droogmans G (1997) Calcium-activated chloride channels in bovine pulmonary artery endothelial cells. J Physiol 498(Pt 2):381–396

Nishimura M, Kakigi A, Takeda T, Takeda S, Doi K (2009) Expression of aquaporins, vasopressin type 2 receptor, and Na^+K^+Cl cotransporters in the rat endolymphatic sac. Acta Otolaryngol 129(8):812–818

Okada Y (1997) Volume expansion-sensing outward-rectifier Cl^- channel: fresh start to the molecular identity and volume sensor. Am J Physiol 273(3 Pt 1):C755–C789

Oppermann M, Mizel D, Huang G, Li C, Deng C, Theilig F, Bachmann S, Briggs J, Schnermann J, Castrop H (2006) Macula densa control of renin secretion and preglomerular resistance in mice with selective deletion of the B isoform of the Na, K, 2Cl co-transporter. J Am Soc Nephrol 17(8):2143–2152

Oppermann M, Mizel D, Kim SM, Chen L, Faulhaber-Walter R, Huang Y, Li C, Deng C, Briggs J, Schnermann J, Castrop H (2007) Renal function in mice with targeted disruption of the A isoform of the Na-K-2Cl co-transporter. J Am Soc Nephrol 18(2):440–448

Orci L, Malaisse W (1980) Hypothesis: single and chain release of insulin secretory granules is related to anionic transport at exocytotic sites. Diabetes 29(11):943–944

Pace AJ, Lee E, Athirakul K, Coffman TM, O'Brien DA, Koller BH (2000) Failure of spermatogenesis in mouse lines deficient in the Na^+-K^+-$2Cl^-$ cotransporter. J Clin Invest 105(4):441–450

Panet R, Eliash M, Atlan H (2006) $Na^+/K^+/Cl^-$ cotransporter activates MAP-kinase cascade downstream to protein kinase C, and upstream to MEK. J Cell Physiol 206(3):578–585

Panten U, Schwanstecher M, Schwanstecher C (1996) Sulfonylurea receptors and mechanism of sulfonylurea action. Exp Clin Endocrinol Diabetes 104(1):1–9

Park MK, Lomax RB, Tepikin AV, Petersen OH (2001) Local uncaging of caged Ca^{2+} reveals distribution of Ca^{2+}-activated Cl^- channels in pancreatic acinar cells. Proc Natl Acad Sci USA 98(19):10948–10953

Parker MD, Boron WF (2013) The divergence, actions, roles, and relatives of sodium-coupled bicarbonate transporters. Physiol Rev 93(2):803–959

Payne JA (1997) Functional characterization of the neuronal-specific K-Cl cotransporter: implications for $[K^+]o$ regulation. Am J Physiol 273(5 Pt 1):C1516–C1525

Perez-Cornejo P, Arreola J (2004) Regulation of Ca^{2+}-activated chloride channels by cAMP and CFTR in parotid acinar cells. Biochem Biophys Res Commun 316(3):612–617

Quinton PM (2007) Too much salt, too little soda: cystic fibrosis. Sheng Li Xue Bao 59(4):397–415

Race JE, Makhlouf FN, Logue PJ, Wilson FH, Dunham PB, Holtzman EJ (1999) Molecular cloning and functional characterization of KCC3, a new K-Cl cotransporter. Am J Physiol 277(6 Pt 1):C1210–C1219

Rasola A, Galietta LJ, Gruenert DC, Romeo G (1992) Ionic selectivity of volume-sensitive currents in human epithelial cells. Biochim Biophys Acta 1139(4):319–323

Reid KH, Guo SZ, Iyer VG (2000) Agents which block potassium-chloride cotransport prevent sound-triggered seizures in post-ischemic audiogenic seizure-prone rats. Brain Res 864(1):134–137

Reilly RF, Ellison DH (2000) Mammalian distal tubule: physiology, pathophysiology, and molecular anatomy. Physiol Rev 80(1):277–313
Reisert J, Bauer PJ, Yau KW, Frings S (2003) The Ca-activated Cl channel and its control in rat olfactory receptor neurons. J Gen Physiol 122(3):349–363
Rhee JS, Ebihara S, Akaike N (1994) Gramicidin perforated patch-clamp technique reveals glycine-gated outward chloride current in dissociated nucleus solitarii neurons of the rat. J Neurophysiol 72(3):1103–1108
Riordan JR (2008) CFTR function and prospects for therapy. Annu Rev Biochem 77:701–726
Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL et al (1989) Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 245(4922):1066–1073
Robinson DS, Nilsson CM, Leonard RF, Horton ES (1981) Effects of loop diuretics on carbohydrate metabolism and electrolyte excretion. J Clin Pharmacol 21(11–12 Pt 2):637–646
Rock JR, O'Neal WK, Gabriel SE, Randell SH, Harfe BD, Boucher RC, Grubb BR (2009) Transmembrane protein 16A (TMEM16A) is a Ca^{2+}-regulated Cl^- secretory channel in mouse airways. J Biol Chem 284(22):14875–14880
Romero MF, Chen AP, Parker MD, Boron WF (2013) The SLC4 family of bicarbonate HCO_3^- transporters. Mol Aspects Med 34(2–3):159–182
Rorsman P, Berggren PO, Bokvist K, Ericson H, Mohler H, Ostenson CG, Smith PA (1989) Glucose-inhibition of glucagon secretion involves activation of GABAA-receptor chloride channels. Nature 341(6239):233–236
Rosario LM, Barbosa RM, Antunes CM, Baldeiras IE, Silva AM, Tome AR, Santos RM (2008) Regulation by glucose of oscillatory electrical activity and 5-HT/insulin release from single mouse pancreatic islets in absence of functional K_{ATP} channels. Endocr J 55(4):639–650
Rowe SM, Verkman AS (2013) Cystic fibrosis transmembrane regulator correctors and potentiators. Cold Spring Harb Perspect Med 3(7):a009761
Roy G, Banderali U (1994) Channels for ions and amino acids in kidney cultured cells (MDCK) during volume regulation. J Exp Zool 268(2):121–126
Russell JM (2000) Sodium-potassium-chloride cotransport. Physiol Rev 80(1):211–276
Rust MB, Alper SL, Rudhard Y, Shmukler BE, Vicente R, Brugnara C, Trudel M, Jentsch TJ, Hubner CA (2007) Disruption of erythroid K-Cl cotransporters alters erythrocyte volume and partially rescues erythrocyte dehydration in SAD mice. J Clin Invest 117(6):1708–1717
Sandstrom PE (1988) Evidence for diabetogenic action of bumetanide in mice. Eur J Pharmacol 150(1–2):35–41
Sandstrom PE (1990) Bumetanide reduces insulin release by a direct effect on the pancreatic β-cells. Eur J Pharmacol 187(3):377–383
Sandstrom PE, Sehlin J (1987) Stereoselective inhibition of chloride transport by loop diuretics in pancreatic β-cells. Eur J Pharmacol 144(3):389–392
Sandstrom PE, Sehlin J (1988a) Furosemide causes acute and long-term hyperglycaemia and reduces glucose tolerance in mice. Acta Physiol Scand 132(1):75–81
Sandstrom PE, Sehlin J (1988b) Furosemide-induced glucose intolerance in mice is associated with reduced insulin secretion. Eur J Pharmacol 147(3):403–409
Sandstrom PE, Sehlin J (1988c) Furosemide reduces insulin release by inhibition of Cl^- and Ca^{2+} fluxes in β-cells. Am J Physiol 255(5 Pt 1):E591–E596
Sandstrom PE, Sehlin J (1993) Evidence for separate Na^+, K^+, Cl^- and K^+, Cl^- co-transport systems in mouse pancreatic β-cells. Eur J Pharmacol 238(2–3):403–405
Schreiber R, Uliyakina I, Kongsuphol P, Warth R, Mirza M, Martins JR, Kunzelmann K (2010) Expression and function of epithelial anoctamins. J Biol Chem 285(10):7838–7845
Schroeder BC, Cheng T, Jan YN, Jan LY (2008) Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. Cell 134(6):1019–1029
Schultheis PJ, Lorenz JN, Meneton P, Nieman ML, Riddle TM, Flagella M, Duffy JJ, Doetschman T, Miller ML, Shull GE (1998) Phenotype resembling Gitelman's syndrome in

mice lacking the apical Na^+-Cl^- cotransporter of the distal convoluted tubule. J Biol Chem 273(44):29150–29155
Scudieri P, Sondo E, Ferrera L, Galietta LJ (2012) The anoctamin family: TMEM16A and TMEM16B as calcium-activated chloride channels. Exp Physiol 97(2):177–183
Sehlin J (1978) Interrelationship between chloride fluxes in pancreatic islets and insulin release. Am J Physiol 235(5):E501–E508
Sehlin J (1981) Are Cl^- mechanisms in mouse pancreatic islets involved in insulin release? Ups J Med Sci 86(2):177–182
Sehlin J, Meissner HP (1988) Effects of Cl^- deficiency on the membrane potential in mouse pancreatic β-cells. Biochim Biophys Acta 937(2):309–318
Semino MC, Gagliardino AM, Bianchi C, Rebolledo OR, Gagliardino JJ (1990) Early changes in the rat pancreatic B cell size induced by glucose. Acta Anat (Basel) 138(4):293–296
Sener A, Malaisse WJ (1976) Measurement of lactic acid in nanomolar amounts. Reliability of such a method as an index of glycolysis in pancreatic islets. Biochem Med 15(1):34–41
Sheader EA, Brown PD, Best L (2001) Swelling-induced changes in cytosolic [Ca^{2+}] in insulin-secreting cells: a role in regulatory volume decrease? Mol Cell Endocrinol 181(1–2):179–187
Shepherd RM, Henquin JC (1995) The role of metabolism, cytoplasmic Ca^{2+}, and pH-regulating exchangers in glucose-induced rise of cytoplasmic pH in normal mouse pancreatic islets. J Biol Chem 270(14):7915–7921
Shepherd RM, Gilon P, Henquin JC (1996) Ketoisocaproic acid and leucine increase cytoplasmic pH in mouse pancreatic B cells: role of cytoplasmic Ca^{2+} and pH-regulating exchangers. Endocrinology 137(2):677–685
Shimizu T, Iehara T, Sato K, Fujii T, Sakai H, Okada Y (2013) TMEM16F is a component of a Ca^{2+}-activated Cl^- channel but not a volume-sensitive outwardly rectifying Cl^- channel. Am J Physiol Cell Physiol 304(8):C748–C759
Shiozaki A, Miyazaki H, Niisato N, Nakahari T, Iwasaki Y, Itoi H, Ueda Y, Yamagishi H, Marunaka Y (2006) Furosemide, a blocker of $Na^+/K^+/2Cl^-$ cotransporter, diminishes proliferation of poorly differentiated human gastric cancer cells by affecting G0/G1 state. J Physiol Sci 56(6):401–406
Simon DB, Lifton RP (1998) Mutations in Na(K)Cl transporters in Gitelman's and Bartter's syndromes. Curr Opin Cell Biol 10(4):450–454
Simon DB, Karet FE, Hamdan JM, DiPietro A, Sanjad SA, Lifton RP (1996) Bartter's syndrome, hypokalaemic alkalosis with hypercalciuria, is caused by mutations in the Na-K-2Cl cotransporter NKCC2. Nat Genet 13(2):183–188
Soejima K, Landing BH (1986) Pancreatic islets in older patients with cystic fibrosis with and without diabetes mellitus: morphometric and immunocytologic studies. Pediatr Pathol 6(1):25–46
Solc CK, Wine JJ (1991) Swelling-induced and depolarization-induced C1-channels in normal and cystic fibrosis epithelial cells. Am J Physiol 261(4 Pt 1):C658–C674
Soleimani M (2013) SLC26 Cl^-/HCO_3^- exchangers in the kidney: roles in health and disease. Kidney Int 84:657–666
Somers G, Sener A, Devis G, Malaisse WJ (1980) The stimulus-secretion coupling of glucose-induced insulin release. XLV. The anion-osmotic hypothesis for exocytosis. Pflugers Arch 388 (3):249–253
Sperelakis N (2012) Origin of resting membrane potentials. In: Sperelakis N (ed) Cell physiology sourcebook: essentials of membrane biophysics, 4th edn. Elsevier/Academic, Amsterdam/ Boston
Strange K, Emma F, Jackson PS (1996) Cellular and molecular physiology of volume-sensitive anion channels. Am J Physiol 270(3 Pt 1):C711–C730
Stutts MJ, Canessa CM, Olsen JC, Hamrick M, Cohn JA, Rossier BC, Boucher RC (1995) CFTR as a cAMP-dependent regulator of sodium channels. Science 269(5225):847–850

Szollosi A, Nenquin M, Aguilar-Bryan L, Bryan J, Henquin JC (2007) Glucose stimulates Ca^{2+} influx and insulin secretion in 2-week-old β-cells lacking ATP-sensitive K^+ channels. J Biol Chem 282(3):1747–1756

Takahashi N, Chernavvsky DR, Gomez RA, Igarashi P, Gitelman HJ, Smithies O (2000) Uncompensated polyuria in a mouse model of Bartter's syndrome. Proc Natl Acad Sci USA 97(10):5434–5439

Takii M, Ishikawa T, Tsuda H, Kanatani K, Sunouchi T, Kaneko Y, Nakayama K (2006) Involvement of stretch-activated cation channels in hypotonically induced insulin secretion in rat pancreatic β-cells. Am J Physiol Cell Physiol 291(6):C1405–C1411

Tamagawa T, Henquin JC (1983) Chloride modulation of insulin release, $^{86}Rb^+$ efflux, and $^{45}Ca^{2+}$ fluxes in rat islets stimulated by various secretagogues. Diabetes 32(5):416–423

Taneera J, Jin Z, Jin Y, Muhammed SJ, Zhang E, Lang S, Salehi A, Korsgren O, Renstrom E, Groop L, Birnir B (2012) gamma-Aminobutyric acid (GABA) signalling in human pancreatic islets is altered in type 2 diabetes. Diabetologia 55(7):1985–1994

Uchida K, Tominaga M (2011) The role of thermosensitive TRP (transient receptor potential) channels in insulin secretion. Endocr J 58(12):1021–1028

Uvarov P, Ludwig A, Markkanen M, Pruunsild P, Kaila K, Delpire E, Timmusk T, Rivera C, Airaksinen MS (2007) A novel N-terminal isoform of the neuron-specific K-Cl cotransporter KCC2. J Biol Chem 282(42):30570–30576

Verkman AS, Synder D, Tradtrantip L, Thiagarajah JR, Anderson MO (2013) CFTR inhibitors. Curr Pharm Des 19(19):3529–3541

Wakade AR, Malhotra RK, Sharma TR, Wakade TD (1986) Changes in tonicity of perfusion medium cause prolonged opening of calcium channels of the rat chromaffin cells to evoke explosive secretion of catecholamines. J Neurosci 6(9):2625–2634

Wall SM, Fischer MP (2002) Contribution of the Na^+-K^+-$2Cl^-$ cotransporter (NKCC1) to transepithelial transport of H^+, NH_4^+, K^+, and Na^+ in rat outer medullary collecting duct. J Am Soc Nephrol 13(4):827–835

Wei WC, Akerman CJ, Newey SE, Pan J, Clinch NW, Jacob Y, Shen MR, Wilkins RJ, Ellory JC (2011) The potassium-chloride cotransporter 2 promotes cervical cancer cell migration and invasion by an ion transport-independent mechanism. J Physiol 589(Pt 22):5349–5359

White MM, Aylwin M (1990) Niflumic and flufenamic acids are potent reversible blockers of Ca^{2+}-activated Cl^- channels in Xenopus oocytes. Mol Pharmacol 37(5):720–724

Wilke M, Buijs-Offerman RM, Aarbiou J, Colledge WH, Sheppard DN, Touqui L, Bot A, Jorna H, de Jonge HR, Scholte BJ (2011) Mouse models of cystic fibrosis: phenotypic analysis and research applications. J Cyst Fibros 10(Suppl 2):S152–S171

Woo NS, Lu J, England R, McClellan R, Dufour S, Mount DB, Deutch AY, Lovinger DM, Delpire E (2002) Hyperexcitability and epilepsy associated with disruption of the mouse neuronal-specific K-Cl cotransporter gene. Hippocampus 12(2):258–268

Worrell RT, Butt AG, Cliff WH, Frizzell RA (1989) A volume-sensitive chloride conductance in human colonic cell line T84. Am J Physiol 256(6 Pt 1):C1111–C1119

Worrell RT, Merk L, Matthews JB (2008) Ammonium transport in the colonic crypt cell line, T84: role for Rhesus glycoproteins and NKCC1. Am J Physiol Gastrointest Liver Physiol 294(2): G429–G440

Wright EM, Diamond JM (1977) Anion selectivity in biological systems. Physiol Rev 57(1):109–156

Wright R, Raimondo JV, Akerman CJ (2011) Spatial and temporal dynamics in the ionic driving force for GABA(A) receptors. Neural Plast 2011:728395

Xue H, Liu S, Ji T, Ren W, Zhang XH, Zheng LF, Wood JD, Zhu JX (2009) Expression of NKCC2 in the rat gastrointestinal tract. Neurogastroenterol Motil 21(10):1068–e1089

Yang YD, Cho H, Koo JY, Tak MH, Cho Y, Shim WS, Park SP, Lee J, Lee B, Kim BM, Raouf R, Shin YK, Oh U (2008) TMEM16A confers receptor-activated calcium-dependent chloride conductance. Nature 455(7217):1210–1215

Yang SS, Lo YF, Yu IS, Lin SW, Chang TH, Hsu YJ, Chao TK, Sytwu HK, Uchida S, Sasaki S, Lin SH (2010) Generation and analysis of the thiazide-sensitive Na^+ -Cl^- cotransporter (Ncc/Slc12a3) Ser707X knockin mouse as a model of Gitelman syndrome. Hum Mutat 31(12):1304–1315

Yang Y, Fu A, Wu X, Reagan JD (2012) GPR35 is a target of the loop diuretic drugs bumetanide and furosemide. Pharmacology 89(1–2):13–17

Zaarour N, Demaretz S, Defontaine N, Zhu Y, Laghmani K (2012) Multiple evolutionarily conserved Di-leucine like motifs in the carboxyl terminus control the anterograde trafficking of NKCC2. J Biol Chem 287(51):42642–42653

Zeuthen T, Macaulay N (2012) Cotransport of water by Na^+-K^+-2Cl^- cotransporters expressed in Xenopus oocytes: NKCC1 versus NKCC2. J Physiol 590(Pt 5):1139–1154

Zhang ZR, Cui G, Liu X, Song B, Dawson DC, McCarty NA (2005) Determination of the functional unit of the cystic fibrosis transmembrane conductance regulator chloride channel. One polypeptide forms one pore. J Biol Chem 280(1):458–468

Zhao C, Wilson MC, Schuit F, Halestrap AP, Rutter GA (2001) Expression and distribution of lactate/monocarboxylate transporter isoforms in pancreatic islets and the exocrine pancreas. Diabetes 50(2):361–366

Zhou Z, Treis D, Schubert SC, Harm M, Schatterny J, Hirtz S, Duerr J, Boucher RC, Mall MA (2008) Preventive but not late amiloride therapy reduces morbidity and mortality of lung disease in βENaC-overexpressing mice. Am J Respir Crit Care Med 178(12):1245–1256

Zhu L, Lovinger D, Delpire E (2005) Cortical neurons lacking KCC2 expression show impaired regulation of intracellular chloride. J Neurophysiol 93(3):1557–1568

Zhu JX, Xue H, Ji T, Xing Y (2011) Cellular localization of NKCC2 and its possible role in the Cl^- absorption in the rat and human distal colonic epithelia. Transl Res 158(3):146–154

Electrical, Calcium, and Metabolic Oscillations in Pancreatic Islets

15

Richard Bertram, Arthur Sherman, and Leslie S. Satin

Contents

Abstract

Oscillations are an integral part of insulin secretion and are due ultimately to oscillations in the electrical activity of pancreatic β-cells, called bursting. We discuss the underlying mechanisms for bursting oscillations in mouse islets and the parallel oscillations in intracellular calcium and metabolism. We present a unified biophysical model, called the dual oscillator model, in which fast electrical oscillations are due to the feedback of Ca^{2+} onto K^+ ion channels and the slow component is due to oscillations in glycolysis. The combination of these mechanisms can produce the wide variety of bursting and Ca^{2+} oscillations

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_10,

observed in islets, including fast, slow, compound, and accordion bursting. We close with a description of recent experimental studies that have tested unintuitive predictions of the model and have thereby provided the best evidence to date that oscillations in glycolysis underlie the slow (~5 min) component of electrical, calcium, and metabolic oscillations in mouse islets.

Keywords
Bursting • Insulin secretion • Islet • Oscillations • Pulsatility

Like many neurons and endocrine cells, pancreatic β-cells are electrically excitable, producing electrical impulses in response to elevations in glucose. The electrical spiking pattern typically comes in the form of bursting and is most well studied in mouse islets. Bursting is characterized as periodic clusters of impulses followed by silent phases when there is a cessation of impulse firing (Fig. 1). In this chapter we discuss the different types of bursting patterns observed in mouse islets and the underlying mechanisms for these oscillations and parallel oscillations in intracellular Ca^{2+} and metabolism.

Bursting electrical activity is important since it leads to oscillations in the intracellular free Ca^{2+} concentration (Santos et al. 1991; Beauvois et al. 2006), which in turn lead to oscillations in insulin secretion (Gilon et al. 1993). Oscillatory insulin levels have been measured in vivo (Lang et al. 1981; Pørksen et al. 1995; Pørksen 2002; Nunemaker et al. 2005), and sampling from the hepatic portal vein in rats, dogs, and humans shows large oscillations with a period of 4–5 min (Song et al. 2000; Matveyenko et al. 2008). Deconvolution analysis demonstrates that the oscillatory insulin level is due to oscillatory secretion of insulin from islets (Pørksen et al. 1997; Matveyenko et al. 2008), and in humans at least 75 % of insulin secretion is in the form of insulin pulses (Pørksen et al. 1997). The amplitude of insulin oscillations in the peripheral blood of human subjects is ~100 times smaller than in the hepatic portal vein (Song et al. 2000). This attenuation is confirmed by findings of hepatic insulin clearance of ~50 % in dogs (Polonsky et al. 1983) and ~40–80 % in humans (Eaton et al. 1983; Meier et al. 2005). It has also been demonstrated that the hepatic insulin clearance rate itself is oscillatory, corresponding to portal insulin oscillations. That is, during the peak of an insulin oscillation, the insulin clearance rate is greater than during the trough (Meier et al. 2005). This illustrates that insulin oscillations are treated differently by the liver than non-pulsatile insulin levels and thus suggests an important role for oscillations in the hepatic processing of insulin and, presumably, of glucose. Additional supporting evidence for this is provided by a study showing that glucose clearance is facilitated when insulin is pulsatile (Matveyenko et al. 2012). Clinical evidence for the importance of pulsatile insulin comes from studies showing that coherent insulin oscillations are disturbed or lost in patients with type II diabetes and their near relatives (Matthews et al. 1983; Weigle 1987; O'Rahilly et al. 1988; Polonsky et al. 1988).

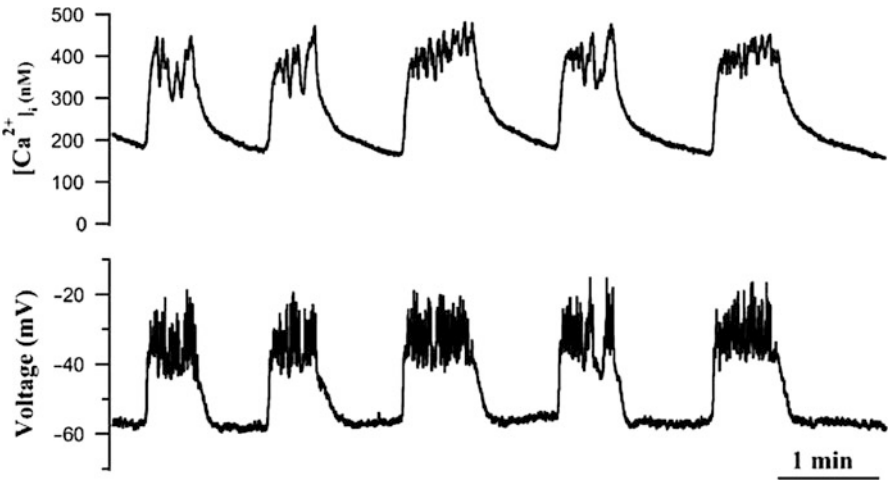

Fig. 1 Intracellular free Ca^{2+} concentration measured using fura-2002FAM (*top*) and electrical bursting (*bottom*) recorded from a mouse islet (Reprinted from Zhang et al. (2003))

Oscillations in insulin have also been observed in the perifused pancreas (Stagner et al. 1980) and in isolated islets (Longo et al. 1991; Bergsten and Hellman 1993; Gilon et al. 1993; Beauvois et al. 2006; Ritzel et al. 2006). The oscillations have two distinct periods; the faster oscillations have a period of 2 min or less (Gilon et al. 1993; Bergsten 1995, 1998; Nunemaker et al. 2005), while the slower oscillations have greater periods of often 4 min or more (Pørksen et al. 1995; Pørksen 2002; Nunemaker et al. 2005). In one recent study, insulin measurements were made in vivo in mice, and it was shown that some mice had insulin oscillations with a period of 1–2 min ("fast mice"), while others exhibited a greater period of 3–5 min ("slow mice"). Interestingly, most of the islets examined in vitro from a given mouse had Ca^{2+} oscillations with similar periods. Most islets from "fast mice" had fast Ca^{2+} oscillations, while most of those examined from "slow mice" exhibited either slow or compound Ca^{2+} oscillations (fast oscillations superimposed on slow ones). Thus, it was conjectured that islets within a single animal are imprinted with a relatively uniform oscillation period that is reflected in the insulin profile in vivo. As we describe later, the two time scales of electrical bursting can explain the two components of oscillatory insulin secretion and their combinations.

The Role of Calcium Feedback

Ca^{2+} enters β-cells through Ca^{2+} channels during the active phase of a burst, during which it accumulates and activates Ca^{2+}-dependent K^+ channels (Göpel et al. 1999; Goforth et al. 2002). The resulting hyperpolarizing current is in many cases sufficient to terminate the active phase of the burst, and the time required to deactivate the current can set the duration of the silent phase of the burst (Chay and Keizer 1983).

Indeed, the first mathematical model for bursting in β-cells was based on this mechanism (Chay and Keizer 1983). The endoplasmic reticulum (ER) plays a major role in shaping the Ca^{2+} dynamics, taking up Ca^{2+} during the active phase of a burst (using the sarco-endoplasmic reticulum Ca^{2+} ATPase or SERCA pump (Ravier et al. 2011)) when Ca^{2+} influx into the cytosolic compartment is large and releasing Ca^{2+} during the silent phase of the burst. These filtering actions have a significant impact on the time dynamics of the cytosolic Ca^{2+} concentration and on the period of bursting. The influence of the ER on cytosolic free Ca^{2+} dynamics was convincingly demonstrated using pulses of KCl to effectively voltage clamp the entire islet (Gilon et al. 1999; Arredouani et al. 2002). Using 30-s pulses, similar to the duration of a fast burst, it was shown that the amplitude of the Ca^{2+} response to depolarization was greater when the ER was drained of Ca^{2+} by pharmacologically blocking ER Ca^{2+} pumps (SERCA). In addition, the slow decline of the cytosolic Ca^{2+} concentration that followed the more rapid declining phase of cytosolic Ca^{2+} was absent when SERCA pumps were blocked. The mechanisms for these effects were determined in mathematical modeling studies (Bertram and Sherman 2004b; Bertram and Arceo II 2008), and it was demonstrated that an active form of Ca^{2+}-induced Ca^{2+} release (CICR) is inconsistent with data from Gilon et al. (1999) and Arredouani et al. (2002). CICR did occur in single β-cells in response to cyclic AMP (Ämmälä et al. 1993), but in this case electrical activity and Ca^{2+} oscillations are out of phase (Keizer and De Young 1993; Zhan et al. 2008), which contrasts with the in-phase oscillations observed in glucose-stimulated islets (Santos et al. 1991; Beauvois et al. 2006). These predictions of the model were confirmed recently by measurements for the first time of Ca^{2+} in the ER during cytoplasmic Ca^{2+} oscillations (Higgins et al. 2006).

In addition to the direct effect on Ca^{2+}-activated K^+ channels, intracellular Ca^{2+} has two opposing effects on glucose metabolism in β-cells. Most of the Ca^{2+} that enters the cell is pumped out of the cell or into the ER by Ca^{2+} ATPases, which utilize ATP and thus decrease the intracellular ATP concentration (Detimary et al. 1998). Ca^{2+} that enters mitochondria through the Ca^{2+} uniporter depolarizes the mitochondrial inner membrane potential and thus reduces the driving force for mitochondrial ATP production (Magnus and Keizer 1997, 1998; Krippeit-Drews et al. 2000; Kindmark et al. 2001). Once inside the mitochondria, free Ca^{2+} stimulates pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase (Civelek et al. 1996; MacDonald et al. 2003), resulting in increased production of NADH, which can increase mitochondrial ATP production. Thus, Ca^{2+} has two opposing effects on the ATP/ADP ratio; one may dominate under some conditions, while the other action dominates in different conditions.

The ATP/ADP ratio is relevant for islet electrical activity due to the presence of ATP-sensitive K^+ channels, which link the potential of the plasma membrane to the metabolic state of the β-cell (Ashcroft et al. 1984). Variations in the nucleotide ratio result in variation of the fraction of open K_{ATP} channels. Thus, oscillations in the intracellular Ca^{2+} concentration can lead to oscillations in the ATP/ADP ratio, which can contribute to bursting through the action of hyperpolarizing K_{ATP} current (Keizer and Magnus 1989; Henquin 1990; Smolen and Keizer 1992; Bertram and Sherman 2004a).

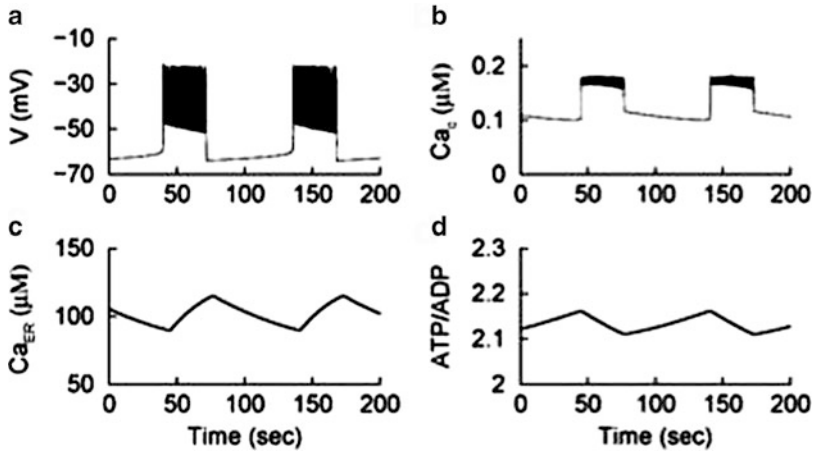

Fig. 2 Model simulation of bursting illustrating the dynamics of membrane potential (V), free cytosolic Ca^{2+} concentration (Ca_c), free ER Ca^{2+} concentration (Ca_{ER}), and the ATP/ADP concentration ratio. The model is described in Bertram and Sherman (2004a) and the computer code can be downloaded from www.math.fsu.edu/~bertram/software/islet

Figure 2 uses a mathematical model (Bertram and Sherman 2004a) to demonstrate the dynamics of the variables described above. (Other models have been developed, postulating different burst mechanisms and highlighting other biochemical pathways (Fridlyand et al. 2003; Diederichs 2006; Cha et al. 2011).) Two bursts are shown in Fig. 2a and the cytosolic free Ca^{2+} concentration (Ca_c) is shown in Fig. 2b. At the beginning of an active phase, Ca_c quickly rises to a plateau that persists throughout the burst. Simultaneously, the ER free Ca^{2+} concentration (Ca_{ER}) slowly increases as SERCA activity begins to fill the ER with Ca^{2+} (Fig. 2c). In contrast, the ATP/ADP ratio during a burst declines (Fig. 2d), since in this model the negative effect of Ca^{2+} on the ATP level dominates the positive effect. Both K_{Ca} and K_{ATP} currents, concomitantly activated by the increased Ca^{2+} and decreased ATP/ADP, respectively, combine to terminate the burst, after which Ca_c slowly declines. This slow decline reflects the passive release of Ca^{2+} from the ER during the silent phase of the burst, along with the removal of Ca^{2+} from the cell by Ca^{2+} pumps in the plasma membrane. Also, ATP/ADP increases during the silent phase, slowly turning off K_{ATP} current. The combined effect of reducing K_{Ca} and K_{ATP} currents eventually leads to the initiation of a new active phase and the cycle restarts.

Metabolic Oscillations

As described above and illustrated in Fig. 2, metabolic oscillations can arise from the effects of Ca^{2+} on the mitochondria and ATP utilization by pumps. In addition, there is considerable evidence for Ca^{2+}-independent metabolic oscillations,

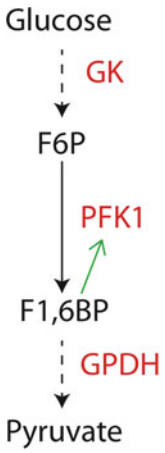

Fig. 3 A few steps in glycolysis, focusing on the positive feedback of F1,6BP onto the allosteric enzyme PFK1 (*green arrow*). *Dashed arrows* indicate several steps in the glycolytic process, one of which is labeled. *GK* glucokinase, *F6P* fructose 6-phosphate, *F1,6BP* fructose 1,6-bisphosphate, *GPDH* glyceraldehyde 3-phosphate dehydrogenase

reviewed in Tornheim (1997) and Bertram et al. (2007b). The leading hypothesis is that glycolysis is oscillatory and is the primary mechanism underlying pulsatile insulin secretion from β-cells (Tornheim 1997). The M-type isoform of the glycolytic enzyme phosphofructokinase-1 (PFK1) is known to exhibit oscillatory activity in muscle extracts, as measured by oscillations in the levels of the PFK1 substrate fructose 6-phosphate (F6P) and the PFK1 product fructose 1,6-bisphosphate (F1,6BP) (Tornheim and Lowenstein 1974; Tornheim et al. 1991). The period of these oscillations, 5–10 min, is similar to the period of slow insulin oscillations (Tornheim 1997). The mechanism for the oscillatory activity of this isoform, which is the dominant PFK1 isoform in islets (Yaney et al. 1995), is the positive feedback of its product F1,6BP on phosphofructokinase activity (Fig. 3) and subsequent depletion of its substrate, F6P (Tornheim 1979; Smolen 1995; Westermark and Lansner 2003). While there is currently no direct evidence for glycolytic oscillations in β-cells, there is substantial evidence for metabolic oscillations. This comes mainly from measurements of oscillations in several key metabolic variables, such as oxygen consumption (Longo et al. 1991; Ortsäter et al. 2000; Bergsten et al. 2002; Kennedy et al. 2002), ATP or the ATP/ADP ratio (Nilsson et al. 1996; Ainscow and Rutter 2002; Juntti-Berggren et al. 2003), the mitochondrial inner membrane potential (Kindmark et al. 2001), lactate release (Chou et al. 1992), and NAD(P)H levels (Luciani et al. 2006). Additionally, it has been demonstrated that patients with homozygous PFK1-M deficiency are predisposed to type II diabetes (Ristow et al. 1997), and in a study on humans with an inherited deficiency of PFK1-M, it was shown that oscillations in insulin secretion were impaired (Ristow et al. 1999). These data suggest that the origin of insulin secretion oscillations is glycolysis. In the second part of this chapter, we discuss additional evidence for glycolytic oscillations, in the context of islet bursting.

There is a long history of modeling of glycolytic oscillations, notably in yeast. Our model has a similar dynamic structure, based on fast positive feedback and slow negative feedback, to some of those models but differs in the identification of

sources of feedback. In the models of Sel'kov (1968) and Goldbeter and Lefever (1972), ATP was considered the substrate, whose depletion provided the negative feedback as F6P does in our model, and ADP was considered the product, which provided the positive feedback, as F1,6BP does in our model.

Such models can be combined with electrical activity to produce many of the patterns described here (Wierschem and Bertram 2004), but the biochemical interpretation is different. In our view, ATP acts rather as a negative modulator, which tends to shut down glycolysis when energy stores are replete, and ADP is a positive modulator, which activates glycolysis when ATP production falls behind metabolic demand. More fundamentally, we argue that β-cells, as metabolic sensors, differ from primary energy-consuming tissues such as the muscle in that they need to activate metabolism whenever glucose is present even if the cell has all the ATP it needs. In this view, ATP and ADP are not suitable to serve as essential dynamic variables but do play significant roles as signaling molecules in regulating activity.

The Dual Oscillator Model for Islet Oscillations

Recent islet data provide the means to disentangle the influences of Ca^{2+} feedback and glycolysis on islet oscillations. Figure 4a shows compound Ca^{2+} oscillations, recorded from islets in 15 mM glucose. There is a slow component (period ~5 min) with much faster oscillations superimposed on the slower plateaus. These compound oscillations have been frequently observed by a number of research groups

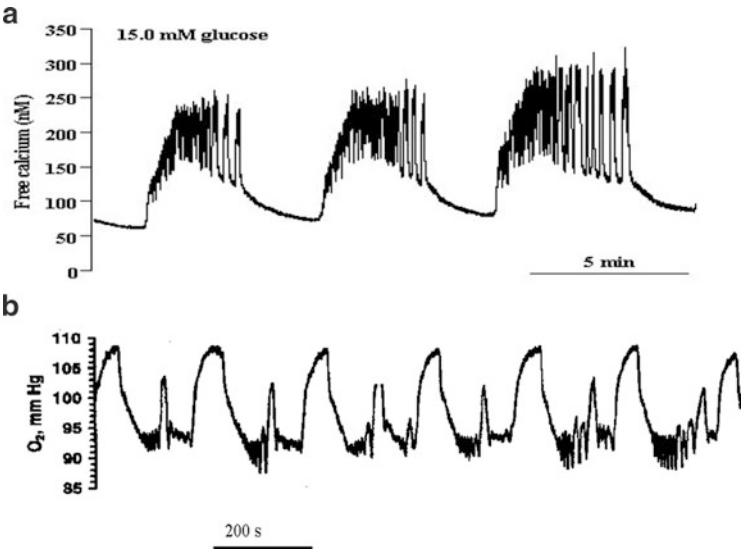

Fig. 4 (**a**) Compound islet Ca^{2+} oscillations measured using fura-2/AM. The oscillations consist of slow episodes of fast oscillations (Reprinted from Bertram et al. (2004)). (**b**) Slow oxygen oscillations with superimposed fast "teeth" (Reprinted from Jung et al. (1999))

(Valdeolmillos et al. 1989; Bergsten et al. 1994; Zhang et al. 2003; Beauvois et al. 2006) and reflect compound bursting oscillations, where fast bursts are clustered together into slower episodes (Henquin et al. 1982; Cook 1983). Figure 4b shows measurements of islet oxygen levels in 10 mM glucose (Jung et al. 1999). Again there are large-amplitude slow oscillations (period of 3–4 min) with superimposed fast oscillations or "teeth." Similar compound oscillations have been observed in intra-islet glucose and in insulin secretion (Jung et al. 2000; Dahlgren et al. 2005), as assayed by Zn^{2+} efflux from β-cells. These data showing compound oscillations in a diversity of cellular variables suggest that compound oscillations are fundamental to islet function.

We have hypothesized that the slow component of the compound oscillations reflects oscillations in glycolysis, while the fast component is due to Ca^{2+} feedback onto ion channels and metabolism. This hypothesis has been implemented as a mathematical model, which we call the "dual oscillator model" (Bertram et al. 2004a, 2007a). The strongest evidence for this model is its ability to account for the wide range of time courses of Ca^{2+} and metabolic variables observed in glucose-stimulated islets both in vitro and in vivo. The fast oscillations introduced above do not have an underlying slow component. An example is shown in Fig. 5a. The dual oscillator model reproduces this pattern (Fig. 5b) when glycolysis is non-oscillatory (Fig. 5c). The fast oscillations are mainly due to the effects of Ca^{2+} feedback onto K^+ channels as discussed earlier. Compound oscillations (Fig. 5d) are also produced by the model (Fig. 5e) and occur when both glycolysis and electrical activity are oscillatory (Fig. 5f) and become phase locked.

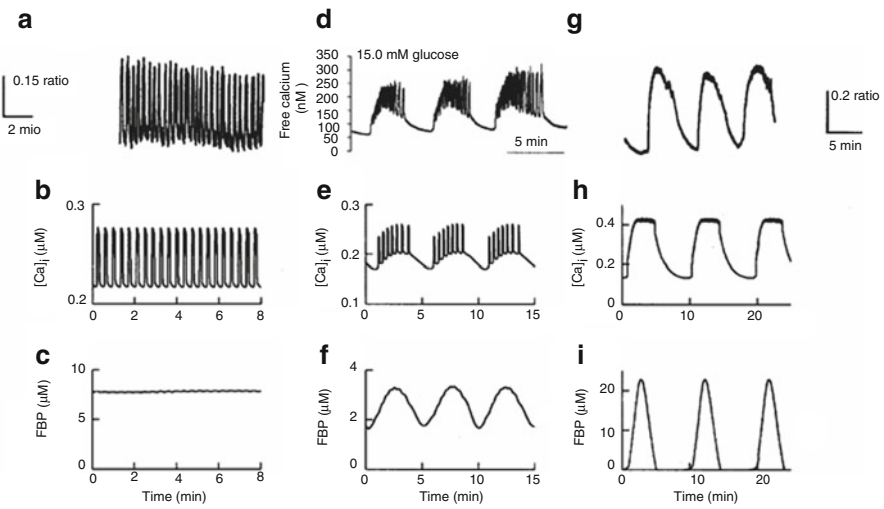

Fig. 5 Three types of oscillations typically observed in islets. *Top row* of *panels* is from islet measurements of Ca^{2+} using fura-2/AM (**a**, **d**, **g**). *Middle row* shows simulations of Ca^{2+} oscillations using the dual oscillator model (**b**, **e**, **h**). *Bottom row* shows simulations of the glycolytic intermediate fructose 1,6-bisphosphate (*FBP*), indicating that glycolysis is either stationary (**c**) or oscillatory (**f**, **i**) (Reprinted from Bertram et al. (2004a, 2007b) and Nunemaker et al. (2005))

The glycolytic oscillations provide the slow envelope, and electrically driven Ca^{2+} oscillations produce the fast pulses of Ca^{2+} that ride on the slow wave. A variant of compound bursting, not shown in Fig. 5, consists of fast bursting with a slowly changing plateau fraction. This pattern, which we call "accordion bursting," has been observed in membrane potential, Ca^{2+}, and oxygen (Henquin et al. 1982; Cook 1983; Bergsten et al. 1994; Kulkarni et al. 2004).

Compound oscillations in Ca^{2+} are accompanied by slow oscillations in O_2 with "teeth," as in Fig. 4b. The slow oscillations in the flux of metabolites from glycolysis to the mitochondria result in slow oscillations in O_2 consumption by the mitochondrial electron transport chain. The Ca^{2+} feedback onto mitochondrial respiration also affects O_2 consumption, resulting in the faster and smaller O_2 oscillations. Pure slow oscillations (Fig. 5g) are also reproduced by the model (Fig. 5h) when glycolysis is oscillatory (Fig. 5i) and the cell is tonically active during the peak of glycolytic activity. Thus, a model that combines glycolytic oscillations with Ca^{2+}-dependent oscillations can produce the three types of oscillatory patterns typically observed in islets, as well as faster oscillations in the O_2 time course when in compound mode.

Accordion bursting, like compound bursting, is accompanied by O_2 oscillations with fast teeth, but now present at all phases of the oscillation both in the model (Bertram et al. 2004a) and in experiments (Kulkarni et al. 2004). The model thus suggests that the compound and accordion modes are just quantitative variants of the same underlying mechanisms. The former can be converted into the latter by reducing the conductance of the K_{ATP} current, limiting its ability to repolarize the islets. It also supports the notion that β-cells have two oscillators that interact but can also occur independently of each other.

Glucose Sensing in the Dual Oscillator Framework

The concept of two semi-independent oscillators can be captured in a diagrammatic scheme (Fig. 6) representing how the two subsystems respond to changes in glucose. Depending on the glucose concentration, glycolysis can be low and steady, oscillatory, or high and steady. Similarly, the electrical activity can be off, oscillatory due to Ca^{2+} feedback, or in a continuous-spiking state. The two oscillators thus have glucose thresholds separating their different activity states. Increasing the glucose concentration can cause both the glycolytic and electrical subsystems to cross their thresholds, but not necessarily at the same glucose concentrations.

The canonical case is for the two oscillators to become activated in parallel. For example, in Case 1 of Fig. 6, when the islet is in 6 mM glucose, both the glycolytic oscillator (GO) and electrical oscillator (EO) are in their low activity states. When glucose is raised to 11 mM, both oscillators are activated, yielding slow Ca^{2+} oscillations. In this scenario, the electrical burst duty cycle or plateau fraction of the slow oscillation, a good indicator of the relative rate of insulin secretion, increases with glucose concentration, as seen in classical studies of fast bursting (Dean and Mathews 1970; Meissner and Schmelz 1974; Beigelman and Ribalet

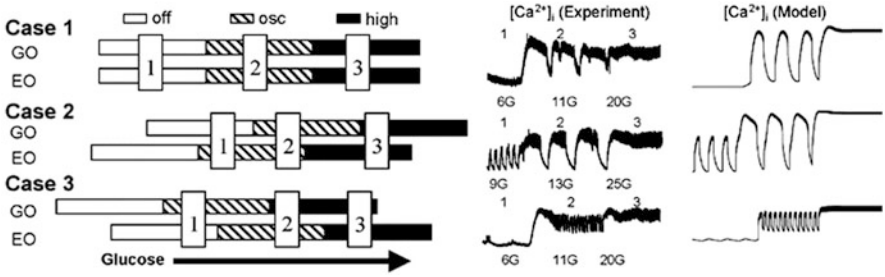

Fig. 6 Schematic diagram illustrating the central hypothesis of the dual oscillator model. In this hypothesis, there is an electrical subsystem that may be oscillatory (*osc*) or in a low (*off*) or high activity state. There is also a glycolytic subsystem that may be in a low or high stationary state or an oscillatory state. The glucose thresholds for the two subsystems need not be aligned, and different alignments can lead to different sequences of behaviors as the glucose concentration is increased (Reprinted from Nunemaker et al. (2006) and Bertram et al. (2007b))

1980). The increase in the glucose concentration in this regime has no effect on the amplitude of Ca^{2+} oscillations and little effect on the oscillation frequency (Nunemaker et al. 2006).

However, some islet responses have been observed to be transformed from fast to slow or compound oscillations when the glucose concentration was increased (Nunemaker et al. 2006). This dramatic increase in the oscillation period was accompanied by a large increase in the oscillation amplitude (Fig. 6, Case 2). We interpreted this as a switch from electrical to glycolytic oscillations and termed this transformation "regime change." The diagrammatic representation in Fig. 6 indicates that this occurs when the threshold for the GO is shifted to the left of that for the EO. This may occur if glucokinase is relatively active or K_{ATP} conductance is relatively low.

At 9 mM glucose the EO is on, but the GO is off, so only fast Ca^{2+} oscillations result, driven by fast bursting electrical activity. When glucose is increased to 13 mM, the lower threshold for glycolytic oscillations is crossed, and the fast Ca^{2+} oscillations combine with glycolytic oscillations to produce much slower and larger-amplitude slow or compound oscillations.

A final example is Case 3. In this islet, subthreshold Ca^{2+} oscillations are produced in 6 mM glucose, which we believe are due to activation of the GO, while the EO is in a low activity (or silent) state. When glucose is increased to 11 mM, the lower threshold for electrical oscillations is crossed, initiating a fast oscillatory Ca^{2+} pattern. However, the upper threshold for glycolytic oscillations is also crossed, so the glycolytic oscillations stop. As a result, a fast oscillatory Ca^{2+} pattern is produced, with only a transient underlying slow component.

In all three cases, when glucose is raised to 20 mM or higher, the system moves past the upper thresholds for both the GO and the EO, so there are neither electrical bursting oscillations nor glycolytic oscillations, and the islet generates a continuous-spiking pattern. The dual oscillator model accounts for each of these regime-change behaviors, as shown in the right column of Fig. 6.

Metabolic Oscillations Can Be Rescued by Calcium

Given that metabolic oscillations can be driven either by Ca^{2+} feedback onto ATP production/utilization or by an independent mechanism such as glycolytic oscillations, experimental tests have been developed to determine which of these occurs in islets. One such test takes the strategy of manipulating the islet so that Ca^{2+} oscillations do not occur. Figure 7 shows that when the islet is hyperpolarized with the K_{ATP} channel agonist diazoxide (250 μM), the oscillations in Ca^{2+} concentration, as measured by fura-2 fluorescence, and metabolism, as measured by NAD(P)H autofluorescence, are both terminated (Luciani et al. 2006; Bertram et al. 2007a). A similar test was performed by Kennedy and colleagues, except that they used an oxygen-sensing electrode to monitor metabolism (Kennedy et al. 2002). They also found that membrane hyperpolarization terminated metabolic oscillations.

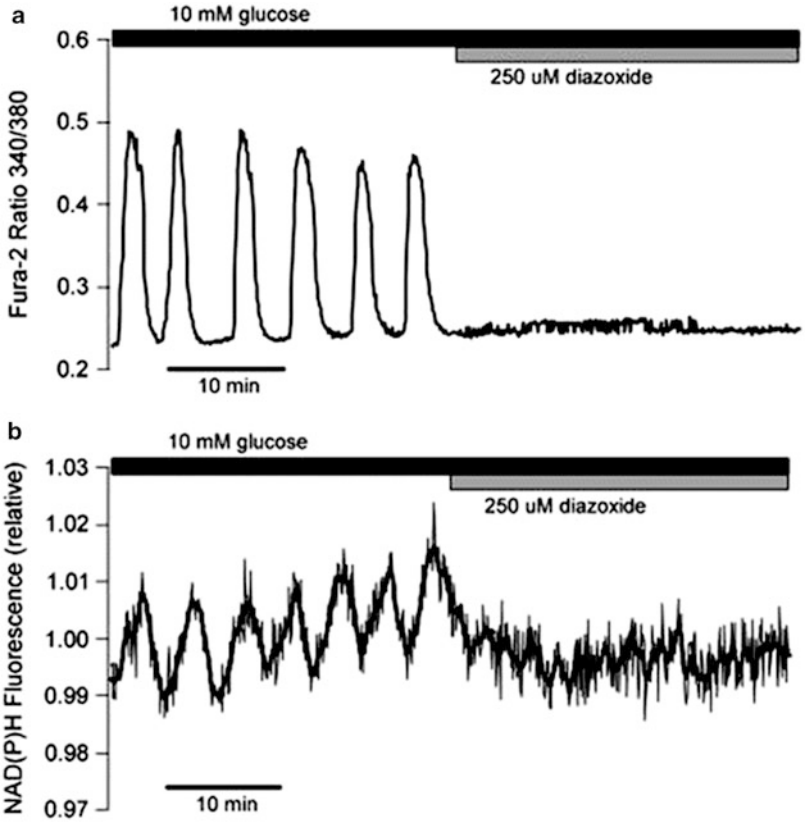

Fig. 7 Measurements of (**a**) fura-2 fluorescence and (**b**) NAD(P)H autofluorescence demonstrate that islet hyperpolarization with diazoxide can terminate metabolic oscillations (Reprinted from Bertram et al. (2007a))

It is tempting to conclude from these data that metabolic oscillations must be driven by Ca^{2+} oscillations, and in the absence of a mathematical model, this seems like a logical conclusion. Surprisingly, though, model simulations we carried out using the dual oscillator model showed that under some conditions islet hyperpolarization, as was done in Fig. 7, would in fact terminate metabolic oscillations *even if they were driven by glycolytic oscillations* (Bertram et al. 2007a). This is because the decline in Ca^{2+} influx that accompanies hyperpolarization reduces ATP utilization by Ca^{2+} pumps. This results in an increase in the cytosolic ATP concentration, and ATP inhibits the enzyme, PFK1, responsible for glycolytic oscillations.

Thus, cessation of metabolic oscillations by blocking Ca^{2+} oscillations does not imply that Ca^{2+} oscillations are necessary for metabolic oscillations, but the converse is valid. If metabolic oscillations are observed when Ca^{2+} is clamped, it must mean that the metabolic oscillations are not merely a reflection of Ca^{2+} oscillations. Indeed, the dual oscillator model predicts that, in many cases, it should be possible to have metabolic oscillations driven by glycolysis even though the Ca^{2+} concentration is clamped. This requires, however, that the level at which Ca^{2+} is clamped be sufficiently high so that the PFK1 enzyme is not inhibited by the elevated ATP that accompanies cessation of Ca^{2+} pumping. With this in mind, the model was used to design an experiment to truly test whether metabolic oscillations can exist in the absence of Ca^{2+} oscillations (Merrins et al. 2010). In the simulation shown in Fig. 8, a model islet exhibiting compound oscillations in stimulatory glucose was hyperpolarized by increasing the fraction of activated K_{ATP} channels (simulating the application of diazoxide). This reduced the cytosolic Ca^{2+} concentration, which in turn increased cytosolic ATP concentration through the reduced activity of Ca^{2+} pumps. Metabolic oscillations, as reflected in the mitochondrial NADH concentration, were thus terminated. The model islet was subsequently depolarized by increasing the Nernst potential for K^+ (simulating the application of KCl). The depolarization activates L-type Ca^{2+} channels in the plasma membrane, raising the level of Ca^{2+} in the cytosol (Fig. 8a). This induced increased activity of the Ca^{2+} pumps, increasing ATP hydrolysis and lowering the ATP level (Fig. 8b). The resultant disinhibition of PFK1 allowed glycolytic oscillations to reemerge (Fig. 8c). Importantly, these oscillations persisted in the absence of Ca^{2+} oscillations. The combination of diazoxide and KCl effectively clamps the Ca^{2+}: the diazoxide cuts the link between metabolism (i.e., ATP) and the membrane potential by opening the K_{ATP} channels, and the KCl inhibits action potential production and the accompanying Ca^{2+} oscillations that would result from spiking-induced Ca^{2+} influx.

The prediction that glycolytic oscillations can be rescued by elevating the intracellular Ca^{2+} level was tested experimentally (Merrins et al. 2010). Metabolic oscillations as measured through NAD(P)H autofluorescence were terminated by hyperpolarization (application of 200 μM diazoxide) in about two-thirds of the islets but persisted in the remainder. Subsequent application of KCl increased the level of intracellular Ca^{2+} while prohibiting Ca^{2+} oscillations (Fig. 9a, with different concentrations of KCl). In about one-half of the islets in which Ca^{2+} oscillations

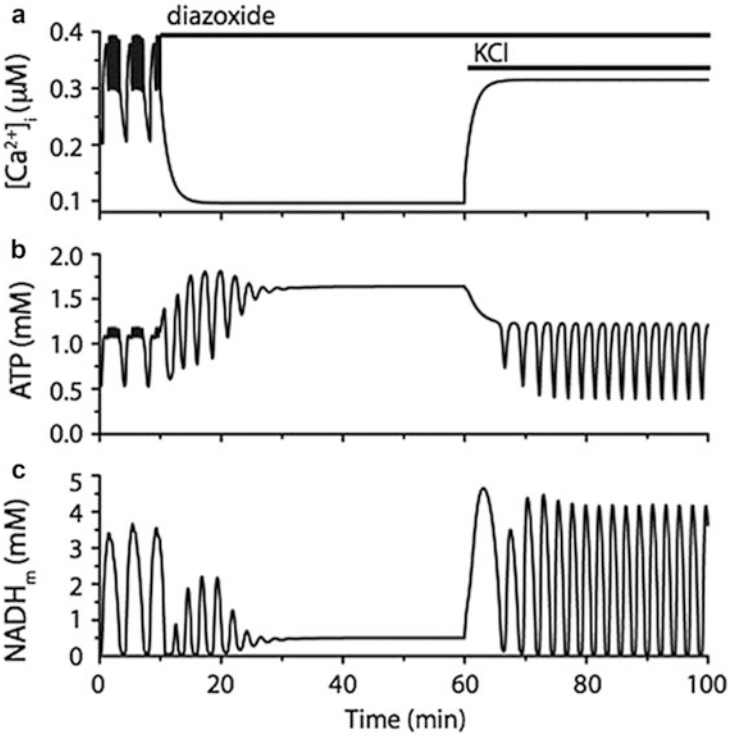

Fig. 8 Mathematical simulations demonstrating how increasing the level of intracellular free Ca^{2+} can rescue metabolic oscillations. Simulated application of diazoxide hyperpolarizes the islet, lowering the intracellular Ca^{2+} concentration (**a**). This greatly reduces the pumping needed to remove Ca^{2+} from the β-cell, thereby decreasing ATP utilization and increasing the ATP concentration in the cytosol (**b**). KCl depolarizes the membrane and subsequently increases the Ca^{2+} concentration, leading to enhanced ATP utilization via Ca^{2+} pumps and a decrease in the ATP concentration. The result of this is disinhibition of PFK1, which allows glycolytic oscillations to emerge (**c**) (Reprinted from Merrins et al. (2010))

had been terminated, metabolic oscillations were restored when the intracellular Ca^{2+} concentration was raised (Fig. 9b). Thus, it was demonstrated that metabolic oscillations can occur in islets in the absence of Ca^{2+} oscillations.

The experiments, however, raised two new questions: Why did slow metabolic oscillations persist in some islets but not in others, and why did raising Ca^{2+} restore oscillations in some islets but not in others? In the latter case, it is possible that oscillations would have been restored if a different KCl concentration had been used, but further mathematical analysis suggests another possibility. The model was found to support yet another type of slow metabolic oscillation that is neither secondary to Ca^{2+} oscillations nor fully independent of Ca^{2+} oscillations. In this regime, neither of the electrical oscillator nor metabolic oscillator is able to oscillate on its own, but only a reciprocal interaction between the two can result in oscillations (Watts et al. 2014).

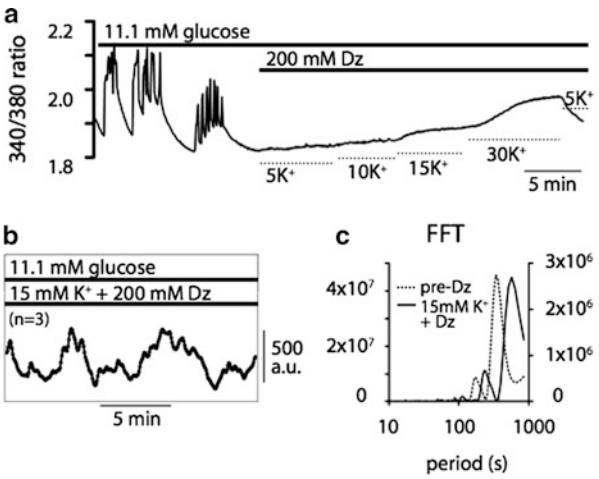

Fig. 9 Experimental test of the model prediction made in Fig. 8. (**a**) Compound Ca^{2+} oscillations are terminated by diazoxide (200 μM). The Ca^{2+} concentration is elevated when KCl is added to the bath, but Ca^{2+} oscillations are not initiated. (**b**) Oscillations in NAD(P)H autofluorescence can be initiated in the presence of diazoxide when KCl has been added to depolarize the cell and increase the level of Ca^{2+}. (**c**) Fourier power spectrum of the NAD(P)H fluorescence prior to the addition of diazoxide (*dotted*) and after diazoxide plus KCl. Large peaks occur at the period of slow metabolic oscillations (Reprinted from Merrins et al. (2010))

Manipulating Glycolysis Alters Oscillations in a Predictable Way

One way to determine if glycolysis is the source of metabolic oscillations is to manipulate it in such a way that glycolytic oscillations, if they exist, are altered in a predictable way. This was done in an indirect way in the experiments described above, through changes in the intracellular Ca^{2+} level, which acts on an inhibitor (ATP) of the key rhythmogenic enzyme PFK1. A second approach is to interfere with the feedback loop that is responsible for the production of putative glycolytic oscillations. This feedback comes from F1,6BP allosterically activating the enzyme that produced it, PFK1. The loop would be broken and the oscillation eliminated if this feedback were removed. In a similar vein, if the feedback were weakened through the actions of another molecule that competes with F1,6BP for the same binding site on PFK1, then the properties of the oscillation (e.g., oscillation frequency and amplitude) would change. A mathematical model of the reaction would allow one to predict the effect of the competitive activator, and if islet Ca^{2+} oscillations were effected in the same way, then this would provide evidence that the Ca^{2+} oscillations are driven by glycolytic oscillations.

This approach was taken in a recent study, which made use of the bifunctional enzyme phosphofructokinase-2/fructobisphosphatase-2, which we call PFK2/FBPase2 or BIF2 (Fig. 10). This enzyme has a kinase on the N-terminal (PFK2) and a phosphatase on the C-terminal (FBPase2). The kinase converts F6P to

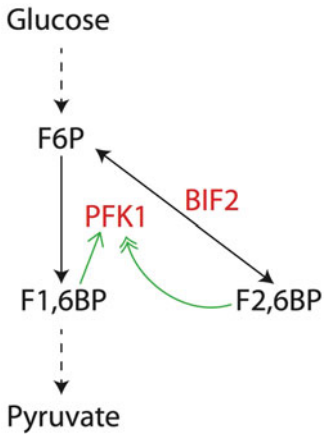

Fig. 10 Illustration showing that fructose 2,6-bisphosphate (F2,6BP) activates PFK1 (*double green arrow*) more strongly than does F1,6BP. The F2,6BP is generated by the enzyme PFK2/FBPase2 (*BIF2*)

fructose 2,6-bisphosphate (F2,6BP) and is the sole source of F2,6BP in the cell. The phosphatase end of the enzyme does the opposite. Importantly, F2,6BP is an allosteric activator of PFK1 and is a more potent activator of PFK1 than is F1,6BP (Malisse et al. 1982; Foe et al. 1983; Sener et al. 1984). It is therefore an ideal molecule for weakening the positive feedback of F1,6BP onto PFK1 and thus changing the properties of putative glycolytic oscillations.

In Merrins et al. (2012), mutants of the islet isoform of PFK2/FBPase2 (Sakurai et al. 1996; Arden et al. 2008) were expressed in mouse islets using an adenoviral delivery system. Four mutants were examined, but we focus here on only two. One mutant (DD-PFK2) contained only the PFK2 domain, tagged at the N-terminal with a degradation domain (DD), which permits transcription and translation, but prevents accumulation of functional protein in the cytosol due to rapid proteasomal degradation. This degradation can be inhibited by a small cell-permeable molecule called Shield1 (Banaszynski et al. 2006). The other mutant (DD-FBPase2) contained only the FBPase2 domain, also tagged at the N-terminal with DD. Adenoviral delivery of the DD-PFK2 mutant in the presence of Shield1 would then result in overproduction of PFK2 and an increase in the concentration of F2,6BP. In the absence of Shield1, functional PFK2 protein would not accumulate, so delivery of DD-PFK2 without Shield1 serves as a control. A similar strategy was used for DD-FBPase2, which when delivered in the presence of Shield1 increases FBPase2 concentration in the cell, resulting in a reduction in the F2,6BP concentration. One advantage of these truncation mutants is that neither activates glucokinase (Merrins et al. 2012), as the full BIF2 molecule is known to do (Langer et al. 2010).

A mathematical model of the allosteric PFK1 reaction was used to predict the effects on glycolytic oscillations of increasing or decreasing the concentration of the competitive allosteric activator F2,6BP. The model predicted that increasing F2,6BP should make oscillations faster and smaller in amplitude, and if F2,6BP was increased too much, the oscillations would be terminated. Decreasing the F2,6BP concentration should have the opposite effect, making glycolytic oscillations

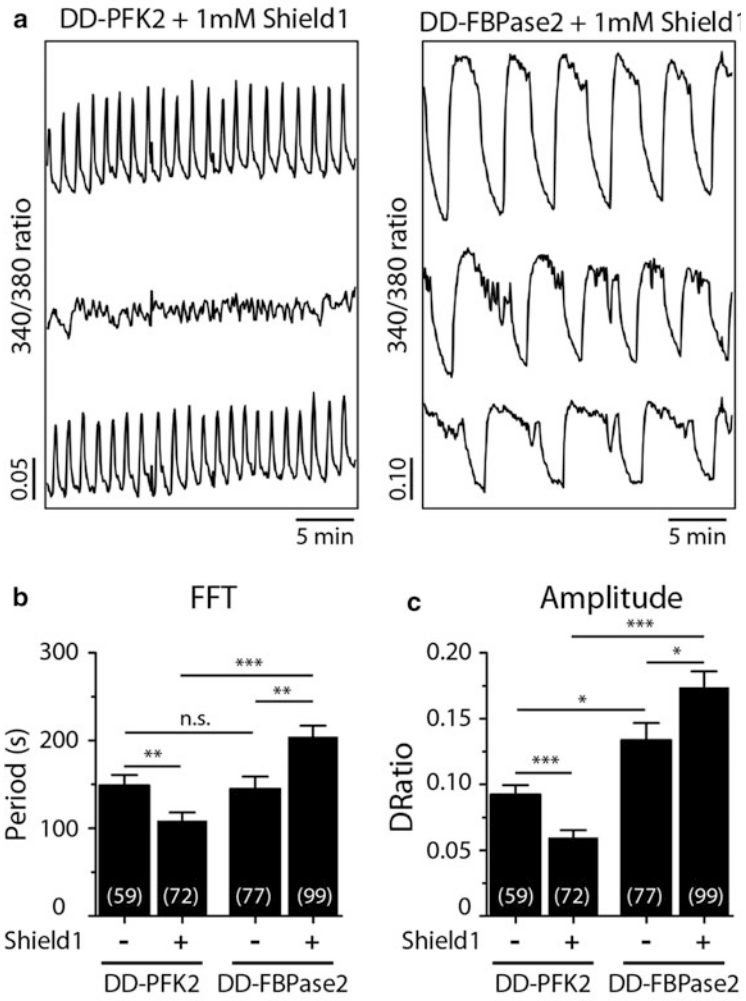

Fig. 11 Expression of DD-PFK2 with Shield1 results in the production of F2,6BP which makes Ca^{2+} oscillations smaller and faster (**a**) compared with islets expressing DD-FBPase2 with Shield1 (**b**). (**c**) Fast Fourier transform of Ca^{2+} oscillation periods in islets expressing DD-PFK2 or DD-FBPase2 (with or without Shield1). (**d**) Amplitude of Ca^{2+} oscillations (Reprinted from Merrins et al. (2012))

slower and larger in amplitude. A similar prediction was made using the full dual oscillator model, where now the final readout was the cytosolic Ca^{2+} concentration.

Model predictions were tested using the DD-PFK2 and DD-FBPase2 mutants. When DD-PFK2 was expressed in mouse islets, the period of the Ca^{2+} oscillations we observed was significantly smaller in the presence of Shield1 than in its absence, and the amplitude of the oscillations was significantly reduced (Fig. 11). That is, when functional PFK2 protein was produced (Shield1

present), which should increase the production of F2,6BP, Ca^{2+} oscillations were faster and smaller compared with islets in which Shield1 was absent. When DD-FBPase2 was expressed, the period of Ca^{2+} oscillations was significantly larger in the presence of Shield1 than in its absence, and the amplitude of the oscillations was significantly increased (Fig. 11). These results match the predictions of the dual oscillator model. Thus, for the first time, it was shown that manipulations that should make Ca^{2+} oscillations faster/slower *if the oscillations are the result of glycolytic oscillations* did indeed make the oscillations faster/slower.

Summary

The dual oscillator model, developed over a period of time from simpler Ca^{2+}-dependent models of fast bursting to account for slower and more complex patterns of islet oscillatory behavior, has done so successfully while also clarifying the complex relationship between intracellular Ca^{2+}, β-cell ion channels, and intrinsic oscillations in islet glucose metabolism. The model also clarifies the results of experiments that would be hard to interpret or open to misinterpretation in the absence of a model. In addition to the studies described above, recent work used the DOM to interpret islet electrophysiological experiments and to understand the role played by gap-junctional coupling between β-cells (Ren et al. 2013). It remains to be determined how functional properties of human islets differ from mouse islets and whether there are similar mechanisms driving oscillations in islets from the two species. It is also yet to be seen what role the model will have in understanding islet dysfunction in models of diabetes.

Acknowledgments The authors thank Bernard Fendler, Pranay Goel, Matthew Merrins, Craig Nunemaker, Morten Gram Pedersen, Brad Peercy, and Min Zhang, who each collaborated on some of the work described herein. RB is supported by NIH grant DK80714. AS is supported by the Intramural Research Program of the NIH (NIDDK). LS is supported by NIH grant RO1 DK 46409.

References

Ainscow EK, Rutter GA (2002) Glucose-stimulated oscillations in free cytosolic ATP concentration imaged in single islet β-cells. Diabetes 51:S162–S170

Ämmälä C, Ashcroft FM, Rorsman P (1993) Calcium-independent potentiation of insulin release by cyclic AMP in single β-cells. Nature 363:356–358

Arden C, Hampson LJ, Huang GC, Shaw JAM, Aldibbiat A, Holliman G, Manas D, Khan S, Lange AJ, Agius L (2008) A role for PFK-2/FBPase-2, as distinct from fructose 2,6-bisphosphate, in regulation of insulin secretion in pancreatic β-cells. Biochem J 411:41–51

Arredouani A, Henquin J-C, Gilon P (2002) Contribution of the endoplasmic reticulum to the glucose-induced $[Ca^{2+}]_c$ response in mouse pancreatic islets. Am J Physiol 282:E982–E991

Ashcroft FM, Harrison DE, Ashcroft SJH (1984) Glucose induces closure of single potassium channels in isolated rat pancreatic β-cells. Nature 312:446–448

Banaszynski LA, Chen L-C, Maynard-Smith LA, Ooi AGL, Wandless TJ (2006) A rapid, reversible, and tunable method to regulate protein function in living cells using synthetic small molecules. Cell 126:995–1004

Beauvois MC, Merezak C, Jonas J-C, Ravier MA, Henquin J-C (2006) Glucose-induced mixed $[Ca^{2+}]_c$ oscillations in mouse β-cells are controlled by the membrane potential and the SERCA3 Ca^{2+}-ATPase of the endoplasmic reticulum. Am J Physiol 290:C1503–C1511

Beigelman PM, Ribalet B (1980) β-cell electrical activity in response to high glucose concentration. Diabetes 29:263–265

Bergsten P (1995) Slow and fast oscillations of cytoplasmic Ca^{2+} in pancreatic islets correspond to pulsatile insulin release. Am J Physiol 268:E282–E287

Bergsten P (1998) Glucose-induced pulsatile insulin release from single islets at stable and oscillatory cytoplasmic Ca^{2+}. Am J Physiol 274:E796–E800

Bergsten P, Hellman B (1993) Glucose-induced amplitude regulation of pulsatile insulin secretion from individual pancreatic islets. Diabetes 42:670–674

Bergsten P, Grapengiesser E, Gylfe E, Tengholm A, Hellman B (1994) Synchronous oscillations of cytoplasmic Ca^{2+} and insulin release in glucose-stimulated pancreatic islets. J Biol Chem 269:8749–8753

Bergsten P, Westerlund J, Liss P, Carlsson P-O (2002) Primary in vivo oscillations of metabolism in the pancreas. Diabetes 51:699–703

Bertram R, Arceo RC II (2008) A mathematical study of the differential effects of two SERCA isoforms on Ca^{2+} oscillations in pancreatic islets. Bull Math Biol 70:1251–1271

Bertram R, Sherman A (2004a) A calcium-based phantom bursting model for pancreatic islets. Bull Math Biol 66:1313–1344

Bertram R, Sherman A (2004b) Filtering of calcium transients by the endoplasmic reticulum in pancreatic β-cells. Biophys J 87:3775–3785

Bertram R, Satin L, Zhang M, Smolen P, Sherman A (2004) Calcium and glycolysis mediate multiple bursting modes in pancreatic islets. Biophys J 87:3074–3087

Bertram R, Satin LS, Pedersen MG, Luciani DS, Sherman A (2007a) Interaction of glycolysis and mitochondrial respiration in metabolic oscillations of pancreatic islets. Biophys J 92:1544–1555

Bertram R, Sherman A, Satin LS (2007b) Metabolic and electrical oscillations: partners in controlling pulsatile insulin secretion. Am J Physiol 293:E890–E900

Cha CY, Nakamura Y, Himeno Y, Wang J, Fujimoto S, Inagaki N, Earm YE, Noma A (2011) Ionic mechanisms and Ca^{2+} dynamics underlying the glucose response of pancreatic β cells: a simulation study. J Gen Physiol 138:21–37

Chay TR, Keizer J (1983) Minimal model for membrane oscillations in the pancreatic β-cell. Biophys J 42:181–190

Chou H-F, Berman N, Ipp E (1992) Oscillations of lactate released from islets of Langerhans: evidence for oscillatory glycolysis in β-cells. Am J Physiol 262:E800–E805

Civelek VN, Deeney JT, Shalosky NJ, Tornheim K, Hansford RG, Prentki M, Corkey BE (1996) Regulation of pancreatic β-cell mitochondrial metabolism: influence of Ca^{2+}, substrate and ADP. Biochem J 318:615–621

Cook DL (1983) Isolated islets of Langerhans have slow oscillations of electrical activity. Metabolism 32:681–685

Dahlgren GM, Kauri LM, Kennedy RT (2005) Substrate effects on oscillations in metabolism, calcium and secretion in single mouse islets of Langerhans. Biochim Biophys Acta 1724:23–36

Dean PM, Mathews EK (1970) Glucose-induced electrical activity in pancreatic islet cells. J Physiol 210:255–264

Detimary P, Gilon P, Henquin JC (1998) Interplay between cytoplasmic Ca^{2+} and the ATP/ADP ratio: a feedback control mechanism in mouse pancreatic islets. Biochem J 333:269–274

Diederichs F (2006) Mathematical simulation of membrane processes and metabolic fluxes of the pancreatic β-cell. Bull Math Biol 68:1779–1818

Eaton RP, Allen RC, Schade DS (1983) Hepatic removal of insulin in normal man: dose response to endogenous insulin secretion. J Clin Endocrinol Metab 56:1294–1300

Foe LG, Latshaw SP, Kemp RG (1983) Binding of hexose bisphosphates to muscle phosphofructokinase. Biochemistry 22:4601–4606

Fridlyand LE, Tamarina N, Phillipson LH (2003) Modeling the Ca^{2+} flux in pancreatic β-cells: role of the plasma membrane and intracellular stores. Am J Physiol 285:E138–E154

Gilon P, Shepherd RM, Henquin JC (1993) Oscillations of secretion driven by oscillations of cytoplasmic Ca^{2+} as evidenced in single pancreatic islets. J Biol Chem 268:22265–22268

Gilon P, Arredouani A, Gailly P, Gromada J, Henquin J-C (1999) Uptake and release of Ca^{2+} by the endoplasmic reticulum contribute to the oscillations of the cytosolic Ca^{2+} concentration triggered by Ca^{2+} influx in the electrically excitable pancreatic β-cell. J Biol Chem 274:20197–20205

Goforth PB, Bertram R, Khan FA, Zhang M, Sherman A, Satin LS (2002) Calcium-activated K^+ channels of mouse β-cells are controlled by both store and cytoplasmic Ca^{2+}: experimental and theoretical studies. J Gen Physiol 114:759–769

Goldbeter A, Lefever R (1972) Dissipative structures for an allosteric model; application to glycolytic oscillations. Biophys J 12:1302–1315

Göpel SO, Kanno T, Barg S, Eliasson L, Galvanovskis J, Renström E, Rorsman P (1999) Activation of Ca^{2+}-dependent K^+ channels contributes to rhythmic firing of action potentials in mouse pancreatic β-cells. J Gen Physiol 114:759–769

Henquin JC (1990) Glucose-induced electrical activity in β-cells: feedback control of ATP-sensitive K^+ channels by Ca^{2+}? Diabetes 39:1457–1460

Henquin JC, Meissner HP, Schmeer W (1982) Cyclic variations of glucose-induced electrical activity in pancreatic β cells. Pflugers Arch 393:322–327

Higgins ER, Cannell MB, Sneyd J (2006) A buffering SERCA pump in models of calcium dynamics. Biophys J 91:151–163

Jung S-K, Aspinwall CA, Kennedy RT (1999) Detection of multiple patterns of oscillatory oxygen consumption in single mouse islets of Langerhans. Biochem Biophys Res Commun 259:331–335

Jung S-K, Kauri LM, Qian W-J, Kennedy RT (2000) Correlated oscillations in glucose consumption, oxygen consumption, and intracellular free Ca^{2+} in single islets of Langerhans. J Biol Chem 275:6642–6650

Juntti-Berggren L, Webb D-L, Arkhammar POG, Schultz V, Schweda EKH, Tornheim K, Berggren P-O (2003) Dihydroxyacetone-induced oscillations in cytoplasmic free Ca^{2+} and the ATP/ADP ratio in pancreatic β-cells at substimulatory glucose. J Biol Chem 278:40710–40716

Keizer J, De Young G (1993) Effect of voltage-gated plasma membrane Ca^{2+} fluxes on IP_3-linked Ca^{2+} oscillations. Cell Calcium 14:397–410

Keizer J, Magnus G (1989) The ATP-sensitive potassium channel and bursting in the pancreatic β cell. Biophys J 56:229–242

Kennedy RT, Kauri LM, Dahlgren GM, Jung S-K (2002) Metabolic oscillations in β-cells. Diabetes 51:S152–S161

Kindmark H, Köhler M, Brown G, Bränström R, Larsson O, Berggren P-O (2001) Glucose-induced oscillations in cytoplasmic free Ca^{2+} concentration precede oscillations in mitochondrial membrane potential in the pancreatic β-cell. J Biol Chem 276:34530–34536

Krippeit-Drews P, Dufer M, Drews G (2000) Parallel oscillations of intracellular calcium activity and mitochondrial membrane potential in mouse pancreatic β-cells. Biochem Biophys Res Commun 267:179–183

Kulkarni RN, Roper MG, Dahlgren GM, Shih DQ, Kauri LM, Peters JL, Stoffel M, Kennedy RT (2004) Islet secretory defect in insulin receptor substrate 1 null mice is linked with reduced calcium signaling and expression of sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA)-2b and −3. Diabetes 53:1517–1525

Lang DA, Matthews DR, Burnett M, Turner RC (1981) Brief, irregular oscillations of basal plasma insulin and glucose concentrations in diabetic man. Diabetes 30:435–439

Langer S, Kaminski MT, Lenzen S, Baltrusch S (2010) Endogenous activation of glucokinase by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase is glucose dependent. Mol Endocrinol 24:1988–1997

Longo EA, Tornheim K, Deeney JT, Varnum BA, Tillotson D, Prentki M, Corkey BE (1991) Oscillations in cytosolic free Ca^{2+}, oxygen consumption, and insulin secretion in glucose-stimulated rat pancreatic islets. J Biol Chem 266:9314–9319

Luciani DS, Misler S, Polonsky KS (2006) Ca^{2+} controls slow NAD(P)H oscillations in glucose-stimulated mouse pancreatic islets. J Physiol 572:379–392

MacDonald MJ, Fahien LA, Buss JD, Hasan NM, Fallon MJ, Kendrick MA (2003) Citrate oscillates in liver and pancreatic β cell mitochondria and in INS-1 insulinoma cells. J Biol Chem 278:51894–51900

Magnus G, Keizer J (1997) Minimal model of β-cell mitochondrial Ca^{2+} handling. Am J Physiol 273:C717–C733

Magnus G, Keizer J (1998) Model of β-cell mitochondrial calcium handling and electrical activity. I. Cytoplasmic variables. Am J Physiol 274:C1158–C1173

Malisse WJ, Malaisse-Lagae F, Sener A (1982) Glucose-induced accumulation of fructose-2,6-bisphosphate in pancreatic islets. Diabetes 31:90–93

Matthews DR, Lang DA, Burnett M, Turner RC (1983) Control of pulsatile insulin secretion in man. Diabetologia 24:231–237

Matveyenko AV, Veldhuis JD, Butler PC (2008) Measurement of pulsatile insulin secretion in the rat: direct sampling from the hepatic portal vein. Am J Physiol 295:E569–E574

Matveyenko AV, Liuwantara D, Gurlo T, Kirakossian D, Dalla Man C, Cobelli C, White MF, Copps KD, Volpi E, Fujita S, Butler PC (2012) Pulsatile portal vein insulin delivery enhances hepatic insulin action and signaling. Diabetes 61:2269–2279

Meier JJ, Veldhuis JD, Butler PC (2005) Pulsatile insulin secretion dictates systemic insulin delivery by regulating hepatic insulin extraction in humans. Diabetes 54:1649–1656

Meissner HP, Schmelz H (1974) Membrane potential of β-cells in pancreatic islets. Pflugers Arch 351:195–206

Merrins MJ, Fendler B, Zhang M, Sherman A, Bertram R, Satin LS (2010) Metabolic oscillations in pancreatic islets depend on the intracellular Ca^{2+} level but not Ca^{2+} oscillations. Biophys J 99:76–84

Merrins MJ, Bertram R, Sherman A, Satin LS (2012) Phosphofructo-2-kinase/fructose-2,6-bisphosphatase modulates oscillations of pancreatic islet metabolism. PLoS One 7(4):e34036

Nilsson T, Schultz V, Berggren P-O, Corkey BE, Tornheim K (1996) Temporal patterns of changes in ATP/ADP ratio, glucose 6-phosphate and cytoplasmic free Ca^{2+} in glucose-stimulated pancreatic β-cells. Biochem J 314:91–94

Nunemaker CS, Zhang M, Wasserman DH, McGuinness OP, Powers AC, Bertram R, Sherman A, Satin LS (2005) Individual mice can be distinguished by the period of their islet calcium oscillations: is there an intrinsic islet period that is imprinted in vivo? Diabetes 54:3517–3522

Nunemaker CS, Bertram R, Sherman A, Tsaneva-Atanasova K, Daniel CR, Satin LS (2006) Glucose modulates $[Ca^{2+}]_i$ oscillations in pancreatic islets via ionic and glycolytic mechanisms. Biophys J 91:2082–2096

O'Rahilly S, Turner RC, Matthews DR (1988) Impaired pulsatile secretion of insulin in relatives of patients with non-insulin-dependent diabetes. N Engl J Med 318:1225–1230

Ortsäter H, Liss P, Lund PE, Åkerman KEO, Bergsten P (2000) Oscillations in oxygen tension and insulin release of individual pancreatic *ob/ob* mouse islets. Diabetologia 43:1313–1318

Polonsky KS, Jaspan J, Emmanouel D, Holmes K, Moossa AR (1983) Differences in the hepatic and renal extraction of insulin and glucagon in the dog: evidence for saturability of insulin metabolism. Acta Endocrinol (Copenh) 102:420–427

Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, Beebe C, Frank BH, Galloway JA, van Cauter E (1988) Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. N Engl J Med 318:1231–1239

Pørksen N (2002) The in vivo regulation of pulsatile insulin secretion. Diabetologia 45:3–20

Pørksen N, Munn S, Steers J, Vore S, Veldhuis J, Butler P (1995) Pulsatile insulin secretion accounts for 70 % of total insulin secretion during fasting. Am J Physiol 269:E478–E488

Pørksen N, Nyholm B, Veldhuis JD, Butler PC, Schmitz O (1997) In humans at least 75 % of insulin secretion arises from punctuated insulin secretory bursts. Am J Physiol 273:E908–E914

Ravier MA, Daro D, Roma LP, Jonas JC, Cheng-Xue R, Schuit FC, Gilon P (2011) Mechanisms of control of the free Ca^{2+} concentration in the endoplasmic reticulum of mouse pancreatic β-cells: interplay with cell metabolism and $[Ca^{2+}]_c$ and role of SERCA2β and SERCA3. Diabetes 60:2533–2545

Ren J, Sherman A, Bertram R, Goforth PB, Nunemaker CS, Waters CD, Satin LS (2013) Slow oscillations of K_{ATP} conductance in mouse pancreatic islets provide support for electrical bursting driven by metabolic oscillations. Am J Physiol 305:E805–E817

Ristow M, Vorgerd M, Möhlig M, Schatz H, Pfeiffer A (1997) Deficiency of phosphofructo-1-kinase/muscle subtype in humans impairs insulin secretion and causes insulin resistance. J Clin Invest 100:2833–2841

Ristow M, Carlqvist H, Hebinck J, Vorgerd M, Krone W, Pfeiffer A, Muller-Wieland D, Ostenson CG (1999) Deficiency of phosphofructo-1-kinase/muscle subtype in humans is associated with impairment of insulin secretory oscillations. Diabetes 48:1557–1561

Ritzel RA, Veldhuis JD, Butler PC (2006) The mass, but not the frequency, of insulin secretory bursts in isolated human islets is entrained by oscillatory glucose exposure. Am J Physiol 290: E750–E756

Sakurai T, Johnson JH, Uyeda K (1996) Islet fructose 6-phosphate, 2-kinase: fructose 2,6-bisphosphatase: isozymic form, expression, and characterization. Biochem Biophys Res Commun 218:159–163

Santos RM, Rosario LM, Nadal A, Garcia-Sancho J, Soria B, Valdeolmillos M (1991) Widespread synchronous $[Ca^{2+}]_i$ oscillations due to bursting electrical activity in single pancreatic islets. Pflugers Arch 418:417–422

Sel'kov EE (1968) Self-oscillations in glycolysis: a simple kinetic model. Eur J Biochem 4:79–86

Sener A, Van Schaftingen E, Van de Winkel M, Pipeleers DG, Malaisse-Lagae F, Malaisse WJ, Hers HG (1984) Effects of glucose and glucagon on the fructose 2,6-bisphosphate content of pancreatic islets and purified pancreatic β-cells. Biochem J 221:759–764

Smolen P (1995) A model for glycolytic oscillations based on skeletal muscle phosphofructokinase kinetics. J Theor Biol 174:137–148

Smolen P, Keizer J (1992) Slow voltage inactivation of Ca^{2+} currents and bursting mechanisms for the mouse pancreatic β-cell. J Membr Biol 127:9–19

Song SH, McIntyre SS, Shah H, Veldhuis JD, Hayes PC, Butler PC (2000) Direct measurement of pulsatile insulin secretion from the portal vein in human subjects. J Clin Endocrinol Metab 85:4491–4499

Stagner JI, Samols E, Weir GC (1980) Sustained oscillations of insulin, glucagon, and somatostatin from the isolated canine pancreas during exposure to a constant glucose concentration. J Clin Invest 65:939–942

Tornheim K (1979) Oscillations of the glycolytic pathway and the purine nucleotide cycle. J Theor Biol 79:491–541

Tornheim K (1997) Are metabolic oscillations responsible for normal oscillatory insulin secretion? Diabetes 46:1375–1380

Tornheim K, Lowenstein JM (1974) The purine nucleotide cycle: IV. Interactions with oscillations of the glycolytic pathway in muscle extracts. J Biol Chem 249:3241–3247

Tornheim K, Andrés V, Schultz V (1991) Modulation by citrate of glycolytic oscillations in skeletal muscle extracts. J Biol Chem 266:15675–15678

Valdeolmillos M, Santos RM, Contreras D, Soria B, Rosario LM (1989) Glucose-induced oscillations of intracellular Ca^{2+} concentration resembling electrical activity in single mouse islets of Langerhans. FEBS Lett 259:19–23

Watts M, Fendler B, Merrins MJ, Satin LS, Bertram R (2014) Calcium and Metabolic Oscillations in Pancreatic Islets: Who's Driving the Bus? SIAM J Appl Dyn Syst 13:683–703

Weigle DS (1987) Pulsatile secretion of fuel-regulatory hormones. Diabetes 36:764–775

Westermark PO, Lansner A (2003) A model of phosphofructokinase and glycolytic oscillations in the pancreatic β-cell. Biophys J 85:126–139

Wierschem K, Bertram R (2004) Complex bursting in pancreatic islets: a potential glycolytic mechanism. J Theor Biol 228:513–521

Yaney GC, Schultz V, Cunningham BA, Dunaway GA, Corkey BE, Tornheim K (1995) Phosphofructokinase isozymes in pancreatic islets and clonal β-cells (INS-1). Diabetes 44:1285–1289

Zhan X, Yang L, Ming Y, Jia Y (2008) RyR channels and glucose-regulated pancreatic β-cells. Eur Biophys J 37:773–782

Zhang M, Goforth P, Sherman A, Bertram R, Satin L (2003) The Ca^{2+} dynamics of isolated mouse β-cells and islets: implications for mathematical models. Biophys J 84:2852–2870

Exocytosis in Islet β-Cells

16

Haruo Kasai, Hiroyasu Hatakeyama, Mitsuyo Ohno, and Noriko Takahashi

Contents

Abstract

The development of technologies that allow for live optical imaging of exocytosis from β-cells has greatly improved our understanding of insulin secretion. Two-photon imaging, in particular, has enabled researchers to visualize the

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_11,

exocytosis of large dense-core vesicles (LDCVs) containing insulin from β-cells in intact islets of Langerhans. These studies have revealed that high glucose levels induce two phases of insulin secretion and that this release is dependent upon cytosolic Ca^{2+} and cAMP. This technology has also made it possible to examine the spatial profile of insulin exocytosis in these tissues and compare that profile with those of other secretory glands. Such studies have led to the discovery of the massive exocytosis of synaptic-like microvesicles (SLMVs) in β-cells. These imaging studies have also helped clarify facets of insulin exocytosis that cannot be properly addressed using the currently available electrophysiological techniques. This chapter provides a concise introduction to the field of optical imaging for those researchers who wish to characterize exocytosis from β-cells in the islets of Langerhans.

Keywords
Insulin secretion • Pancreatic islet • Sequential exocytosis • Two-photon microscopy

Introduction

Insulin is a growth hormone promoting cellular storage of carbohydrates, fats, and proteins. Insulin is the only hormone that reduces the blood glucose level, and it is selectively secreted from β-cells in the islets of Langerhans in response to elevated blood glucose and nutrient levels. The process of insulin secretion involves the formation of membrane-encased insulin granules, also referred to as large dense-core vesicles (LDCVs), which have diameters of 200–500 nm (Dean 1973; Takahashi et al. 2004; Kasai et al. 2005a). These granules are then transported to the plasma membrane, where the granule membrane fuses with the plasma membrane. This exocytotic fusion is characterized by the opening of a fusion pore connecting the two membranes. The granules are called LDCVs because in chemically fixed β-cells visualized via electron microscopy (EM), they appear to have a dense core surrounded by a halo (see Fig. 9). The observed dense core, however, is mostly an artifact of chemical fixation and staining, as it is not observed in quick-frozen β-cells (Dudek and Boyne 1986) or adrenal chromaffin cells (Plattner et al. 1997).

It is generally accepted that high glucose levels increase cytosolic ATP, Ca^{2+}, and cAMP, which triggers exocytotic fusion of insulin granules (Fig. 1a, section "Actions of Glucose and cAMP"). Such insulin exocytosis occurs in two phases (Fig. 1b; Kahn 2004; Henquin 2000). The first phase of insulin secretion (2–5 min), which is particularly impaired in patients with type 2 diabetes mellitus, directly triggers glycogen synthesis in the liver and plays an important role in the control of the blood glucose level, while the second phase facilitates the utilization of glucose in a variety of tissues (Vaag et al. 1995).

In addition to granules, β-cells also contain many synaptic-like microvesicles (SLMV, 50–100 nm) (Fig. 1; Thomas-Reetz and De Camilli 1994), which undergo

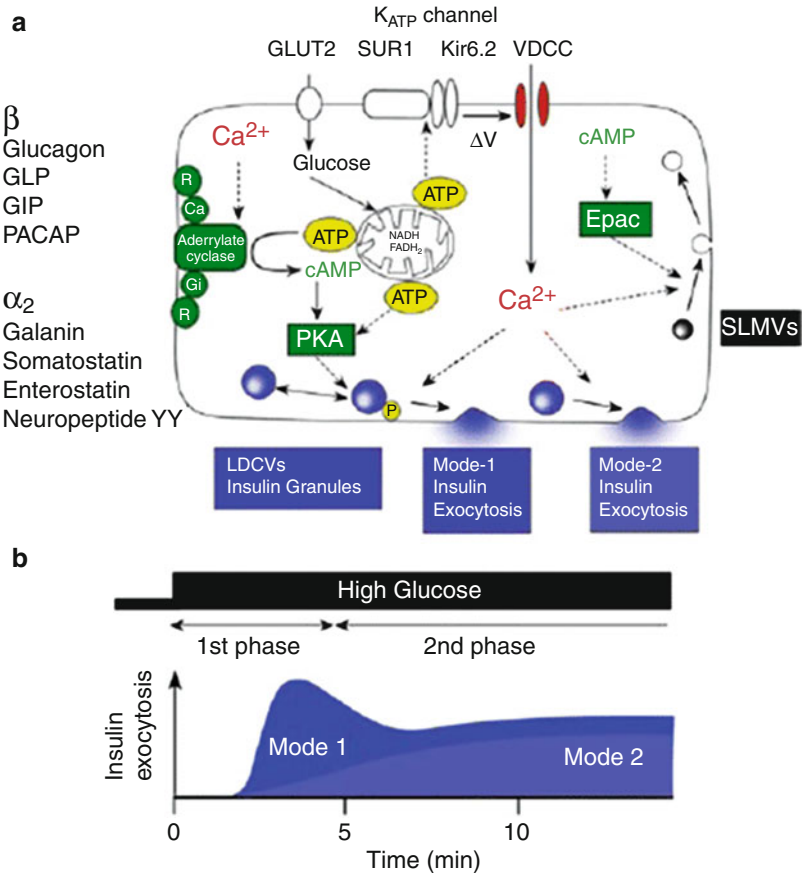

Fig. 1 Exocytosis in β-cells. (**a**) A simplified scheme for the exocytosis of LDCVs and SLMVs induced by glucose and hormones. Mode-1 insulin exocytosis requires the phosphorylation of targets by PKA, while Mode-2 exocytosis does not. See section "Regulation of Insulin Exocytosis" for a more detailed explanation. (**b**) The two phases of exocytosis of LDCVs (insulin exocytosis) and the differential involvement of Mode-1 and Mode-2 insulin exocytosis in the two phases

Ca^{2+}-dependent exocytosis (Hatakeyama et al. 2007) in a manner that is similar to that observed in other cell types (Dan and Poo 1992; Borgonovo et al. 2002; Kasai 1999; McNeil and Steinhardt 2003). SLMVs in synaptic terminals, namely, synaptic vesicles, are utilized for ultrafast secretion of neurotransmitters such as glutamate and γ-aminobutyric acid (GABA). The physiological role of SLMVs in nonneuronal cells, in contrast, may include housekeeping functions, such as membrane repair (Steinhardt et al. 1994). It is also possible that SLMVs may indirectly regulate the exocytosis of LDCVs (Maritzen et al. 2008). As such, SLMV exocytosis may play a specific and unique role in different cell types, and thus the role of this process in islet cells needs to be elucidated in both physiological and pathological contexts (section "Exocytosis of Synaptic-Like Microvesicles (SLMVs)").

It has been 20 years since the key molecules involved in exocytosis were identified (Rothman 1994; Sudhof 1995; Mochida 2000; Jahn et al. 2003), and still, the molecular mechanisms of exocytosis are only partly understood. This is because a single exocytotic event involves multiple copies of a variety of different proteins, including the core fusion complex, soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein receptors (SNAREs), putative Ca^{2+} sensors, synaptotagmins, and a myriad of membrane lipid molecules. β-cells express the full complement of proteins identified as playing a key role in neurotransmitter release, including the SNAREs, syntaxin-1, SNAP-25, and VAMP2; the SNARE-interacting proteins, Munc18-1, Munc13-1, and complexin 1; and the GTPase Rab3A (Lang 1999; Gerber and Sudhof 2002). In addition to these proteins, insulin secretion requires the actions of nonneuronal proteins, such as granuphilin, Noc2, and Rab27A, which are selectively enriched in endocrine cells (Gomi et al. 2005; Kasai et al. 2005b) and whose expression is regulated by microRNAs (section "Molecular Mechanisms of Insulin Exocytosis").

Elucidation of the molecular mechanisms underlying insulin exocytosis therefore depends on a combination of modern biophysical and molecular biological analysis techniques. In this chapter, we first introduce methodologies to investigate exocytosis in β-cells, with emphasis placed on two-photon excitation imaging, which is the only imaging methodology available that allows for the examination of exocytosis in intact tissue preparations (Takahashi et al. 2002a, 2004; Kasai et al. 2005a; Nemoto et al. 2001, 2004; Oshima et al. 2005; Kishimoto et al. 2005, 2006; Liu et al. 2005; Hatakeyama et al. 2006). We then proceed to characterize insulin exocytosis from β-cells compared to exocytosis by other secretory cells, explaining the molecular bases of the two phases of glucose-induced insulin secretion, and we finally describe small vesicle exocytosis in β-cells. Throughout this process, we will address apparent discrepancies in data that have been obtained using different methodologies and discuss the rationale behind the notions of "docking," "priming," and the "readily releasable pool of vesicles."

Measurements of Exocytosis

Electrophysiological Approaches and Quantification

The classic methods of quantifying insulin secretion involve the measurement of the amount of insulin secreted from the islets using, for example, radioimmunoassays (RIAs) or enzyme-linked immunosorbent assays (ELISAs). More analytical approaches, however, are necessary to elucidate the mechanisms underlying this secretion. One such approach, membrane capacitance measurement, is based upon the assumption that the capacitance per membrane area is constant and thus employs the patch-clamp method to detect the increases in capacitance that result from the exocytosis-mediated increases in the membrane area (Fig. 2a; Neher and Marty 1982). This approach is particularly useful when stepwise changes in capacitance can be detected, as this makes it possible to estimate the diameters of vesicles

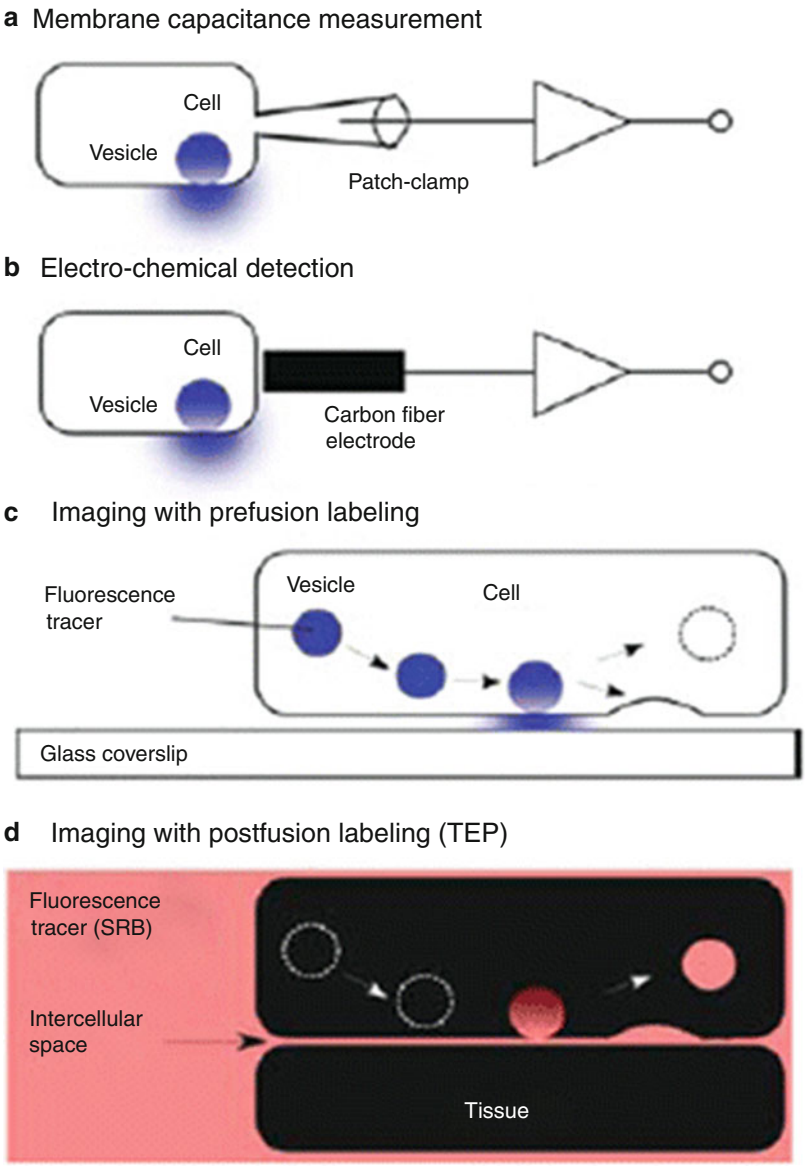

Fig. 2 Analytical methods used to study exocytosis and endocytosis. TEP represents two-photon extracellular polar-tracer imaging (Adapted from Fukui et al. (2005). With permission from Elsevier)

(Neher and Marty 1982; Klyachko and Jackson 2002; MacDonald et al. 2005) and to characterize fusion pore properties with temporal resolution in the millisecond range (Klyachko and Jackson 2002; Alvarez de Toledo et al. 1993; Albillos et al. 1997). When capacitance changes are not stepwise, however, exocytosis of

LDCVs and SLMVs cannot be readily distinguished with this method (Takahashi et al. 1997). Furthermore, concurrent exocytosis and endocytosis are also not distinguishable using this technique (Liu et al. 2005; Smith and Betz 1996; Kasai et al. 1996).

Electrochemical measurement of serotonin release from serotonin-loaded β-cells using carbon fiber electrodes (amperometric measurement) has also been employed to analyze the exocytosis of insulin granules (Fig. 2b; Takahashi et al. 1997; Zhou and Misler 1996). Unlike capacitance measurement, amperometric measurement is unaffected by concurrent endocytosis. However, this approach generally assumes that serotonin is loaded selectively into insulin granules, which is a critical assumption that has not been validated experimentally. Differences between amperometric signals and capacitance increases have been reported in various secretory cells (Kirillova et al. 1993; Oberhauser et al. 1996; Ninomiya et al. 1997; Haller et al. 1998), including β-cells (Takahashi et al. 1997). These differences likely reflect the delay associated with the diffusion of monoamines (Haller et al. 1998) and the involvement of SLMV exocytosis in the observed release (Hatakeyama et al. 2007; Takahashi et al. 1997; Ninomiya et al. 1997). Amperometric measurements also contain an artifact caused by the effects of UV irradiation on the carbon fiber electrode, which is particularly disruptive when rapid events are examined (Karanauskaite et al. 2009).

The release of fast neurotransmitters, such as ATP and GABA, can be detected via a biosensor method that uses chromaffin (Hollins and Ikeda 1997), INS-1 (Obermuller et al. 2005), and β-cells (Braun et al. 2004) exogenously expressing receptors for these transmitters. In these cells, the currents evoked by transmitter binding to the exogenously expressed receptors can be measured electrophysiologically as an indicator of transmitter release. This biosensor approach was used previously to characterize SLMV exocytosis based upon the assumption that GABA is selectively stored in SLMVs (Braun et al. 2004); however, it has since been found that GABA is also stored in insulin granules (Braun et al. 2007), complicating the situation considerably. It is also likely that ATP is found in both types of vesicles, as ATP is an established neurotransmitter in synaptic vesicles. In support of this theory, the uncaging of caged Ca^{2+} in β-cells induced rapid ATP-mediated currents (Karanauskaite et al. 2009), which occur with a time constant that is similar to that of SLMV exocytosis measured with two-photon imaging (Hatakeyama et al. 2007) (section "Exocytosis of Synaptic-Like Microvesicles (SLMVs)"). In short, electrophysiological methodologies are convenient tools for the quantification of exocytosis parameters, but they often lack the specificity necessary to provide a complete picture of the events involved.

TIRF Imaging and "Docking" of Granules

Live imaging is necessary to study dynamic structural processes like exocytosis. A popular live imaging methodology involves the staining of vesicles before fusion (Fig. 2c) and the visualization of exocytosis via total internal reflection fluorescence

(TIRF or evanescent-field) microscopy, which illuminates preparations less than 100 nm from the surface of a glass cover slip with high spatial resolution (Steyer et al. 1997). This approach is particularly well suited to investigations of the process of vesicle attachment or "docking" to the plasma membrane, which has often been considered to be a prerequisite for exocytosis (Oheim et al. 1998; Tsuboi et al. 2002). The first phase of insulin secretion has been reported in one study (Ohara-Imaizumi et al. 2007) to be mostly mediated by insulin granules "docked" to the plasma membrane; however, conflicting data have been presented in other studies (Kasai et al. 2008; Shibasaki et al. 2007). These latter studies demonstrate that fusion of granules with the plasma membrane can occur without apparent "docking" during insulin exocytosis in β-cells. Such "crash fusion" has also been reported in chromaffin cells (Allersma et al. 2004; Toonen et al. 2006; Verhage and Sorensen 2008). Furthermore, EM studies on β-cells have revealed that the apparent "docking" observed in TIRF imaging does not represent the actual attachment of vesicles to the plasma membrane (Kasai et al. 2008) and that differences in the depth of penetration of TIRF microscopy might provide one explanation for the inconsistent detection of "docking" across laboratories (Ohara-Imaizumi et al. 2007; Kasai et al. 2008; Shibasaki et al. 2007). In fact, experiments with granuphilin have even indicated that "docking" may actually delay the fusion of insulin granules (Gomi et al. 2005; Kasai et al. 2008).

TIRF imaging detects only a small portion of individual LDCVs, as the diameters of LDCVs are larger than the depth of penetration of TIRF microscopy. Thus, in principle, fluorescence changes are not fully interpretable without additional information. For example, it is impossible to measure the distance between the vesicle and the plasma membrane without invoking a number of assumptions, for example, on the constancies in size, shape, and brightness of vesicle. TIRF imaging also cannot detect exocytotic events, such as compound exocytosis, that involve structures deeper than the evanescent illumination can penetrate (section "Spatial Organization of Exocytosis: Docking, Priming, and the Readily Releasable Pool of Vesicles"). In fact, TIRF microscopy can only be used to study vesicles associated with portions of the plasma membrane that are attached to the glass cover slip, which is an important consideration in light of the fact that these regions do not actually correspond to the sites of physiological exocytosis. If these points are carefully addressed, however, the high spatial resolution of TIRF microscopy can be useful in studies aiming to elucidate the molecular bases of exocytosis.

Two-Photon Imaging and the Spatial Organization of Exocytosis

TEP (Two-Photon Extracellular Polar-Tracer) Imaging

In general, imaging coupled with prefusion labeling (Fig. 2c) does not allow for estimation of vesicle diameters or tracking of vesicle fates after exocytosis and is subject to a selection bias for the subset of vesicles that are well labeled (Michael et al. 2004). More seriously, methods that involve labeling vesicles with GFP-based probes can actually alter secretion kinetics (Michael et al. 2004). Postfusion

labeling, in contrast, does not suffer from the abovementioned issues of selection bias and interference with secretion processes and provides an ideal method to study postfusion vesicle fates (Fig. 2d). We have found that such postfusion labeling experiments are ideally performed by immersing the secretory preparations in a solution containing fluid-phase polar tracers and visualizing the inside of the tissue using the two-photon excitation microscope (Kasai et al. 2006; Fig. 4a). We have screened a number of such polar tracers and have found sulforhodamine B (SRB) to be the best tracer available in terms of size, solubility, brightness, and cost-effectiveness. The intercellular space of tissues is normally quite narrow at 20–40 nm, which is less than the diameter of most secretory vesicles (Fig. 2d), and thus allows for a high signal-to-noise ratio when employing imaging techniques. Also, TEP imaging overcomes many of the shortcomings discussed above for TIRF microscopy, as the intercellular space within tissue is very clean, unlike the space between a cell and a glass cover slip, and is also the physiological site of exocytosis. Since staining with TEP imaging is nonselective, it will reveal all exocytotic events in the visual field (Hatakeyama et al. 2006) and thus can also be used to characterize abnormal secretion patterns in diseased or mutant animals with little selection bias.

Two-photon excitation can simultaneously excite multiple tracers with a single laser source because of the broader two-photon excitation spectra (Kasai et al. 2006), making this technology ideal for simultaneous multicolor imaging. To date, it has been possible to perform up to four-color imaging using an 830-nm laser for excitation. Two-photon imaging thus provides the best method to correlate fluorescence resonance energy transfer (FRET)-based protein signals with cellular events, such as exocytosis and endocytosis. TEP imaging can also be combined with capacitance measurements (Liu et al. 2005), investigations involving photolysis of caged compounds coupled with electron microscopy (Kishimoto et al. 2005; Liu et al. 2005), and prefusion labeling of specific molecules (Takahashi et al. 2004; Kishimoto et al. 2006). TEP imaging is also a good compliment for molecular biological techniques, as it allows for direct study of the tissues of mutant animals. We have succeeded in using TEP imaging to reproduce RIA findings regarding insulin secretion in three mutant mice: the *ashen* mouse, which lacks Rab27a (Kasai et al. 2005b); the collectrin transgenic mice (Fukui et al. 2005); and the HNF-4α knockout mice (Miura et al. 2006). The effects of knocking out CAPSs have also been similarly evaluated with both RIA and TEP imaging in another laboratory (Speidel et al. 2008).

The major disadvantage of two-photon microscopy is the costly femtosecond laser and the difficulty involved in maintaining the laser and the microscope, which are unfamiliar technologies for most biologists and even many biophysicists. We hope that these difficulties will be overcome with time and that more laboratories will thus be able to enjoy the benefits of TEP imaging.

Spatial Organization of Exocytosis: Docking, Priming, and the Readily Releasable Pool of Vesicles

We have used TEP imaging to study a number of representative secretory cell types and have found that in each secretory cell type, the exocytotic machinery and the

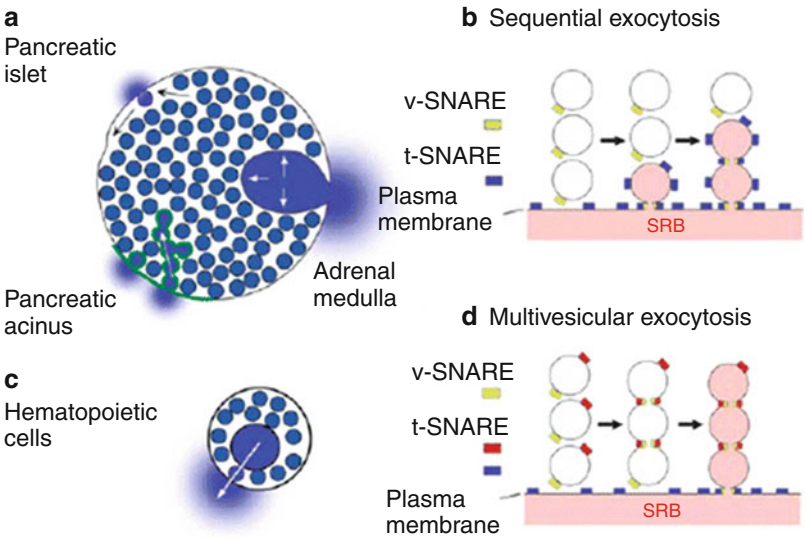

Fig. 3 Different forms of compound exocytosis. (**a**) Full fusion exocytosis, sequential exocytosis, and vacuolar sequential exocytosis in the pancreatic islet, the acinus, and the adrenal medulla, respectively. *Green outlines* in the pancreatic acinus indicate the actin coating. (**b**) Sequential exocytosis is supported by lateral diffusion of t-SNAREs from the plasma membrane. (**c**) Multivesicular exocytosis of eosinophils and basophils. (**d**) Multivesicular exocytosis may utilize two distinct t-SNAREs (Adapted from Kasai et al. (2006). With permission from Elsevier)

processes involved exhibit a unique pattern of spatial organization (Fig. 3). Full fusion of a vesicle with the plasma membrane is the dominant form of exocytosis in β-cells in the pancreatic islets (Takahashi et al. 2002a, 2004; Kasai et al. 2005a; Hatakeyama et al. 2006; Ma et al. 2004; Michael et al. 2006; Fig. 3a), though other forms of exocytosis do exist in these cells (section "Insulin Exocytosis"). In contrast, exocrine acinar cells frequently exhibit sequential exocytosis (Nemoto et al. 2001, 2004; Oshima et al. 2005; Fig. 3a), and adrenal chromaffin cells exhibit vacuolar sequential exocytosis (Kishimoto et al. 2006; Fig. 3a), where swelling of the granule contents facilitates sequential exocytosis. Sequential exocytosis is most suitable for those secretory cells that undergo massive exocytosis under the control of secretagogues. Sequential progression of exocytosis in these cells is indicative that some factors necessary for fusion diffuse from the plasma membrane into the vesicle membrane (Fig. 3b), an idea that has been confirmed via direct imaging (see Fig. 6). This may be one physiological function of the observed tight "docking" of granules to the plasma membrane in sequential exocytosis, as this process has been shown to require stable attachment of the outermost granules to the plasma membrane, even after exocytosis (Kishimoto et al. 2005, 2006; Kasai et al. 2006).

There is another type of compound exocytosis, called multivesicular (or multigranular) compound exocytosis, where vesicles fuse with each other in the cytosol before fusing with the plasma membrane (Fig. 3c). Multivesicular exocytosis has been described in eosinophils (Scepek and Lindau 1993;

Hafez et al. 2003), where secretion can be explosive. This is in contrast to the sequential exocytosis described above, where secretion is under the specific control of regulatory agents (Fig. 3a, b). The term "compound exocytosis" has been used to describe sequential exocytosis in an early study of mast cells (Alvarez de Toledo and Fernandez 1990) but for multivesicular exocytosis in eosinophils (Scepek and Lindau 1993; Hafez et al. 2003). It should be noted, however, that these two forms of "compound exocytosis" most likely have distinct molecular mechanisms (Fig. 3b, d) and physiological functions.

In line with the "crash fusion" events observed via TIRF imaging of LDCVs (Kasai et al. 2008; Shibasaki et al. 2007; Verhage and Sorensen 2008), TEP imaging has revealed that SLMVs undergo massive exocytosis without the "docking" of vesicles to the plasma membrane prior to stimulation in PC12 cells (Liu et al. 2005) and β-cells (Hatakeyama et al. 2007). Studies of sequential exocytosis have also indicated that prior "docking" of vesicles to the plasma membrane is unnecessary for exocytosis, because the vesicles in the deep cytosolic layer do not dock to the plasma membrane but do undergo rapid exocytosis and participate in this sequential replenishment process (Nemoto et al. 2004; Kishimoto et al. 2006). These findings thus suggest that a "priming" step may occur before the attachment of vesicles to the plasma membrane (Klenchin and Martin 2000) and that the "readily releasable pool of vesicles" is not necessarily comprised of "docked" vesicles (Shibasaki et al. 2007; Rizzoli and Betz 2004).

Insulin Exocytosis

Single Insulin Granule Exocytosis

In TEP imaging, insulin exocytosis occurs as sudden appearance of a fluorescent spot close to the intercellular space in the islets (Fig. 4a–c). The increase in the fluorescence indicates the backfilling of granules with extracellular SRB (Fig. 4c), as these fluorescent spots are immunoreactive to insulin (Takahashi et al. 2002a). Two-photon extracellular polar-tracer imaging-based quantification (TEPIQ) analysis has also indicated that the observed spots in islets have an estimated diameter that is consistent with that of insulin granules (Fig. 5a; Kasai et al. 2006). TEPIQ has been used to estimate the diameters of exocytotic granules in various secretory cells based on the intensity of fluorescent spots relative to that of the extracellular medium, and these estimates have been consistent with the values obtained via EM measurements, supporting the accuracy of this method.

Fluorescence decays within several seconds during most release events (Fig. 4c), reflecting the full fusion of granules with the plasma membrane. In support of this, flattening of the Ω-shaped profile has been directly detected in en face events (Fig. 4c). Such exocytotic events are induced by an increase in the cytosolic Ca^{2+} concentration, which often occurs in an oscillatory manner (Fig. 4d). TEP imaging can be used to visualize most insulin exocytosis in a focal plane of the islet, as the amount of insulin secretion predicted based upon the number of glucose-induced

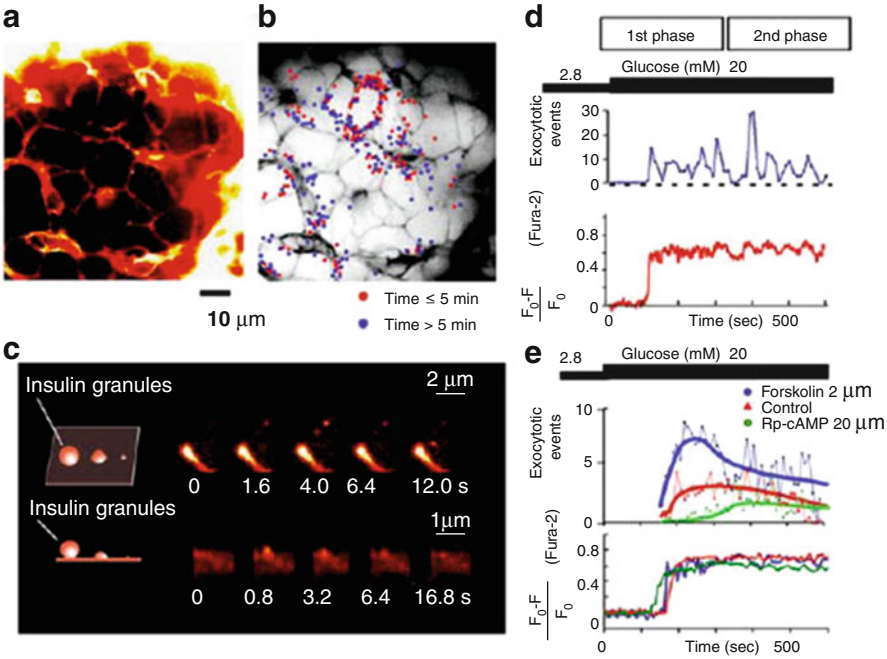

Fig. 4 Two-photon excitation imaging of exocytotic events in β-cells in mouse pancreatic islets. (**a**) A single islet immersed in a solution containing SRB. Large vessels can be seen at the *bottom left portion* of the panel. (**b**) Distribution of exocytotic events in an islet stimulated with 20 mM glucose. *Red* and *blue dots* represent sites at which exocytotic events were observed in the first and second phases of glucose-induced insulin secretion, respectively. The underlying *gray image* is the inverse image of the SRB fluorescence shown in (**a**). (**c**) Successive images of the glucose-induced abrupt appearance of SRB fluorescent spots. As illustrated by the schemes on the *left*, the *top images* were obtained from the interstitial space parallel to the imaging plane, while the *bottom images* show an en face view of exocytosis toward the vertically oriented interstitial space, and flattening of vesicles can be directly visualized. Glucose-induced exocytosis in a single islet (**d**) or averaged for several islets (**e**). The data in (**e**) were acquired from islets treated in the absence (*red*, $n = 5$) or the presence (*blue*, $n = 3$) of forskolin (2 μM) or Rp-cAMP (20 μM) (*green*, $n = 4$). Exocytotic events were measured within an arbitrary area (2,000 μm^2) of islets. The *bottom* traces show the increase in the cytosolic Ca^{2+} concentration recorded from single islets. The cytosolic Ca^{2+} concentration is represented by $(F_0-F)/F_0$, where F_0 and F stand for resting and poststimulation fluorescence, respectively (Adapted from Takahashi et al. (2002a). With permission from AAAS)

exocytotic events observed via TEP imaging is in accord with the amount of insulin release measured by RIA (Hatakeyama et al. 2006). The number of exocytotic events observed via TEP imaging per 800-μm^2 field correlates well with the reported number released by a single cell (Hatakeyama et al. 2006), which amounts to 6–12 LDCVs min^{-1} $cell^{-1}$. TEP imaging has also been used to show that the number of exocytotic events can be greatly potentiated, most prominently in the first phase, by treatment with forskolin, an activator of adenylate cyclase that increases the cytosolic concentration of cAMP (Fig. 4e). These features of

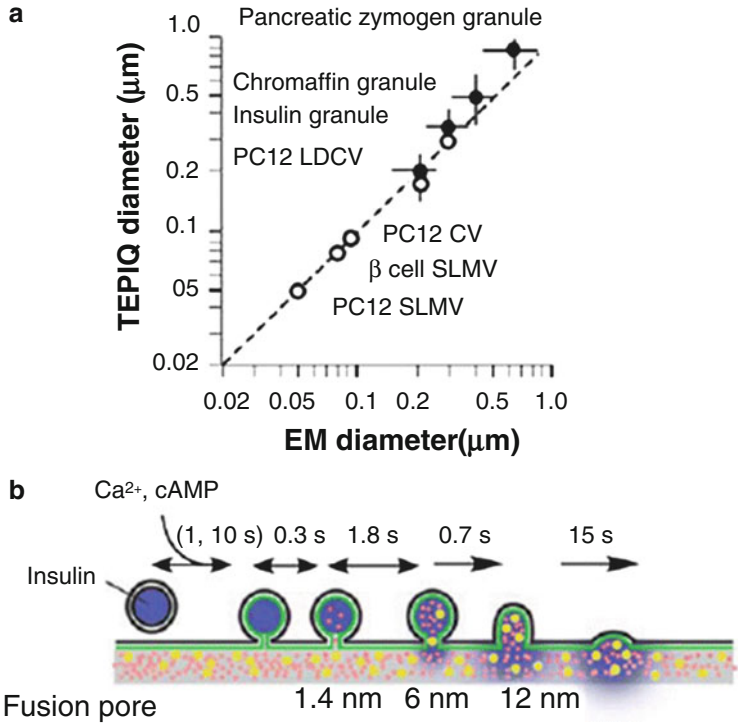

Fig. 5 Diameters of exocytotic vesicles and the fusion pore. (**a**) Diameters of exocytotic and endocytotic vesicles estimated via electron microscopy (EM) and TEPIQ analysis. The diameter of the zymogen vesicles in pancreatic acinar cells and those of the large vesicles in chromaffin, insulin, and PC12 cells were estimated via TEPIQ analysis of ΔV (Kasai et al. 2006). Bars represent the SD. The diameters of clathrin vesicles (CV) and SLMVs (*open circles*) in PC12 cells and β-cells were estimated via TEPIQ analysis of $\Delta V/\Delta S$. (**b**) Gradual opening of fusion pores estimated by staining of polar tracers of different diameters: *magenta points* represent SRB with a diameter of 1.4 nm and *yellow points* represent 10-kDa dextran fluorescein of a diameter of 6 nm. These conclusions are supported by the results of experiments with various fluorescent polar tracers (Takahashi et al. 2002a)

exocytosis observed via TEP imaging are consistent with those observed via RIA (Kahn 2004) and TIRF imaging (Shibasaki et al. 2007).

It is notable that exocytosis has been observed all over the plasma membrane of β-cells in TEP studies (Fig. 4b), though in accord with the findings of previous studies (Bonner-Weir 1988), there appears to be a slight trend toward exocytosis in the direction of blood vessels (Takahashi et al. 2002a). Given that no tight-junction structure is seen in islets (In't et al. 1984) or endocrine cells in general, this indicates that the intercellular space of the gland is the major pathway for secretion of the hormone.

In our study, β-cells underwent full flattening with the plasma membrane in 92 % of events (Takahashi et al. 2002a). This type of exocytosis, termed "full

fusion exocytosis," is the simplest form of exocytosis and has been assumed to exist for a long time (Heuser and Reese 1973), but TEP imaging has provided the first definitive evidence of this process in mammalian secretory cells. Experiments with both SRB and FM1-43 have shown that full fusion occurs with two time constants, 1.5 and 15 s in β-cells (Supplementary Fig. S2 of (Takahashi et al. 2002a)). The time course of decay was somewhat slower than in chromaffin cells, where the mean lifetime of the Ω-shaped profile was about 0.25 s (Kishimoto et al. 2006). As discussed below, this may reflect the time required for the insulin crystal to dissolve. Consistent with the above data, a recent study using Zn-sensitive dyes demonstrated that most vesicles (60 %) completely released insulin within 0.2–10 s of the onset of exocytosis in primary β-cells (Michael et al. 2006).

It has become evident that β-cells are rather exceptional in predominantly utilizing full fusion exocytosis with minor contributions by two other forms of exocytosis: kiss-and-run exocytosis (6 %; see below) and sequential exocytosis (2 %). Sequential or compound exocytosis has been observed in some studies of β-cells (Kwan and Gaisano 2005), particularly in cases where the islets were strongly stimulated, but this is far less common in these cells than in pancreatic acinar cells (Nemoto et al. 2001), adrenal chromaffin cells (Kishimoto et al. 2006), and hematopoietic cells. We hypothesize that sequential exocytosis is suppressed in β-cells to prevent hypersecretion of insulin, which would result in hypoglycemic coma. One mechanism for the suppression of sequential exocytosis in these cells is that most vesicles undergo full fusion, and there is thus no chance for secondary exocytosis. There must, however, be an additional mechanism responsible for this blockade of hypersecretion (described in section "Fusion Pore Compositions and Fusion Mechanisms"), as stimulation with a caged Ca^{2+}compound greatly prolonged the lifetime of the Ω-shaped profile without affecting the occurrence of sequential exocytosis (Takahashi et al. 2004).

Fusion Pore Kinetics and "Kiss-and-Run" Exocytosis

The fusion pore is the initial semi-stable aqueous pore that is formed during the fusion of two membranes. Time-resolved membrane capacitance measurement has been used to estimate the diameter of the pore to be about 0.3–2 nm, where it is stable for a period and can be reversibly closed (Klyachko and Jackson 2002; Alvarez de Toledo et al. 1993; Breckenridge and Almers 1987). Capacitance measurement, however, cannot estimate the diameters of pores larger than 2 nm for small vesicles or 6 nm for large vesicles (Klyachko and Jackson 2002; Alvarez de Toledo et al. 1993), possibly leading to underestimation of pore sizes.

To overcome this limitation, we used fluorescent polar tracers as nanometer-sized probes in TEP imaging experiments (Fig. 5b; Kasai et al. 2006). In these experiments, we used two polar tracers: SRB (0.3–0.7 mM) and dextrans conjugated with fluorescein of different molecular weights (0.5–2 mM). Based on the

molecular structures and light scattering, we estimated the hydrodynamic diameters of SRB and 10-kDa fluorescein dextran (FD) to be 1.4 and 6 nm, respectively (Kasai et al. 2006), and found that large dense-core vesicles of adrenal chromaffin cells and PC12 cells were nearly simultaneously stained with the two compounds, with a time lag of less than 50 ms. This time lag is consistent with the 10–50-ms lifetime of the fusion pore observed in these cells in studies using capacitance measurements and amperometry (Zhou et al. 1996).

In β-cells, there were significant time lags of 1–2 s between the SRB and 10-kDa FD signals, with a mean value of 1.8 s (Fig. 5b; Takahashi et al. 2002a; Hatakeyama et al. 2006), suggesting that dilation of the fusion pore is exceptionally slow for insulin vesicles in β-cells. There are a number of observations that suggest that this is due to crystallization of insulin in the vesicles, which prevents the dilation of vesicle contents and, consequently, the fusion pore. First, pore dilation was further slowed by the addition of zinc (3 mM), which is known to stabilize insulin crystals by binding to the insulin (Dodson and Steiner 1998), to the extracellular solution (Takahashi et al. 2002a). Second, pore dilation is significantly faster in guinea pig islets, where crystallization of insulin is known to be less prevalent (Dodson and Steiner 1998). Finally, flattening of vesicles has been found to start after the fusion pores were dilated to more than 12 nm, as confirmed by experiments with 70-kDa FD (Takahashi et al. 2002a; Fig. 5b), which may be interpreted to mean that the flattening of vesicles occurs when the fusion pore allows permeation of the 36-kDa insulin hexamer. Thus, our study suggests that secretion of insulin takes several seconds, which is in line with the results obtained via Zn imaging (Michael et al. 2006). In contrast, the decay of insulin-GFP release occurred within 1 s in TIRF imaging experiments (Ohara-Imaizumi et al. 2007; Kasai et al. 2008; Shibasaki et al. 2007), likely due to the lack of crystallization of insulin-GFP in these experiments.

Another interesting finding is that though the closure of the fusion pore can be reversible, transient openings are not larger than 6 nm in β-cells (Fig. 5b), which is similar to what has been reported in other cells (Takahashi et al. 2002a; Liu et al. 2005; Kishimoto et al. 2006). Some granules at such transient pore sites also subsequently move away from the site of exocytosis (Movie 2 of Takahashi et al. (2002a)), suggesting that these granules were engaged in "kiss-and-run" secretion. Since insulin supposedly cannot be secreted through such a transient narrow fusion pore (Takahashi et al. 2002a; Barg et al. 2002), such kiss-and-run events are considered to be failures of insulin secretion. In fact, such kiss-and-run exocytosis represents only 7 % of exocytotic events in β-cells, a number that is further reduced at higher cytosolic concentrations of cAMP (Hatakeyama et al. 2006). It is notable that while experiments employing confocal imaging of islets (Ma et al. 2004) and Zn imaging of insulin release (Michael et al. 2006) also indicate that most insulin granules undergo full fusion exocytosis in β-cells, "kiss-and-run" exocytosis was detected more frequently in a TIRF imaging experiment (Tsuboi et al. 2004). This suggests that the process of exocytosis significantly differs at plasma membranes on glass cover slips and in cells expressing exogenous proteins.

Fusion Pore Compositions and Fusion Mechanisms

The slow dilation of the fusion pore of insulin vesicles has facilitated the examination of the molecular composition of such pores. This has been done by observing the time course of the staining of insulin vesicles with the lipidic dye FM1-43. Because the pore size of these vesicles remains too small for FM1-43 to pass through for a period of time preceding full fusion and because of the lipidic nature of FM1-43, if the fusion pore was proteinaceous, the aqueous pore would be the only pathway for FM1-43 staining (Fig. 5b), and the rate of staining of vesicles with FM1-43 would be similar to that of SRB. In contrast, if the fusion pore was lipidic, FM1-43 should stain insulin vesicles via lateral diffusion from the previously stained plasma membrane (Fig. 5b, green), and since these vesicles can be as small as 350 nm, this lateral diffusion could be quite rapid, depending on the geometry of the fusion pore.

When we performed these experiments, we found that FM1-43 stained the vesicles with an earlier onset and faster time course than did SRB (Takahashi et al. 2002a; Hatakeyama et al. 2006). More precisely, FM1-43 had stained more than 64 % of a given vesicle when the SRB signal started to appear. We used this information and estimated the diffusion constant of the FM1-43 molecule to be 3.3 $\mu m^2/s$ along the fusion pore (Takahashi et al. 2002a), which is within the range of values expected for a pure lipid bilayer. This value is also greater than that observed in the plasma membrane ($<1\ \mu m^2/s$), where lateral diffusion of membrane lipids is prevented by the actin-based membrane skeleton, which blocks diffusion via the "picket" mechanism (Fujiwara et al. 2002). These data indicate that the fusion pore is already lipidic when the pore size is about 1.4 nm and thus that the purely proteinaceous fusion pore model does not apply in this case. The data also indicate that any proteins that might be present do not substantially disturb the flow of a lipidic molecule like FM1-43. It is notable that the faster staining of vesicles by FM1-43 compared to SRB is seen only in insulin vesicles and not in the large dense-core vesicles of PC12 cells (Kishimoto et al. 2005) and adrenal chromaffin cells (Kishimoto et al. 2006). This, however, is most likely because the pore expands rapidly in these cells, thus allowing SRB to rapidly stain a vesicle, and does not necessarily indicate a difference in fusion pore composition across cell lines. The lipidic nature of the fusion pore has also been indicated in other studies employing entirely different experimental approaches (Taraska et al. 2003; Chernomordik and Kozlov 2005) (but see Thorn et al. 2004; Han et al. 2004). Thus, our data suggest that SNARE proteins do not directly form the fusion pore in β-cells but rather that they trigger the formation of a lipidic fusion pore to initiate exocytosis.

Lateral Diffusion of SNARE Proteins

Sequential exocytosis has been observed in β-cells, though only as a small proportion of overall exocytosis (2 %) and only with a long latency of 6.5 s (Takahashi et al. 2004). We examined whether such sequential exocytosis was associated with

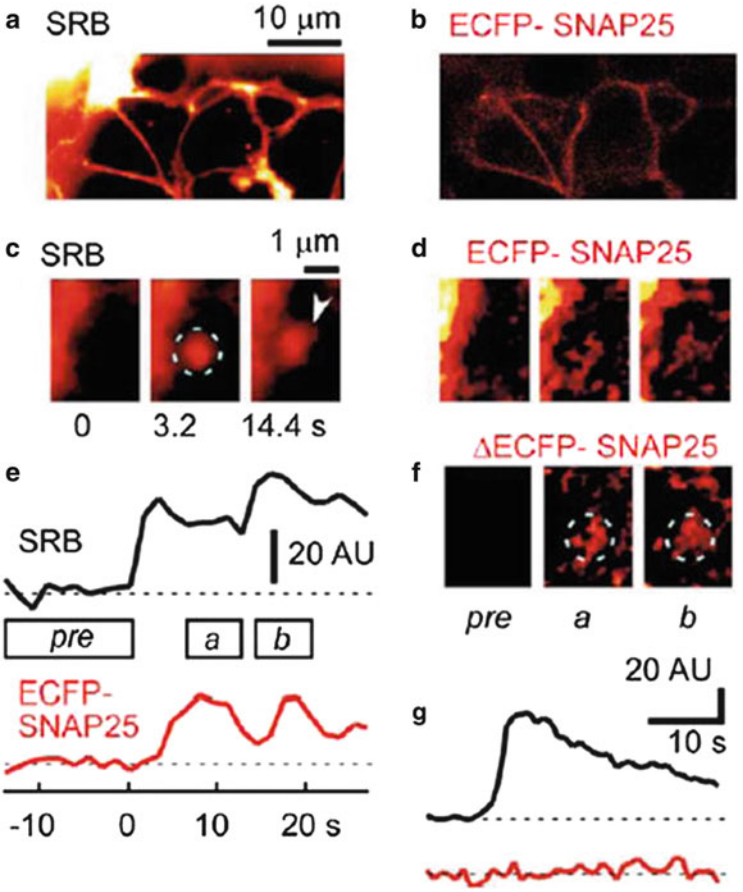

Fig. 6 Redistribution of SNAP-25 during sequential exocytosis. SRB (**a**) and ECFP-SNAP-25 (**b**) fluorescence images of an islet. The islet was transfected with an adenoviral vector encoding ECFP-SNAP-25 and then immersed in a solution containing the polar tracer SRB. Simultaneous measurement of SRB (**c**) and ECFP-SNAP-25 (**d**) fluorescence during a sequential exocytotic event. The number below each image in (**c**) represents the time after the onset of exocytosis. The *blue dashed circle* represents the region of interest. Each image in (**d**) was obtained by averaging 5–10 images in the three time periods shown in (**e**). (**e**) Time courses of fluorescence of SRB (*black*) and ECFP-SNAP-25 (*red*) in the region of interest shown in (**c**). *Open horizontal bars* represent time periods between −14.4 and 0 s after the onset of exocytosis (*pre*), between 6.4 and 12.8 s (**a**), and between 14.4 and 20.8 s (**b**), respectively. *Dashed horizontal lines* show baseline fluorescence levels. (**f**) Difference images obtained by subtracting image *pre* from three images in (**d**). (**g**) Time courses of fluorescence for solitary exocytotic events in a control cell (Adapted from Takahashi et al. (2004). With permission from the Rockefeller University Press)

lateral diffusion of SNAP-25 by expressing ECFP-SNAP-25 in the islets and performing TEP imaging together with ECFP imaging (Fig. 6a–d). We observed lateral diffusion of SNAP-25 in 6 % of exocytotic events (Fig. 6e). Importantly, the lateral diffusion of SNAP-25 was still detected in 54 % of vesicles undergoing

sequential exocytosis in β-cells but only in 5 % of vesicles involved in solitary exocytotic events (Fig. 6g). Furthermore, when islets were treated with cyclodextrin to remove lipid rafts that might trap SNAP-25, sequential exocytosis was hastened and its proportion increased to 8.9 % of total exocytosis, and lateral diffusion of SNAP-25 was detected in 15 % of all events (Takahashi et al. 2004).

When we performed the same experiment in adrenal chromaffin cells, where sequential exocytosis has been found to occur, and occur rapidly, in 73 % of exocytotic events (Kishimoto et al. 2006), lateral diffusion of SNAP-25 was detected in 43 % of exocytotic events. Furthermore, these sequential exocytosis events occurred with a short latency of only 1.2 s. Since the fluorescence signal from SNAP-25 was relatively small, it is likely that the actual diffusion occurred more frequently than we observed. Thus, lateral diffusion of SNAP-25 appears to occur in parallel with the sequential progression of exocytosis in both chromaffin cells and β-cells. Furthermore, the suppression of the lateral diffusion of SNAP-25 may account for the infrequency of sequential exocytosis in β-cells. Sequential exocytosis also involves other SNAREs, as redistribution of syntaxin-2 has been demonstrated via immunohistochemistry in pancreatic acinar cells (Pickett et al. 2005).

Regulation of Insulin Exocytosis

Biphasic Insulin Exocytosis and Protein Kinase A

Insulin secretion in the islet is known to occur in two phases in response to glucose stimulation or increases in cytosolic Ca^{2+} concentrations (Fig. 4d). There are likely multiple mechanisms underlying the two phases of insulin secretion. Using TEP imaging, we have revealed that protein kinase A (PKA) is specifically involved in the first phase of insulin exocytosis (Hatakeyama et al. 2006), as a series of PKA inhibitors (PKI, Rp-cAMPS, H89, KT5720) all potently and specifically blocked the initiation of the first phase of insulin exocytosis from the islet. This is in contrast to previous reports based on RIA studies that PKA antagonists have no or only a small effect on insulin exocytosis (Persaud et al. 1990; Lester et al. 1997; Harris et al. 1997). This apparent contradiction may be due to the lack of time resolution of RIA and the fact that membrane-permeable inhibitors often do not penetrate into tissues when applied via superfusion (Takahashi et al. 2002b). In support of this second hypothesis, we have demonstrated certain PKA inhibitors (H89 and KT5720) to be effective only in small cluster preparations, but not in the whole islets (Hatakeyama et al. 2006). It is also notable that these inhibitors did not affect the increases in cytosolic Ca^{2+} induced by glucose (Hatakeyama et al. 2006). It is therefore suggested that PKA plays a role in modulating insulin exocytosis, with this role being particularly important for the first phase of the process (Fig. 1).

The functions of Ca^{2+} and cAMP in insulin exocytosis have been intensively studied using both TEP imaging (Fig. 7d–g; Hatakeyama et al. 2006) and amperometry to measure serotonin release in whole-cell-clamped cells (Fig. 7a–c;

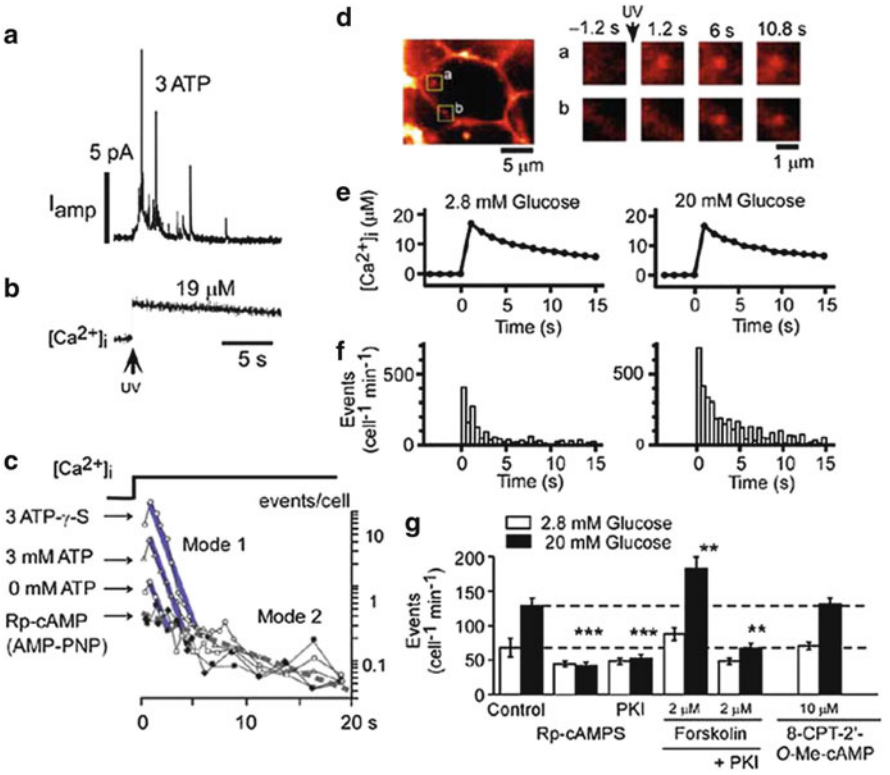

Fig. 7 Effects of cytosolic cAMP, ATP, and extracellular glucose on Ca^{2+}-induced exocytosis in β-cells. (**a**) Amperometric measurements of LDCV exocytosis from single β-cells in the presence of cytosolic ATP (3 mM). (**b**) $[Ca^{2+}]_i$ measured in the same cells. I_{amp}, amperometric current; UV, ultraviolet irradiation. (**c**) The regulation of insulin exocytosis by ATP is shown with semilogarithmic plots of amperometric latency histograms. Data shown are from cells perfused with 3 mM ATP-γS (*open circles*), 3 mM ATP (*open triangles*), 0 mM ATP (*open squares*), or AMP-PNP (*closed diamonds*) as well as from those pretreated with Rp-cAMP before perfusion with 3 mM ATP (*closed squares*). *Blue and dashed lines* indicate the Mode-1 and Mode-2 components, respectively. (**d**) TEP imaging of LDCV exocytosis in a β-cell within an islet loaded with a caged Ca^{2+} compound, nitrophenyl-EGTA (NPE), and perfused with a solution containing SRB. Exocytosis was triggered by UV-induced photolysis of NPE at time 0. The *boxed regions* (*a*) and (*b*) in the micrograph on the *left* are sites of individual exocytotic events after UV irradiation and are shown at higher magnification on the *right*. (**e**) Increases in $[Ca^{2+}]_i$ induced by the uncaging of NPE at time 0 in islets loaded with the Ca^{2+} indicator fura-2FF and exposed to 2.8 or 20 mM glucose. The high glucose solution was applied 1 min before UV irradiation. Traces represent average time courses obtained from four to six islets. (**f**) Latency histograms of insulin exocytosis induced by the uncaging of NPE in islets exposed to 2.8 or 20 mM glucose. The bin width is 0.5 s, and the data represent averages from nine to six islets, respectively. (**g**) The pharmacology of Ca^{2+}-dependent insulin exocytosis in the presence of 2.8 and 20 mM glucose. Exocytotic events were counted during the first 15 s after the uncaging of NPE. Inhibitors were applied for 40 min, forskolin and 8-CPT-2′–*O*-Me-cAMP for 10 min, and 20 mM glucose for 1 min before UV irradiation. Data are mean ± SEM. The actions of the various compounds were tested relative to the control values with the Dunnett test for 2.8 and 20 mM glucose, as indicated by dashed lines.

Takahashi et al. 1999). We examined the exocytosis induced via flash photolysis of caged Ca^{2+} compounds (Takahashi et al. 2004; Hatakeyama et al. 2006). Rapid and large increases in cytosolic Ca^{2+} released via the uncaging of a caged Ca^{2+} compound induced the exocytosis of LDCVs with two time constants, 1 and 10 s (Fig. 7c). Interestingly, the first component (Mode-1 exocytosis) was strongly dependent upon cytosolic ATP levels and was more potently augmented in response to the addition of ATP-γS (Fig. 7c), suggesting that the effect is dependent upon ATP-mediated phosphorylation rather than the cleavage of ATP itself. The ATP effect was mediated by PKA, the activity of which was found to depend upon both ATP and cAMP levels in the cytosol (Takahashi et al. 1999; Fig. 1a). We also used TEP imaging of intact islets of Langerhans to investigate whether PKA plays a similar role under glucose stimulation because whole-cell perfusion alters cytosolic environments.

Actions of Glucose and cAMP

The major effects of glucose on β-cells are thought to be mediated by mitochondrial generation of ATP, which results in the closure of K_{ATP} channels, the depolarization of the cell, and the activation of voltage-dependent Ca^{2+} channels (VDCC, Fig. 1a; Kahn 2004; Henquin 2000). It is known, however, that glucose has stimulatory effects on insulin secretion beyond those mediated by the closure of K_{ATP} channels, an effect referred to as the K_{ATP}-independent action of glucose (Gembal et al. 1992; Aizawa et al. 1994; Ravier et al. 2009). We thus examined the effect of glucose on LDCV exocytosis using TEP imaging in islets loaded with an AM ester of a caged Ca^{2+} compound (NP-EGTA) (Fig. 7d) and found that glucose stimulation produced a twofold increase in uncaging-induced exocytosis (Hatakeyama et al. 2006; Fig. 7e, f). Interestingly, the glucose effect was completely eliminated by PKA inhibitors (Fig. 7g; Hatakeyama et al. 2006). Furthermore, forskolin, which activates PKA by increasing cytosolic concentrations of cAMP, potentiated exocytosis at high glucose concentrations, but not at low glucose concentrations (Fig. 7g), indicating that another factor, in addition to cAMP, is required for the PKA effect. One possible candidate for this "other factor" is cytosolic ATP, which is known to increase in response to glucose and which is known, as noted above, to potentiate insulin exocytosis in a manner that is dependent upon PKA (Takahashi et al. 1999; Kasai et al. 2001; Fig. 7c). These experiments indicate that PKA is also involved in the glucose-sensing mechanism of islets and likely contributes to the K_{ATP}-independent action of glucose on insulin exocytosis (Henquin 2000; Gembal et al. 1992; Aizawa et al. 1994; Ravier et al. 2009).

Fig. 7 (continued) ** and *** represent $P < 0.01$ and $P < 0.001$, respectively (**a**–**c**: Adapted from Takahashi et al. (1999). With permission from National Academy of Sciences, USA, and **d**–**g**: from Hatakeyama et al. (2006). With permission from the Physiological Society)

Although glucose stimulation does not always increase cytosolic concentrations of cAMP (Gembal et al. 1992; Charles et al. 1973; Valverde et al. 1979; Hellman et al. 1974), the mechanisms described above can explain the observed increase in insulin exocytosis in response to glucose, even in the absence of increases in cAMP (Takahashi et al. 1999; Ravier et al. 2009; Kasai et al. 2001). In addition, recent studies employing cellular imaging of cAMP have revealed that cytosolic concentrations of cAMP may actually be increased by glucose in an oscillatory manner (Dyachok et al. 2008), providing one potential explanation for the oscillatory exocytosis of insulin observed during glucose stimulation (Tengholm and Gylfe 2009; Fig. 4d), as the effect of cAMP/PKA on insulin exocytosis occurs within a few seconds (Hatakeyama et al. 2007).

It is notable that the actions of cAMP on insulin release can be blocked by PKI (Fig. 7g) or H89 (Shibasaki et al. 2007) but are not mimicked by 10 μM 8-CPT-2′–*O*-Me-cAMP (Fig. 7g), which activates Epac (Enserink et al. 2002) and enhances exocytosis of SLMVs (Fig. 10b) but does not activate PKA. This further indicates that the acute action of cAMP is mediated by PKA. It is possible, however, that Epac may regulate exocytosis of LDCVs under basal conditions (Shibasaki et al. 2007). Unlike the whole-cell clamp experiment with forskolin or ATP (Fig. 7c), the slow component of insulin exocytosis was also facilitated by forskolin in AM ester-loaded intact islets (Fig. 7f). This is probably due to the rapid recovery of Ca^{2+} concentrations in the intact AM ester-loaded cells and delayed Mode-1 exocytosis in these experiments.

Beyond glucose, there are many hormones that also influence cytosolic cAMP levels and insulin exocytosis (Fig. 1a), and these hormones thus play a crucial role in the regulation of blood glucose levels (Henquin 2000). Furthermore, in addition to its direct effects on exocytosis, cAMP may enhance insulin exocytosis by potentiating VDCCs (Yaekura et al. 1996) and Ca^{2+} release from internal Ca^{2+} stores (Kang et al. 2005; Islam 2002). We have also revealed that cAMP reduces the amount of kiss-and-run exocytosis (Hatakeyama et al. 2006) and further facilitates PKA-mediated insulin secretion. In contrast, transient opening of very small fusion pores, which cannot be detected via TEP imaging, was reportedly increased by forskolin in cell-attached capacitance measurement experiments (MacDonald et al. 2006), though insulin secretion is unlikely to be significantly affected by the opening of such small fusion pores.

Molecular Mechanisms of Insulin Exocytosis

In spite of the confirmed role of cytosolic Ca^{2+} and PKA in mediating exocytosis, their target molecules have not been identified. In β-cells, the absence of synaptotagmins I and II suggests a role for vesicular synaptotagmins VII (Gustavsson et al. 2009; Gauthier and Wollheim 2008) and IX (Monterrat et al. 2007; Grise et al. 2007) as the major Ca^{2+} sensors. One candidate target of cAMP-dependent phosphorylation by PKA is a plasma membrane SNARE, SNAP-25, as threonine-138 of this protein is phosphorylatable by PKA (Nagy et al. 2004),

and this protein has been implicated in the early phase of Ca^{2+}-triggered exocytosis of large dense-core vesicles in chromaffin cells (Nagy et al. 2004). Snapin, a protein that binds to SNARE complexes, is also a target of PKA in chromaffin cells (Chheda et al. 2001), and while this protein is also expressed in β-cells, its role in the exocytosis of large dense-core vesicles is unknown. Rab-interacting molecule-2 (RIM2) (Kashima et al. 2001; Ozaki et al. 2000) also contains a PKA phosphorylation site that regulates its binding to Munc13, which plays a priming role in vesicle exocytosis (Kwan et al. 2007). Furthermore, another RIM protein, RIM1, has been shown to regulate neurotransmitter release at synapses in a PKA-dependent manner (Lonart et al. 2003).

The first phase of insulin exocytosis is markedly augmented by increased PKA in the islet (Figs. 1a and 4e; Hatakeyama et al. 2006) and is also enhanced by cAMP/Epac2/Rap1 signaling via mobilization of insulin granules toward the plasma membrane in isolated β-cells (Shibasaki et al. 2007). On the other hand, it has been reported that the second phase of insulin exocytosis is preferentially affected by CAPS (Speidel et al. 2008), myosin Va (Ivarsson et al. 2005), Cdc42 (Wang et al. 2007; Nevins and Thurmond 2005), and $Ca_v2.3$ (Jing et al. 2005).

Many molecules regulating insulin secretion upstream of the fusion reaction have been identified. PKA promotes the translation of various proteins necessary for the biogenesis of insulin granules via phosphorylation of polypyrimidine tract-binding protein 1 (PTB1) (Knoch et al. 2006). Islet antigen-2 (IA-2) and IA-2b (or phogrin) are required for proper cargo loading and stabilization of LDCVs (Henquin et al. 2008). The mobility of insulin granules is then facilitated by Rap1 (Shibasaki et al. 2007) and myosin Va (Varadi et al. 2005), and a small G protein Rab27 facilitates the transport of insulin granules to the plasma membrane (Kasai et al. 2008), while its target granuphilin facilitates docking but prevents fusion by forming the fusion-incompetent syntaxin-Munc18 complex (Gomi et al. 2005; Tomas et al. 2008). In contrast, a target of Rab3, Noc2 facilitates insulin secretion via its interaction with Munc13 (Cheviet et al. 2004) or by inhibition of Gi/Go signaling (Matsumoto et al. 2004), while another small G protein RalA plays a central role in the biphasic insulin secretion by regulating the mobilization of granules (Lopez et al. 2008).

Interestingly, the protein affected in maturity-onset diabetes of the young (MODY3), hepatocyte nuclear factor-1a (HNF-1a), has been observed to reduce the expression of collectrin, which facilitates SNARE complex formation via its interaction with snapin (Fukui et al. 2005), while MAPKp38 inhibits protein kinase D1 (PKD1), which regulates both insulin secretion and β-cell survival (Sumara et al. 2009). Finally, a metabolite of inositol phosphate $InsP_7$ has been reported to promote depolarization-induced capacitance increases in β-cells (Illies et al. 2007). It should also be noted here that insulin secretion is also very sensitive to cholesterol contents in the plasma membrane (Takahashi et al. 2004; Reese et al. 2005; Larsson et al. 2008).

Short noncoding RNAs (microRNAs) have been reported to directly or indirectly regulate the expression of proteins involved in secretion. A certain set of microRNAs, including miR375 and miR124a, are particularly enriched in β-cells.

Furthermore, miR375 has been reported to suppress insulin secretion, possibly via the activity of myotrophin (Poy et al. 2004), and to maintain β-cell mass (Poy et al. 2009). In addition, miR124a increases the expression of SNAREs and reduces Rab27A mRNA levels, thus suppressing glucose-induced insulin secretion (Lovis et al. 2008a). Some of the effects of miR124a may be mediated by the transcription factor Foxa2, which inhibits a number of signaling pathways affecting insulin secretion in mature β-cells (Gao et al. 2007). In addition, miR9 and miR96 increase the levels of granuphilin and reduce Noc2, thereby negatively regulating insulin exocytosis (Plaisance et al. 2006). Elevation of the microRNAs may also contribute to the detrimental effects of palmitate on insulin exocytosis (Lovis et al. 2008b).

Exocytosis of Synaptic-Like Microvesicles (SLMVs)

Historical Perspective

The presence of SLMVs in β-cells was first indicated with the identification of synaptophysin-positive small vesicles in such cells (Thomas-Reetz and De Camilli 1994). Several years later, the exocytosis of small vesicles with a mean diameter of 80 nm was demonstrated via cell-attached capacitance measurements (MacDonald et al. 2005). Additional evidence for the existence of SLMV exocytosis was provided by experiments employing whole-cell capacitance measurements that demonstrated a rapid increase in the plasma membrane area that was not associated with the exocytosis of insulin granules (Takahashi et al. 1997). Building on these discoveries, SLMV exocytosis in β-cells was further characterized via TEP imaging combined with EM using the membrane tracer FM1-43 instead of a volume tracer (SRB). These experiments revealed rapid increases in fluorescence upon uncaging of caged Ca^{2+} (Fig. 8a–c) as well as similar increases after glucose stimulation (Hatakeyama et al. 2007). These increases in FM1-43 fluorescence were diffuse (Fig. 8b) and rapid (Fig. 8d), unlike those associated with LDCV release (Figs. 4c and 7f). The diffuse fluorescence increases could also be detected using SRB, but the signals were far weaker than those obtained with FM1-43, suggesting that the fluorescence increases in these experiments were mediated by vesicles smaller than LDCVs. The diameter of vesicles was estimated via TEPIQ analysis to be about 80 nm (Fig. 5a; Hatakeyama et al. 2007), which is in accord with the size predicted via capacitance measurements (Vaag et al. 1995). The TEP/EM experiments also revealed that after washout of the dyes, the fluorescence was mostly retained in the cells (Fig. 8e) and even moved into the cytosol (Fig. 8f), indicating that the vesicles were internalized.

The conclusions drawn from the imaging analyses above were tested in studies employing electron microscopy of islet cell clusters in which DAB was photoconverted by the fluorescence of an aldehyde-fixable analog of FM1-43, FM1-43FX (Rebois et al. 1980; Harata et al. 2001; Brumback et al. 2004). As a control for this study, the constitutive endocytotic pathway was first labeled by immersing cells in FM1-43FX for 30 min without stimulation. This treatment

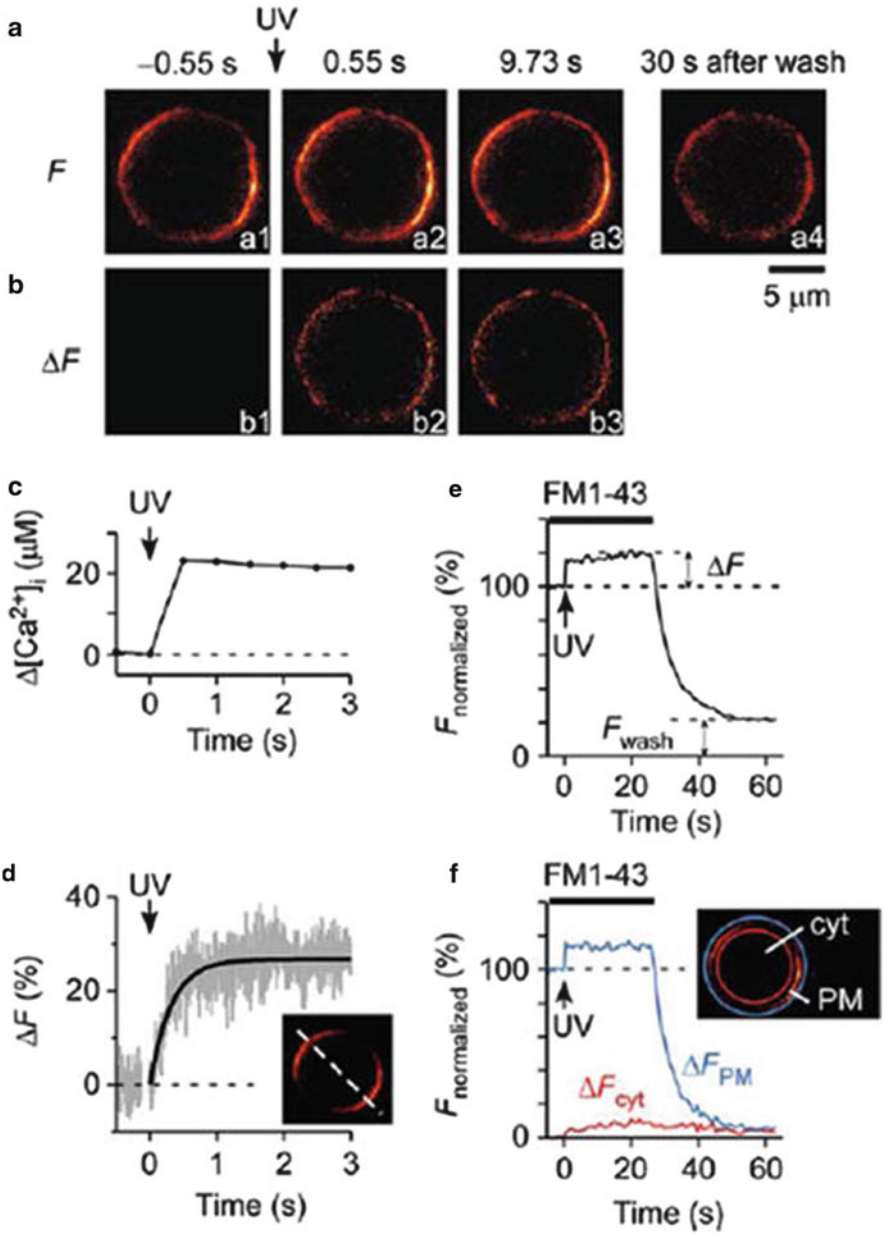

Fig. 8 TEP imaging of exocytosis and endocytosis using FM1-43 in isolated β-cells. (**a**) FM1-43 fluorescence (F) images of a cell loaded with NPE-AM are shown in frames a1–a4. Photolysis was induced at a time 0 between frames a1 and a2 (UV). The dye was washed out 30 s before frame a4. (**b**) Difference images (ΔF) obtained by subtracting the image at rest (a1) from frames a1 to a3 in (**a**) are shown in frames b1–b3. (**c**) Increase in $[Ca^{2+}]_i$ induced by photolysis of NPE in cells loaded

produced many small DAB-positive vesicles as well as DAB-positive endosome- and lysosome-like structures (Fig. 9a), similar to those observed in studies of PC12 cells (Liu et al. 2005). Notably, no staining of intracellular organelles was detected in cells exposed to FM1-43FX for 90 s before fixation (Fig. 9b). In contrast, many small DAB-positive vesicles were apparent in cells fixed within 15 s after stimulation via NPE photolysis during TEP imaging (Fig. 9c). In these samples, whereas some DAB-positive vesicles were still attached to the plasma membrane, many were scattered in the cytoplasm (Fig. 9c), which is consistent with the results obtained in the TEP imaging studies (Fig. 8f). The diameter of the stained vesicles in this series was about 70 nm, which is similar to the value estimated via both TEPIQ analysis and electrophysiology. The number of DAB-positive vesicles was 0.6 μm^{-2}, corresponding to a total of 4,000 vesicles per cell or 13 % of the original area of the plasma membrane. These results are consistent with the observed diffuse fluorescence increase of 20 % and with the fact that most of the membrane added to the cell surface during exocytosis events was recaptured by endocytosis events (Fig. 8e).

It is very interesting to note that images of LDCV exocytosis are rarely captured via EM, even though exocytosis of LDCVs is frequently detected by TEP imaging. This apparent discrepancy can be explained by our estimation of the rate of insulin exocytotic events as 20 $cell^{-1}$ within 15 s after photolysis of NPE (Hatakeyama et al. 2006), which means that the predicted number of LDCVs undergoing exocytosis in the thin sections required for electron microscopy is 0.009 μm^{-2} (Hatakeyama et al. 2007). This value is only 1.5 % of the corresponding value for SLMVs (0.6 μm^{-2}), which means that it is very challenging to detect insulin exocytosis in β-cells via EM (Orci et al. 1973). The fact that SLMV exocytosis could be readily identified via EM (Fig. 9c) supports our conclusion that the frequency of SLMV exocytosis is far greater than that of LDCV exocytosis in β-cells.

Abundant Ca^{2+}-dependent exocytosis of SLMVs has been observed in every type of cell examined to date. In PC12 cells, massive exocytosis of SLMVs has been experimentally associated with a rapid increase in membrane capacitance (Liu et al. 2005; Kasai et al. 1996; Ninomiya et al. 1997). Similar rapid capacitance increases have been reported in other cell types, including mast cells (Kirillova et al. 1993), fibroblasts (Coorssen et al. 1996; Ninomiya et al. 1996), pancreatic acinar cells (Ito et al. 1997), and adrenal chromaffin cells (Ninomiya et al. 1997;

Fig. 8 (continued) with the Ca^{2+} indicator fura-2FF. (**d**) Time course of the change in FM1-43 fluorescence induced by photolysis of NPE during line scanning along the *dashed line* shown in the inset. Average values from six cells (*gray trace*) and the single-exponential fit (*black line*) are shown. (**e**) Time course of the change in FM1-43 fluorescence for the entire section of the cell shown in (**a**). The zero level of fluorescence was obtained before the application of FM1-43 to the cell. Fluorescence was normalized to that of the entire section of the cell before photolysis and is expressed as a percentage of the control value ($F_{normalized}$). (**f**) Time courses of the changes in fluorescence in the plasma membrane (PM) region (*blue*) and in the cytoplasmic (cyt) region (*red*) of the cell depicted in the inset (Adapted from Hatakeyama et al. (2007). With permission from the Physiological Society)

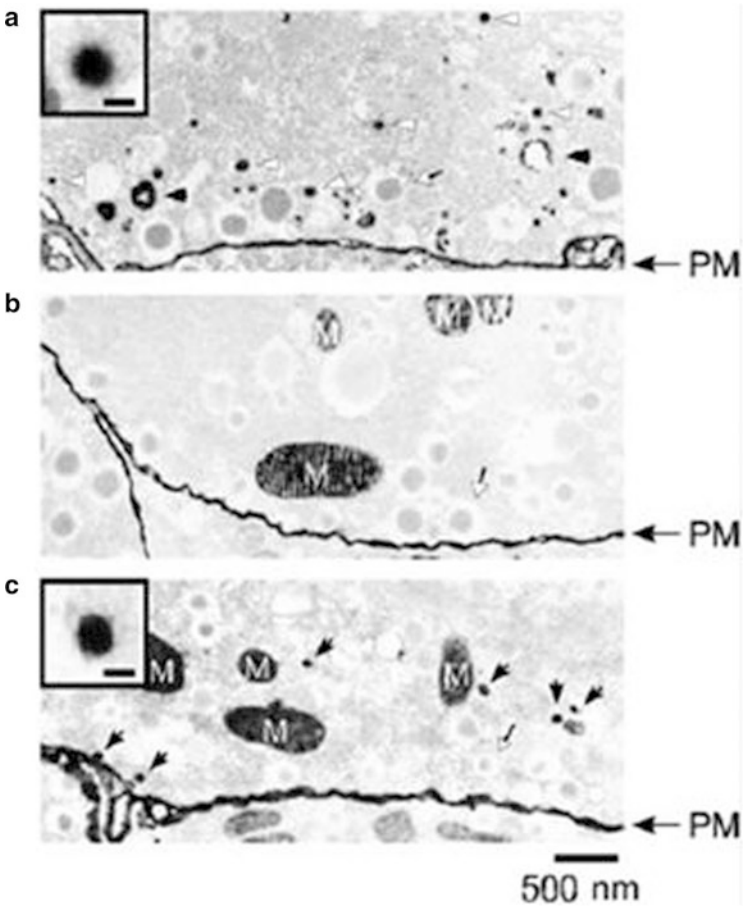

Fig. 9 Ultrastructural identification of endocytotic vesicles in β-cells. Endocytotic vesicles were examined via electron microscopy in cells loaded with FM1-43FX. Photoconversion of DAB was induced by the fluorescence of FM1-43FX remaining after extensive washout. Some FM1-43FX molecules remained in the plasma membrane (PM) despite washout, resulting in its staining with DAB (*long solid arrows*). (**a**) A cluster of cells immersed in a solution containing FM1-43FX for 30 min at rest. *Open arrows*, *open arrowheads*, and *closed arrowheads* indicate LVs, constitutive endocytotic vesicles, and lysosomes or endosomes, respectively. (**b**) A cluster of cells immersed in a solution containing FM1-43FX for 90 s without stimulation. M – mitochondria. (**c**) A cluster of cells immersed in a solution containing FM1-43FX for 1 min before photolysis of NPE and fixed with glutaraldehyde within ~15 s after photolysis. *Short solid arrows* indicate small endocytotic vesicles. The external scale bar (500 nm) applies to all panels with the exception of the insets in (**a**) and (**c**), which show magnified images of DAB-positive endocytotic vesicles and for which the associated scale bars represent 50 nm (Adapted from Hatakeyama et al. (2007). With permission from the Physiological Society)

Haller et al. 1998). Thus, mammalian cells may commonly possess numerous SLMVs that can undergo rapid Ca^{2+}-dependent exocytosis (Borgonovo et al. 2002; Steinhardt et al. 1994; McNeil and Steinhardt 1997).

Regulation by cAMP

We examined the effect of cAMP on the Ca^{2+}-dependent exocytosis of SLMVs in β-cells. Forskolin increased SLMV exocytosis induced by photolysis of NPE by 39 %, which was detected as an increase in the diffuse FM1-43 fluorescence signal (Fig. 10a). The potentiation of SLMV exocytosis by cAMP was not blocked by antagonists of PKA, including PKI and Rp-cAMPS (Fig. 10b). A portion of the PKA-independent cAMP signaling is mediated by guanine nucleotide exchange factors that are directly activated by cAMP (Epac) and are also specifically activated by 10 μM 8-CPT-2′–*O*-Me-cAMP (Enserink et al. 2002). The finding that 10 μM 8-CPT-2′–*O*-Me-cAMP mimicked the effect of forskolin on SLMV exocytosis (Fig. 10b) suggests that the potentiation of Ca^{2+}-dependent exocytosis of SLMVs by cAMP is dependent upon Epac, but not PKA.

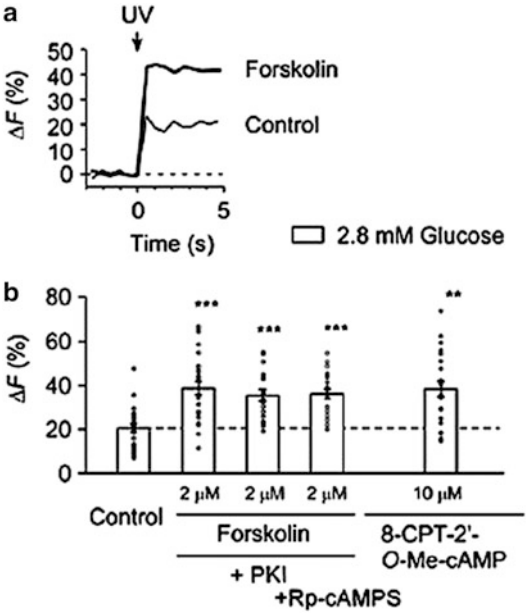

Fig. 10 Pharmacology of Ca^{2+}-dependent exocytosis of SLMVs in β-cells. (**a**) Time courses of FM1-43 fluorescence after photolysis of NPE in representative β-cells either maintained under control conditions or pretreated with forskolin (2 μM) for 10 min. (**b**) Cells were exposed (or not) to PKI (5 μM) or Rp-cAMPS (200 μM) for 30 min before incubation for 10 min with forskolin (2 μM) or 8-CPT-2′–*O*-Me-cAMP (10 μM), as indicated. The cells were then stimulated by NPE photolysis, and the diffuse increase in FM1-43 fluorescence was measured. *Circles* represent data obtained from individual cells, with columns and error bars indicating mean ± SEM. The *dashed line* shows the control level (NPE photolysis alone). Statistical analyses were performed using the Kruskal-Wallis test ($P < 0.001$), followed by the Steel test for comparison with control values. ** and *** represent $P < 0.01$ and $P < 0.001$, respectively (Adapted from Hatakeyama et al. (2007). With permission from the Physiological Society)

The regulation of SLMV exocytosis by cAMP is strikingly different from that of LDCV exocytosis (Hatakeyama et al. 2006). First, Ca^{2+}-dependent exocytosis of LDCVs was significantly enhanced by forskolin at a high glucose concentration (20 mM), but not at a low glucose concentration (2.8 mM) (Fig. 7g), whereas SLMV exocytosis was potentiated by forskolin at a low glucose concentration (Fig. 10b). Second, the effect of forskolin was inhibited by PKI (Fig. 7g), while 10 μM 8-CPT-2′–*O*-Me-cAMP did not increase the extent of insulin exocytosis (Fig. 7g; Hatakeyama et al. 2006). These observations indicate that the acute effects of Epac and PKA are specific to Ca^{2+}-dependent exocytosis of SLMVs and LDCVs, respectively, in β-cells. The fact that rapid exocytosis of SLMVs is selectively modulated by Epac is consistent with reports that the observed rapid capacitance increase was found to be resistant to Rp-cAMPS and PKI (Renstrom et al. 1997) and that 8-CPT-2′–*O*-Me-cAMP potentiated the rapid component of such capacitance increases (Eliasson et al. 2003). Similar effects of Epac on increases in the membrane capacitance were reported in melanotroph (Sedej et al. 2005).

The potentiation of SLMV exocytosis by cAMP was faster than that of LDCV exocytosis (Hatakeyama et al. 2007). The latency to augmentation of LDCV exocytosis (~5 s) may reflect the time required for protein phosphorylation and activation by activated PKA to take place. In contrast, the action of Epac may be faster because it requires only nucleotide exchange, which occurs within a fraction of a second (John et al. 1990; Itzen et al. 2007). Epac has also been proposed to regulate exocytosis via direct binding to Rim2 (Ozaki et al. 2000). Rim proteins are putative effectors of Rab3 and are thought to serve as Rab3-dependent regulators of synaptic vesicle fusion (Wang et al. 1997), a role that they may also play in the exocytosis of SLMVs in β-cells. Activation of rap by Epac might also be involved in this process (Shibasaki et al. 2007; Enserink et al. 2002).

Functional Role of SLMV Exocytosis in β-Cells

Glucose stimulation also induces massive exocytosis of SLMVs in β-cells (Hatakeyama et al. 2007). If we assume that the diameters of SLMVs and β-cells are 80 nm and 12 μm, respectively, an increase in FM1-43 fluorescence of 3 % $cell^{-1}$ min^{-1} represents exocytosis of 675 SLMVs $cell^{-1}$ min^{-1}, a rate that is more than 100-fold greater than that previously reported for LDCV exocytosis (6.4 LDCVs $cell^{-1}$ min^{-1}) (Hatakeyama et al. 2006). Exocytosis of SLMVs in β-cells thus likely plays a significant physiological role. Although SLMVs in β-cells contain GABA (Thomas-Reetz and De Camilli 1994), fewer than 100 GABA-induced quantal currents were detected after photolysis of a caged Ca^{2+} compound in β-cells expressing recombinant $GABA_A$ receptors (Braun et al. 2004), whereas exocytosis of ~4,500 SLMVs was detected via TEPIQ analysis (a 20 % increase in membrane area) and, similarly, 4,000 endocytotic vesicles were detected via electron microscopy in response to this stimulus. These observations suggest that GABA is present in, at most, only ~1 % of SLMVs in β-cells, which is similar to the situation observed with acetylcholine in PC12 cells, where only a small

proportion of SLMVs contain detectable acetylcholine (Liu et al. 2005; Ninomiya et al. 1997). It is therefore unlikely that the physiological role of SLMV exocytosis lies in the secretion of vesicle contents (Hatakeyama et al. 2007).

The trafficking of proteins and lipids between the plasma membrane and endosomes is another possible function of SLMV exo-/endocytosis. SLMVs likely contribute more efficiently to membrane trafficking than they do to secretion because of their large surface-to-volume ratio. Membrane area added to the plasma membrane via SLMV exocytosis is ~3 % $cell^{-1}$ min^{-1} during glucose stimulation, a rate that is about five times greater than that for LDCV exocytosis (0.54 % $cell^{-1}$ min^{-1}). Furthermore, SLMVs may play a similar membrane trafficking role in "nonsecretory" cells that nonetheless exhibit a substantial amount of SLMV exocytosis (Borgonovo et al. 2002; Steinhardt et al. 1994; McNeil and Steinhardt 1997).

The finding that the actions of both Ca^{2+} and cAMP were faster for SLMVs than for LDCVs indicates that SLMV exocytosis-mediated trafficking of molecules precedes LDCV exocytosis in response to a stimulatory event. Exocytosis of SLMVs may thus precondition the plasma membrane for LDCV exocytosis and endocytosis. An example of a molecule that may be trafficked in this manner is a sialylated form of the cell adhesion molecule NCAM (PSA-NCAM), which is expressed specifically in β-cells and is mobilized to the cell surface in an activity-dependent manner (Bernard-Kargar et al. 2001). Moreover, the surface expression of PSA-NCAM is correlated with glucose-stimulated insulin secretion (Bernard-Kargar et al. 2001). In pancreatic islets, NCAM is thought to contribute to the maintenance of cell-cell interactions and is required for normal turnover of secretory granules (Langley et al. 1989; Esni et al. 1999). As such, increased surface expression of PSA-NCAM might therefore facilitate contact between β-cells and other islet cells in order to preserve islet integrity in the face of secretion of reactive substances stored in insulin granules, such as insulin, Zn, protons, ATP, GABA, carboxypeptidase E, and islet amyloid polypeptide (Hutton et al. 1983; Hutton 1989; Gammelsaeter et al. 2004). Impairment of such SLMV-mediated preconditioning might thus result in islet dysfunction.

Perspectives

Various methodologies have been employed to analyze the various types of exocytosis from β-cells, and considerable progress has recently been made in elucidating the mechanistic underpinnings of these processes. Our knowledge of exocytosis, however, still remains incomplete because none of the available methodologies are ideal for investigating all facets of these processes. Genetic approaches can correlate molecules with phenotypes, but the knowledge gap between molecular and cellular phenomena is immense, and imaging and reconstruction approaches, though very powerful and full of potential, are still unable to completely bridge this gap. Thus, these techniques should be further developed to gain more direct insight into the complex cellular processes involved. We need to fully characterize the molecular basis of the fusion reaction itself and its preparatory reactions, without relying on the

arbitrary assumptions used in the age of electrophysiology. In this vein, it should also be kept in mind that the quantitative characteristics of exocytosis differ considerably between neurons and secretory cells (Kasai 1999), and thus the molecular mechanisms must also differ. We also need to identify and characterize the functions of and physiological roles played by SLMVs in both secretory and "nonsecretory" cells.

We hope that the studies described in this review provide a thorough introduction to the power of two-photon imaging in investigating exocytosis and endocytosis in secretory tissues. TEP imaging is the only method currently available that allows for the investigation of intact secretory tissues, and it can be used to address almost every important parameter related to exocytosis, including vesicle diameter, fusion readiness, fusion pore properties, lifespan of the Ω-shaped profile, swelling of vesicles, compound exocytosis, endocytosis, the spatial organization of exocytosis, and the proteins involved in exocytosis, such as SNARE proteins and the actin cytoskeleton. TEP imaging is also well suited to the study of mutant animals and even human specimens, because intact tissue preparations may be examined. Another benefit of TEP imaging is its compatibility with electron microscopy via the photoconversion of DAB using FM1-43. The powerful biophysical tool of caged compounds can also be utilized to quantify the processes of exocytosis. In the future, the power of this technology will only increase as it will be possible to perform TEP imaging simultaneously with other methods, such as prefusion labeling, to track the entire life cycle of vesicles, both before and after exocytosis, and to reveal the molecular mechanisms underlying exocytosis. Thus, we predict that two-photon imaging will play an increasingly important role in the full characterization of exocytosis and endocytosis in β-cells as well as other cell types.

Acknowledgments This work was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan and the Global COE Program (Integrative Life Science Based on the Study of Biosignaling Mechanisms) of MEXT.

References

Aizawa T, Sato Y, Ishihara F, Taguchi N, Komatsu M, Suzuki N, Hashizume K, Yamada T (1994) ATP-sensitive K^+ channel-independent glucose action in rat pancreatic β-cell. Am J Physiol 266:C622–C627

Albillos A, Dernick G, Horstmann H, Almers W, Alvarez de Toledo G, Lindau M (1997) The exocytotic event in chromaffin cells revealed by patch amperometry. Nature 389:509–512

Allersma MW, Wang L, Axelrod D, Holz RW (2004) Visualization of regulated exocytosis with a granule-membrane probe using total internal reflection microscopy. Mol Biol Cell 15:4658–4668

Alvarez de Toledo G, Fernandez JM (1990) Compound versus multigranular exocytosis in peritoneal mast cells. J Gen Physiol 95:397–409

Alvarez de Toledo G, Fernadez-Chacon R, Fernandez JM (1993) Release of secretory products during transient vesicle fusion. Nature 363:554–558

Barg S, Olofsson CS, Schriever-Abeln J, Wendt A, Gebre-Medhin S, Renstrom E, Rorsman P (2002) Delay between fusion pore opening and peptide release from large dense-core vesicles in neuroendocrine cells. Neuron 33:287–299

Bernard-Kargar C, Kassis N, Berthault MF, Pralong W, Ktorza A (2001) Sialylated form of the neural cell adhesion molecule (NCAM): a new tool for the identification and sorting of β-cell subpopulations with different functional activity. Diabetes 50(Suppl 1):S125–S130

Bonner-Weir S (1988) Morphological evidence for pancreatic polarity of β-cell within islets of Langerhans. Diabetes 37:616–621

Borgonovo B, Cocucci E, Racchetti G, Podini P, Bachi A, Meldolesi J (2002) Regulated exocytosis: a novel, widely expressed system. Nat Cell Biol 4:955–962

Braun M, Wendt A, Birnir B, Broman J, Eliasson L, Galvanovskis J, Gromada J, Mulder RP (2004) Regulated exocytosis of GABA-containing synaptic-like microvesicles in pancreatic β-cells. J Gen Physiol 123:191–204

Braun M, Wendt A, Karanauskaite J, Galvanovskis J, Clark A, MacDonald PE, Rorsman P (2007) Corelease and differential exit via the fusion pore of GABA, serotonin, and ATP from LDCV in rat pancreatic β cells. J Gen Physiol 129:221–231

Breckenridge LJ, Almers W (1987) Currents through the fusion pore that forms during exocytosis of a secretory vesicle. Nature 328:814–817

Brumback AC, Lieber JL, Angleson JK, Betz WJ (2004) Using FM1-43 to study neuropeptide granule dynamics and exocytosis. Methods 33:287–294

Charles MA, Fanska R, Schmid FG, Forsham PH, Grodsky GM (1973) Adenosine 3′, 5′-monophosphate in pancreatic islets: glucose-induced insulin release. Science 179:569–571

Chernomordik LV, Kozlov MM (2005) Membrane hemifusion: crossing a chasm in two leaps. Cell 123:375–382

Cheviet S, Coppola T, Haynes LP, Burgoyne RD, Regazzi R (2004) The Rab-binding protein Noc2 is associated with insulin-containing secretory granules and is essential for pancreatic β-cell exocytosis. Mol Endocrinol 18:117–126

Chheda MG, Ashery U, Thakur P, Rettig J, Sheng ZH (2001) Phosphorylation of Snapin by PKA modulates its interaction with the SNARE complex. Nat Cell Biol 3:331–338

Coorssen JR, Schmitt H, Almers W (1996) Ca^{2+} triggered massive exocytosis in Chinese hamster ovary cells. EMBO J 15:3787–3791

Dan Y, Poo M (1992) Quantal transmitter secretion from myocytes loaded with acetylcholine. Nature 359:733–736

Dean PM (1973) Ultrastructural morphometry of the pancreatic β-cell. Diabetologia 9:115–119

Dodson G, Steiner D (1998) The role of assembly in insulin's biosynthesis. Curr Opin Struct Biol 8:189–194

Dudek RW, Boyne AF (1986) An excursion through the ultrastructural world of quick-frozen pancreatic islets. Am J Anat 175:217–243, 354

Dyachok O, Idevall-Hagren O, Sagetorp J, Tian G, Wuttke A, Arrieumerlou C, Akusjarvi G, Gylfe E, Tengholm A (2008) Glucose-induced cyclic AMP oscillations regulate pulsatile insulin secretion. Cell Metab 8:26–37

Eliasson L, Ma X, Renstrom E, Barg S, Berggren PO, Galvanovskis J, Gromada J, Jing X, Lundquist I, Salehi A, Sewing S, Rorsman P (2003) SUR1 regulates PKA-independent cAMP-induced granule priming in mouse pancreatic β-cells. J Gen Physiol 121:181–197

Enserink JM, Christensen AE, De RJ, Van TM, Schwede F, Genieser HG, Doskeland SO, Blank JL, Bos JL (2002) A novel Epac-specific cAMP analogue demonstrates independent regulation of Rap1 and ERK. Nat Cell Biol 4:901–906

Esni F, Taljedal IB, Perl AK, Cremer H, Christofori G, Semb H (1999) Neural cell adhesion molecule (N-CAM) is required for cell type segregation and normal ultrastructure in pancreatic islets. J Cell Biol 144:325–337

Fujiwara T, Ritchie K, Murakoshi H, Jacobson K, Kusumi A (2002) Phospholipids undergo hop diffusion in compartmentalized cell membrane. J Cell Biol 157:1071–1082

Fukui K, Yang Q, Cao Y, Takahashi N, Hatakeyama H, Wang H, Wada J, Zhang Y, Marselli L, Nammo T, Yoneda K, Onishi M, Higashiyama S, Matsuzawa Y, Gonzalez FJ, Weir GC, Kasai H, Shimomura I, Miyagawa J, Wollheim CB, Yamagata K (2005) The HNF-1 target collectrin controls insulin exocytosis by SNARE complex formation. Cell Metab 2:373–384

Gammelsaeter R, Froyland M, Aragon C, Danbolt NC, Fortin D, Storm-Mathisen J, Davanger S, Gundersen V (2004) Glycine, GABA and their transporters in pancreatic islets of Langerhans: evidence for a paracrine transmitter interplay. J Cell Sci 117:3749–3758

Gao N, White P, Doliba N, Golson ML, Matschinsky FM, Kaestner KH (2007) Foxa2 controls vesicle docking and insulin secretion in mature β cells. Cell Metab 6:267–279

Gauthier BR, Wollheim CB (2008) Synaptotagmins bind calcium to release insulin. Am J Physiol Endocrinol Metab 295:E1279–E1286

Gembal M, Gilon P, Henquin J (1992) Evidence that glucose can control insulin release independently from its action on ATP-sensitive K^+ channels in mouse B cells. J Clin Invest 89:1288–1295

Gerber SH, Sudhof TC (2002) Molecular determinants of regulated exocytosis. Diabetes 51(Suppl 1):S3–S11

Gomi H, Mizutani S, Kasai K, Itohara S, Izumi T (2005) Granuphilin molecularly docks insulin granules to the fusion machinery. J Cell Biol 171:99–109

Grise F, Taib N, Monterrat C, Lagree V, Lang J (2007) Distinct roles of the C_2A and the C_2B domain of the vesicular Ca^{2+} sensor synaptotagmin 9 in endocrine β-cells. Biochem J 403:483–492

Gustavsson N, Wei SH, Hoang DN, Lao Y, Zhang Q, Radda GK, Rorsman P, Sudhof TC, Han W (2009) Synaptotagmin-7 is a principal Ca^{2+}-sensor for Ca^{2+}-induced glucagon exocytosis in pancreas. J Physiol 587:1169–1178

Hafez I, Stolpe A, Lindau M (2003) Compound exocytosis and cumulative fusion in eosinophils. J Biol Chem 278:44921–44928

Haller M, Heinemann C, Chow RH, Heidelberger R, Neher E (1998) Comparison of secretory responses as measured by membrane capacitance and by amperometry. Biophys J 74:2100–2113

Han X, Wang CT, Bai J, Chapman ER, Jackson MB (2004) Transmembrane segments of syntaxin line the fusion pore of Ca^{2+}-triggered exocytosis. Science 304:289–292

Harata N, Ryan TA, Smith SJ, Buchanan J, Tsien RW (2001) Visualizing recycling synaptic vesicles in hippocampal neurons by FM 1–43 photoconversion. Proc Natl Acad Sci USA 98:12748–12753

Harris TE, Persaud SJ, Jones PM (1997) Pseudosubstrate inhibition of cyclic AMP-dependent protein kinase in intact pancreatic islets: effects on cyclic AMP-dependent and glucose-dependent insulin secretion. Biochem Biophys Res Com 232:648–651

Hatakeyama H, Kishimoto T, Nemoto T, Kasai H, Takahashi N (2006) Rapid glucose sensing by protein kinase A for insulin exocytosis in mouse pancreatic islets. J Physiol 570:271–282

Hatakeyama H, Takahashi N, Kishimoto T, Nemoto T, Kasai H (2007) Two cAMP-dependent pathways differentially regulate exocytosis of large dense-core and small vesicles in mouse β-cells. J Physiol 582:1087–1098

Hellman B, Idahl LA, Lernmark A, Taljedal IB (1974) The pancreatic β-cell recognition of insulin secretagogues: does cyclic AMP mediate the effect of glucose? Proc Natl Acad Sci USA 71:3405–3409

Henquin JC (2000) Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 49:1751–1760

Henquin JC, Nenquin M, Szollosi A, Kubosaki A, Louis NA (2008) Insulin secretion in islets from mice with a double knockout for the dense core vesicle proteins islet antigen-2 (IA-2) and IA-2β. J Endocrinol 196:573–581

Heuser JE, Reese TS (1973) Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. J Cell Biol 57:315–344

Hollins B, Ikeda SR (1997) Heterologous expression of a P2x-purinoceptor in rat chromaffin cells detects vesicular ATP release. J Neurophysiol 78:3069–3076

Hutton JC (1989) The insulin secretory granule. Diabetologia 32:271–281

Hutton JC, Peshavaria M, Tooke NE (1983) 5-Hydroxytryptamine transport in cells and secretory granules from a transplantable rat insulinoma. Biochem J 210:803–810

Illies C, Gromada J, Fiume R, Leibiger B, Yu J, Juhl K, Yang SN, Barma DK, Falck JR, Saiardi A, Barker CJ, Berggren PO (2007) Requirement of inositol pyrophosphates for full exocytotic capacity in pancreatic β cells. Science 318:1299–1302

In't VP, Pipeleers DG, Gepts W (1984) Evidence against the presence of tight junctions in normal endocrine pancreas. Diabetes 33:101–104

Islam MS (2002) The ryanodine receptor calcium channel of β-cells: molecular regulation and physiological significance. Diabetes 51:1299–1309

Ito K, Miyashita Y, Kasai H (1997) Micromolar and submicromolar Ca^{2+} spikes regulating distinct cellular functions in pancreatic acinar cells. EMBO J 16:242–251

Itzen A, Rak A, Goody RS (2007) Sec2 is a highly efficient exchange factor for the Rab protein Sec4. J Mol Biol 365:1359–1367

Ivarsson R, Jing X, Waselle L, Regazzi R, Renstrom E (2005) Myosin 5a controls insulin granule recruitment during late-phase secretion. Traffic 6:1027–1035

Jahn R, Lang T, Sudhof TC (2003) Membrane fusion. Cell 112:519–533

Jing X, Li DQ, Olofsson CS, Salehi A, Surve VV, Caballero J, Ivarsson R, Lundquist I, Pereverzev A, Schneider T, Rorsman P, Renstrom E (2005) $Ca_v2.3$ calcium channels control second-phase insulin release. J Clin Invest 115:146–154

John J, Sohmen R, Feuerstein J, Linke R, Wittinghofer A, Goody RS (1990) Kinetics of interaction of nucleotides with nucleotide-free H-ras p21. Biochemistry 29:6058–6065

Kahn CR (2004) Joslin's diabetes mellitus. Lippincott Williams & Wilkins, Philadelphia

Kang G, Chepurny OG, Rindler MJ, Collis L, Chepurny Z, Li WH, Harbeck M, Roe MW, Holz GG (2005) A cAMP and Ca^{2+} coincidence detector in support of Ca^{2+}-induced Ca^{2+} release in mouse pancreatic β cells. J Physiol 566:173–188

Karanauskaite J, Hoppa MB, Braun M, Galvanovskis J, Rorsman P (2009) Quantal ATP release in rat β-cells by exocytosis of insulin-containing LDCVs. Pflugers Arch 458:389–401

Kasai H (1999) Comparative biology of exocytosis: implications of kinetic diversity for secretory function. Trends Neurosci 22:88–93

Kasai H, Takagi H, Ninomiya Y, Kishimoto T, Ito K, Yoshida A, Yoshioka T, Miyashita Y (1996) Two components of exocytosis and endocytosis in PC12 cells studied using caged-Ca^{2+} compounds. J Physiol (Lond) 494:53–65

Kasai H, Suzuki T, Liu T, Kishimoto T, Takahashi T (2001) Fast and cAMP-sensitive mode of Ca^{2+}-dependent insulin exocytosis in pancreatic β-cells. Diabetes 51:S19–S24

Kasai H, Hatakeyama H, Kishimoto T, Liu T-T, Nemoto T, Takahashi N (2005a) A new quantitative (two-photon extracellular polar-tracer imaging-based quantification (TEPIQ)) analysis for diameters of exocytic vesicles and its application to mouse pancreatic islets. J Physiol 568:891–903

Kasai K, Ohara-Imaizumi M, Takahashi N, Mizutani S, Zhao S, Kikuta T, Kasai H, Nagamatsu S, Gomi H, Izumi T (2005b) Rab27a mediates the tight docking of insulin granules onto the plasma membrane during glucose stimulation. J Clin Invest 115:388–396

Kasai H, Kishimoto T, Nemoto T, Hatakeyama H, Liu TT, Takahashi N (2006) Two-photon excitation imaging of exocytosis and endocytosis and determination of their spatial organization. Adv Drug Deliv Rev 58:850–877

Kasai K, Fujita T, Gomi H, Izumi T (2008) Docking is not a prerequisite but a temporal constraint for fusion of secretory granules. Traffic 9:1191–1203

Kashima Y, Miki T, Shibasaki T, Ozaki N, Miyazaki M, Yano H, Seino S (2001) Critical role of cAMP-GEFII-Rim2 complex in incretin-potentiated insulin secretion. J Biol Chem 276:46046–46053

Kirillova J, Thomas P, Almers W (1993) Two independently regulated secretory pathways in mast cells. J Physiol Paris 87:203–208

Kishimoto T, Liu TT, Hatakeyama H, Nemoto T, Takahashi N, Kasai H (2005) Sequential compound exocytosis of large dense-core vesicles in PC12 cells studied with TEPIQ analysis. J Physiol 568:905–915

Kishimoto T, Kimura R, Liu T-T, Nemoto T, Takahashi N, Kasai H (2006) Vacuolar sequential exocytosis of large dense-core vesicles in adrenal medulla. EMBO J 25:673–682
Klenchin VA, Martin TF (2000) Priming in exocytosis: attaining fusion-competence after vesicle docking. Biochimie 82:399–407
Klyachko VA, Jackson MB (2002) Capacitance steps and fusion pores of small and large-dense-core vesicles in nerve terminals. Nature 418:89–92
Knoch KP, Meisterfeld R, Kersting S, Bergert H, Altkruger A, Wegbrod C, Jager M, Saeger HD, Solimena M (2006) CAMP-dependent phosphorylation of PTB1 promotes the expression of insulin secretory granule proteins in β cells. Cell Metab 3:123–134
Kwan EP, Gaisano HY (2005) Glucagon-like peptide 1 regulates sequential and compound exocytosis in pancreatic islet β-cells. Diabetes 54:2734–2743
Kwan EP, Xie L, Sheu L, Ohtsuka T, Gaisano HY (2007) Interaction between Munc13-1 and RIM is critical for glucagon-like peptide-1 mediated rescue of exocytotic defects in Munc13-1 deficient pancreatic β-cells. Diabetes 56:2579–2588
Lang J (1999) Molecular mechanisms and regulation of insulin exocytosis as a paradigm of endocrine secretion. Eur J Biochem 259:3–17
Langley OK, Etsee-Ufrecht MC, Grant NJ, Gratzl M (1989) Expression of the neural cell adhesion molecule NCAM in endocrine cells. J Histochem Cytochem 37:781–791
Larsson S, Wierup N, Sundler F, Eliasson L, Holm C (2008) Lack of cholesterol mobilization in islets of hormone-sensitive lipase deficient mice impairs insulin secretion. Biochem Biophys Res Commun 376:558–562
Lester LB, Langeberg LK, Scott JD (1997) Anchoring of protein kinase A facilitates hormone-mediated insulin secretion. Proc Natl Acad Sci USA 94:14942–14947
Liu TT, Kishimoto T, Hatakeyama H, Nemoto T, Takahashi N, Kasai H (2005) Exocytosis and endocytosis of small vesicles in PC12 cells studied with TEPIQ analysis. J Physiol 568:917–929
Lonart G, Schoch S, Kaeser PS, Larkin CJ, Sudhof TC, Linden DJ (2003) Phosphorylation of RIM1α by PKA triggers presynaptic long-term potentiation at cerebellar parallel fiber synapses. Cell 115:49–60
Lopez JA, Kwan EP, Xie L, He Y, James DE, Gaisano HY (2008) The RalA GTPase is a central regulator of insulin exocytosis from pancreatic islet β cells. J Biol Chem 283:17939–17945
Lovis P, Gattesco S, Regazzi R (2008a) Regulation of the expression of components of the exocytotic machinery of insulin-secreting cells by microRNAs. Biol Chem 389:305–312
Lovis P, Roggli E, Laybutt DR, Gattesco S, Yang JY, Widmann C, Abderrahmani A, Regazzi R (2008b) Alterations in microRNA expression contribute to fatty acid-induced pancreatic β-cell dysfunction. Diabetes 57:2728–2736
Ma L, Bindokas VP, Kuznetsov A, Rhodes C, Hays L, Edwardson JM, Ueda K, Steiner DF, Philipson LH (2004) Direct imaging shows that insulin granule exocytosis occurs by complete vesicle fusion. Proc Natl Acad Sci USA 101:9266–9271
MacDonald PE, Obermuller S, Vikman J, Galvanovskis J, Rorsman P, Eliasson L (2005) Regulated exocytosis and kiss-and-run of synaptic-like microvesicles in INS-1 and primary rat β-cells. Diabetes 54:736–743
MacDonald PE, Braun M, Galvanovskis J, Rorsman P (2006) Release of small transmitters through kiss-and-run fusion pores in rat pancreatic β cells. Cell Metab 4:283–290
Maritzen T, Keating DJ, Neagoe I, Zdebik AA, Jentsch TJ (2008) Role of the vesicular chloride transporter ClC-3 in neuroendocrine tissue. J Neurosci 28:10587–10598
Matsumoto M, Miki T, Shibasaki T, Kawaguchi M, Shinozaki H, Nio J, Saraya A, Koseki H, Miyazaki M, Iwanaga T, Seino S (2004) Noc2 is essential in normal regulation of exocytosis in endocrine and exocrine cells. Proc Natl Acad Sci USA 101:8313–8318
McNeil PL, Steinhardt RA (1997) Loss, restoration, and maintenance of plasma membrane integrity. J Cell Biol 137:1–4
McNeil PL, Steinhardt RA (2003) Plasma membrane disruption: repair, prevention, adaptation. Annu Rev Cell Dev Biol 19:697–731

Michael DJ, Geng X, Cawley NX, Loh YP, Rhodes CJ, Drain P, Chow RH (2004) Fluorescent cargo proteins in pancreatic β-cells: design determines secretion kinetics at exocytosis. Biophys J 87:L03–L05

Michael DJ, Ritzel RA, Haataja L, Chow RH (2006) Pancreatic β-cells secrete insulin in fast- and slow-release forms. Diabetes 55:600–607

Miura A, Yamagata K, Kakei M, Hatakeyama H, Takahashi N, Fukui K, Nammo T, Yoneda K, Inoue Y, Sladek FM, Magnuson MA, Kasai H, Miyagawa J, Gonzalez FJ, Shimomura I (2006) Hepatocyte nuclear factor-4α is essential for glucose-stimulated insulin secretion by pancreatic β-cells. J Biol Chem 281:5246–5257

Mochida S (2000) Protein-protein interactions in neurotransmitter release. Neurosci Res 36:175–182

Monterrat C, Grise F, Benassy MN, Hemar A, Lang J (2007) The calcium-sensing protein synaptotagmin 7 is expressed on different endosomal compartments in endocrine, neuroendocrine cells or neurons but not on large dense core vesicles. Histochem Cell Biol 127:625–632

Nagy G, Reim K, Matti U, Brose N, Binz T, Rettig J, Neher E, Sorensen JB (2004) Regulation of releasable vesicle pool sizes by protein kinase A-dependent phosphorylation of SNAβ-25. Neuron 41:417–429

Neher E, Marty A (1982) Discrete changes of cell membrane capacitance observed under conditions of enhanced secretion in bovine adrenal chromaffin cells. Proc Natl Acad Sci USA 79:6712–6716

Nemoto T, Kimura R, Ito K, Tachikawa A, Miyashita Y, Iino M, Kasai H (2001) Sequential-replenishment mechanism of exocytosis in pancreatic acini. Nat Cell Biol 3:253–258

Nemoto T, Kojima T, Oshima A, Bito H, Kasai H (2004) Stabilization of exocytosis by dynamic F-actin coating of zymogen granules in pancreatic acini. J Biol Chem 279:37544–37550

Nevins AK, Thurmond DC (2005) A direct interaction between Cdc42 and vesicle-associated membrane protein 2 regulates SNARE-dependent insulin exocytosis. J Biol Chem 280:1944–1952

Ninomiya Y, Kishimoto T, Miyashita Y, Kasai H (1996) Ca^{2+}-dependent exocytotic pathways in CHO fibroblasts revealed by capacitance measurement and a caged-Ca^{2+} compound. J Biol Chem 271:17751–17754

Ninomiya Y, Kishimoto T, Yamazawa T, Ikeda H, Miyashita Y, Kasai H (1997) Kinetic diversity in the fusion of exocytotic vesicles. EMBO J 16:929–934

Oberhauser AF, Robinson I, Fernandez JM (1996) Simultaneous capacitance and amperometric measurements of exocytosis: a comparison. Biophys J 71:1131–1139

Obermuller S, Lindqvist A, Karanauskaite J, Galvanovskis J, Rorsman P, Barg S (2005) Selective nucleotide-release from dense-core granules in insulin-secreting cells. J Cell Sci 118:4271–4282

Ohara-Imaizumi M, Fujiwara T, Nakamichi Y, Okamura T, Akimoto Y, Kawai J, Matsushima S, Kawakami H, Watanabe T, Akagawa K, Nagamatsu S (2007) Imaging analysis reveals mechanistic differences between first- and second-phase insulin exocytosis. J Cell Biol 177:695–705

Oheim M, Loerke D, Stuhmer W, Chow RH (1998) The last few milliseconds in the life of a secretory granule. Docking, dynamics and fusion visualized by total internal reflection fluorescence microscopy (TIRFM). Eur Biophys J 27:83–98

Orci L, Malaisse-Lagae F, Ravazzola M, Amherdt M, Renold AE (1973) Exocytosis-endocytosis coupling in the pancreatic β cell. Science 181:561–562

Oshima A, Kojima T, Dejima K, Hisa I, Kasai H, Nemoto T (2005) Two-photon microscopic analysis of acetylcholine-induced mucus secretion in guinea pig nasal glands. Cell Calcium 37:349–357

Ozaki N, Shibasaki T, Kashima Y, Miki T, Takahashi K, Ueno H, Sunaga Y, Yano H, Matsuura Y, Iwanaga T, Takai Y, Seino S (2000) CAMP-GEFII is a direct target of cAMP in regulated exocytosis. Nat Cell Biol 2:805–811

Persaud SJ, Jones PM, Howell SL (1990) Glucose-stimulated insulin secretion is not dependent on activation of protein kinase A. Biochem Biophys Res Com 173:833–839
Pickett JA, Thorn P, Edwardson JM (2005) The plasma membrane Q-SNARE syntaxin 2 enters the zymogen granule membrane during exocytosis in the pancreatic acinar cell. J Biol Chem 280:1506–1511
Plaisance V, Abderrahmani A, Perret-Menoud V, Jacquemin P, Lemaigre F, Regazzi R (2006) MicroRNA-9 controls the expression of Granuphilin/Slp4 and the secretory response of insulin-producing cells. J Biol Chem 281:26932–26942
Plattner H, Artalejo AR, Neher E (1997) Ultrastructural organization of bovine chromaffin cell cortex-analysis by cryofixation and morphometry of aspects pertinent to exocytosis. J Cell Biol 139:1709–1717
Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, Pfeffer S, Tuschl T, Rajewsky N, Rorsman P, Stoffel M (2004) A pancreatic islet-specific microRNA regulates insulin secretion. Nature 432:226–230
Poy MN, Hausser J, Trajkovski M, Braun M, Collins S, Rorsman P, Zavolan M, Stoffel M (2009) miR-375 maintains normal pancreatic α- and β-cell mass. Proc Natl Acad Sci USA 106:5813–5818
Ravier MA, Nenquin M, Miki T, Seino S, Henquin JC (2009) Glucose controls cytosolic Ca^{2+} and insulin secretion in mouse islets lacking adenosine triphosphate-sensitive K^+ channels owing to a knockout of the pore-forming subunit Kir6.2. Endocrinology 150:33–45
Rebois RV, Reynolds EE, Toll L, Howard BD (1980) Storage of dopamine and acetylcholine in granules of PC12, a clonal pheochromocytoma cell line. Biochemistry 19:1240–1248
Reese C, Heise F, Mayer A (2005) Trans-SNARE pairing can precede a hemifusion intermediate in intracellular membrane fusion. Nature 436:410–414
Renstrom E, Eliasson L, Rorsman P (1997) Protein kinase A-dependent and -independent stimulation of exocytosis by cAMP in mouse pancreatic β-cells. J Physiol 502:105–118
Rizzoli SO, Betz WJ (2004) The structural organization of the readily releasable pool of synaptic vesicles. Science 303:2037–2039
Rothman JE (1994) Mechanisms of intracellular protein transport. Nature 372:55–63
Scepek S, Lindau M (1993) Focal exocytosis by eosinophils - compound exocytosis and cumulative fusion. EMBO J 12:1811–1817
Sedej S, Rose T, Rupnik M (2005) CAMP increases Ca^{2+}-dependent exocytosis through both PKA and Epac2 in mouse melanotrophs from pituitary tissue slices. J Physiol 567:799–813
Shibasaki T, Takahashi H, Miki T, Sunaga Y, Matsumura K, Yamanaka M, Zhang C, Tamamoto A, Satoh T, Miyazaki J, Seino S (2007) Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP. Proc Natl Acad Sci USA 104:19333–19338
Smith CB, Betz WJ (1996) Simultaneous independent measurement of endocytosis and exocytosis. Nature 380:531–534
Speidel D, Salehi A, Obermueller S, Lundquist I, Brose N, Renstrom E, Rorsman P (2008) CAPS1 and CAPS2 regulate stability and recruitment of insulin granules in mouse pancreatic β cells. Cell Metab 7:57–67
Steinhardt RA, Bi G, Alderton JM (1994) Cell membrane resealing by a vesicular mechanism similar to neurotransmitter release. Science 263:390–393
Steyer JA, Horstmann H, Almers W (1997) Transport, docking and exocytosis of single secretory granules in live chromaffin cells. Nature 388:474–478
Sudhof TC (1995) The synaptic vesicle cycle: a cascade of protein-protein interactions. Nature 375:645–653
Sumara G, Formentini I, Collins S, Sumara I, Windak R, Bodenmiller B, Ramracheya R, Caille D, Jiang H, Platt KA, Meda P, Aebersold R, Rorsman P, Ricci R (2009) Regulation of PKD by the MAPK p38delta in insulin secretion and glucose homeostasis. Cell 136:235–248
Takahashi N, Kadowaki T, Yazaki Y, Miyashita Y, Kasai H (1997) Multiple exocytotic pathways in pancreatic β cells. J Cell Biol 138:55–64

Takahashi N, Kadowaki T, Yazaki Y, Elis-Davies GCR, Miyashita Y, Kasai H (1999) Post-priming actions of ATP in the Ca^{2+} dependent exocytosis in pancreatic β-cells. Proc Natl Acad Sci USA 96:760–765

Takahashi N, Kishimoto T, Nemoto T, Kadowaki T, Kasai H (2002a) Fusion pore dynamics and insulin granule exocytosis in the pancreatic islet. Science 297:1349–1352

Takahashi N, Nemoto T, Kiumra R, Tachikawa A, Miwa A, Okado H, Miyashita Y, Iino M, Kadowaki T, Kasai H (2002b) Two-photon excitation imaging of pancreatic islets with various fluorescent probes. Diabetes 51(Suppl 1):S25–S28

Takahashi N, Hatakeyama H, Okado H, Miwa A, Kishimoto T, Kojima T, Abe T, Kasai H (2004) Sequential exocytosis of insulin granules is associated with redistribution of SNAP25. J Cell Biol 165:255–262

Taraska JW, Perrais D, Ohara-Imaizumi M, Nagamatsu S, Almers W (2003) Secretory granules are recaptured largely intact after stimulated exocytosis in cultured endocrine cells. Proc Natl Acad Sci USA 100:2070–2075

Tengholm A, Gylfe E (2009) Oscillatory control of insulin secretion. Mol Cell Endocrinol 297:58–72

Thomas-Reetz AC, De Camilli P (1994) A role for synaptic vesicles in non-neuronal cells: clues from pancreatic β cells and from chromaffin cells. FASEB J 8:209–216

Thorn P, Fogarty KE, Parker I (2004) Zymogen granule exocytosis is characterized by long fusion pore openings and preservation of vesicle lipid identity. Proc Natl Acad Sci USA 101:6774–6779

Tomas A, Meda P, Regazzi R, Pessin JE, Halban PA (2008) Munc 18–1 and granuphilin collaborate during insulin granule exocytosis. Traffic 9:813–832

Toonen RF, Kochubey O, De WH, Gulyas-Kovacs A, Konijnenburg B, Sorensen JB, Klingauf J, Verhage M (2006) Dissecting docking and tethering of secretory vesicles at the target membrane. EMBO J 25:3725–3737

Tsuboi T, Terakawa S, Scalettar BA, Fantus C, Roder J, Jeromin A (2002) Sweeping model of dynamin activity. Visualization of coupling between exocytosis and endocytosis under an evanescent wave microscope with green fluorescent proteins. J Biol Chem 277:15957–15961

Tsuboi T, McMahon HT, Rutter GA (2004) Mechanisms of dense core vesicle recapture following "kiss and run" ("cavicapture") exocytosis in insulin-secreting cells. J Biol Chem 279:47115–47124

Vaag A, Henriksen JE, Madsbad S, Holm N, Beck-Nielsen H (1995) Insulin secretion, insulin action, and hepatic glucose production in identical twins discordant for non-insulin-dependent diabetes mellitus. J Clin Invest 95:690–698

Valverde I, Vandermeers A, Anjaneyulu R, Malaisse WJ (1979) Calmodulin activation of adenylate cyclase in pancreatic islets. Science 206:225–227

Varadi A, Tsuboi T, Rutter GA (2005) Myosin Va transports dense core secretory vesicles in pancreatic MIN6 β-cells. Mol Biol Cell 16:2670–2680

Verhage M, Sorensen JB (2008) Vesicle docking in regulated exocytosis. Traffic 9:1414–1424

Wang Y, Okamoto M, Schmitz F, Hofmann K, Sudhof TC (1997) Rim is a putative Rab3 effector in regulating synaptic-vesicle fusion. Nature 388:593–598

Wang Z, Oh E, Thurmond DC (2007) Glucose-stimulated Cdc42 signaling is essential for the second phase of insulin secretion. J Biol Chem 282:9536–9546

Yaekura K, Kakei M, Yada T (1996) CAMP-signaling pathway acts in selective synergism with glucose or tolbutamide to increase cytosolic Ca^{2+} in rat pancreatic β-cells. Diabetes 45:295–301

Zhou Z, Misler S (1996) Amperometric detection of quantal secretion from patch-clamped rat pancreatic β-cells. J Biol Chem 270:270–277

Zhou Z, Misler S, Chow RH (1996) Rapid fluctuations in transmitter release from single vesicles in bovine adrenal chromaffin cells. Biophys J 70:1543–1552

Zinc Transporters in the Endocrine Pancreas

17

Mariea Dencey Bosco, Chris Drogemuller, Peter Zalewski, and Patrick Toby Coates

Contents

Abstract

The pancreas is composed of two types of cells: the exocrine (acinar) cells and endocrine (pancreatic islet) cells. Pancreatic islets have a high content of zinc (Zn) compared to exocrine tissue. Zinc is especially high in the pancreatic β cells, where it is involved in the maturation, synthesis, and secretion of

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_42,

insulin. Zn in the islet is regulated by zinc-buffering proteins such as metallothionein, membrane Zn transporters, and Zn-permeable ion channels such as TRPM3. There are two families of membrane protein Zn transporters: ZnT proteins lower cytosolic Zn by transporting it into organelles or out of cells while ZIP proteins increase cytosolic Zn by transporting zinc from the extracellular fluids or out of organelles into the cytosol. Some zinc transporters play specific roles in influencing insulin maturation, synthesis, and secretion. For example, ZnT8 is predominantly localized to the membranes of secretory granules in the pancreatic β cells where it is involved in incorporating Zn into crystalline structures of insulin. In both type 1 and 2 diabetes, Zn metabolism is altered and there are changes in ZnT8. A polymorphic variant of ZnT8 is associated with increase in the risk of type 2 diabetes while ZnT8 is an autoantigen in type 1 diabetes. The mechanisms by which ZnT8 is regulated and the role of other Zn transporters in pancreatic islet function are topics of much current interest, with potential implications as future therapeutic targets in diabetes.

Keywords
Zinc • Zinc transporters • β cell • α cell • Type 2 diabetes • Polymorphism

Introduction

It is 75 years since the observation that the zinc content of the pancreas is abnormally low in diabetic humans was first made (Scott and Fisher 1938). In recent years, details of the mechanisms of Zn homeostasis in the normal pancreas and the diabetic pancreas have begun to be deciphered. Central to the normal metabolism of insulin is the availability of labile Zn ions. Zn metabolism is closely linked to three major families of proteins. The metallothionein proteins (MT) act to buffer cytosolic zinc ions and thereby control intracellular zinc availability (Hijova 2004). The ZnT and ZIP families control movement of Zn in and out of cells and their organelles (Cousins et al. 2006). The relevance to diabetes of these transporters and ZnT8, in particular, has been shown by two clinical studies. Achenbach and colleagues, studying a large cohort of children with family history of type 1 diabetes, were the first to report that ZnT8 was an autoantigen in type 1 diabetes (Achenbach et al. 2009). In 2010, a genome wide association study in diabetes was conducted by Weijers and colleagues. This study found that a single-nucleotide polymorphism in ZnT8 is linked to increased risk of type 2 diabetes and altered transport of Zn into the insulin-containing secretory granules (Weijers 2010). The purpose of this chapter is to describe the gene and protein expression of Zn transporters in the α and β cells of pancreatic islets, based on data from cell lines as well as human and rodent pancreatic islets.

Zinc and Normal Pancreatic Islets

Pancreatic islets are composed of several different cell types including glucagon-producing α cells, insulin-producing β cells, somatostatin-producing delta cells, and pancreatic polypeptide-producing pp cells (Skelin et al. 2010). Pancreatic islets have an important role in regulating glucose levels in the blood and they contain the only cell type (β cells) in the body capable of releasing insulin in response to rising serum glucose levels. The release of insulin stimulates the uptake of glucose in various cells and tissues in the body, thereby maintaining glucose homeostasis. Zn is an essential trace element that is found abundantly in the pancreas and, in particular, in the pancreatic islets (Fig. 1). β cells contain high concentrations of Zn and this metal is essential for insulin production; less is known about the other pancreatic islet cellular constituents (Zalewski et al. 1994).

Within the body, Zn is found in both tightly bound and loose (labile) forms (Truong-Tran et al. 2000). Tightly bound Zn is complexed in metalloproteins, including over 300 types of metalloenzymes. Interestingly, this form of Zn is not usually altered during periods of zinc deficiency, unless the tissue or metalloprotein is subject to rapid turnover. The pool of labile zinc is involved in signal transduction, binding of transcription factors and gene regulation, and regulation of apoptosis; in the β cell, labile Zn is critical for insulin maturation, synthesis, and secretion (Emdin et al. 1980). This form of Zn is most susceptible to zinc deficiency and disease (Zalewski et al. 2006). Labile Zn exits at very low (pM) levels in cytosol but is concentrated in organelles and especially in the insulin-containing granules, reaching estimated concentrations of 20 mM (Foster et al. 1993). Zn may be important at several stages in the synthesis and storage of insulin in the β cell (Fig. 2), especially in the formation of proinsulin hexamers within the Golgi apparatus and in the formation of the mature insulin crystals in the secretory vesicles (Emdin et al. 1980). During glucose stimulation, zinc and insulin are co-secreted into the extracellular space, where the insulin structure (hexamers) breaks up into monomers of insulin and Zn ions are released. Whether the released

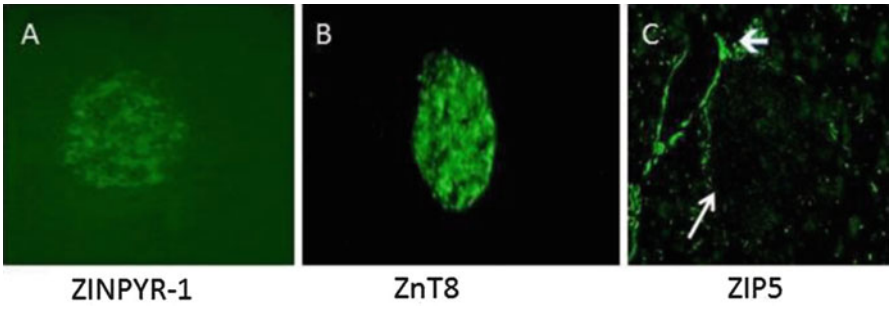

Fig. 1 Examples of zinc and zinc transporter staining in murine pancreatic islets: Figure shows strong and homogeneous staining of (**a**) Zn by ZinPyr1 fluorescence and (**b**) ZnT8 by immunofluorescence in typical islets. (**c**) Shows typical predominant exocrine acinar localization for ZIP5 (*arrow head*) although some peripheral islet localization of ZIP 5 can also be seen (*arrow*)

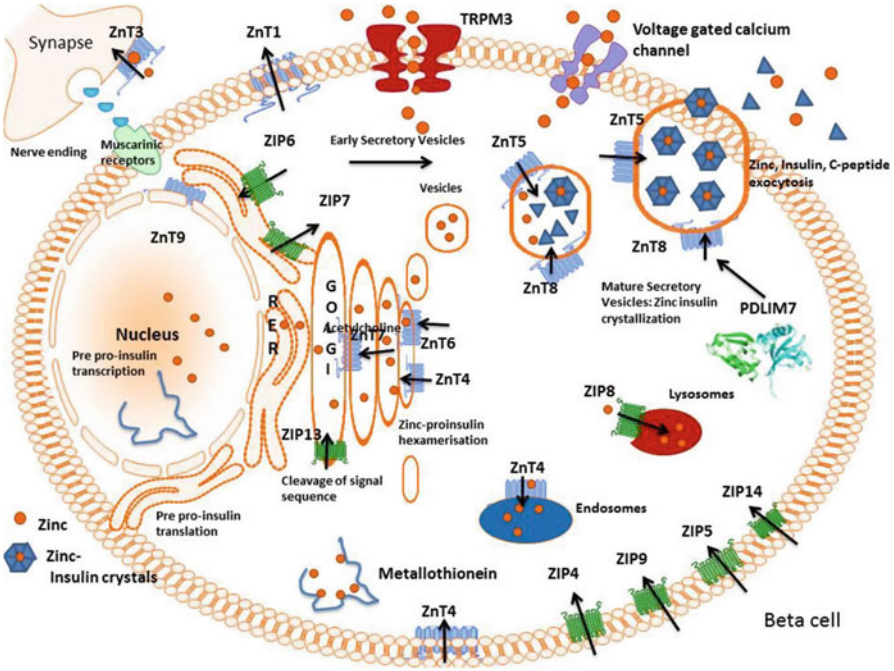

Fig. 2 Cartoon showing distribution and roles of Zn and Zn transporters inβ cell metabolism: This figure presents a speculative view of the roles of Zn and its transporters at various stages of insulin production. (1) ZIP4, 5, 9, and possibly 14 are expressed in the plasma membrane and are involved in bringing zinc from the extracellular space into the cytoplasm of the β cell. Whether the major efflux transporter ZnT1 is present in the plasma membrane of β cells is unclear. (2) Pre-proinsulin transcription occurs in the nucleus. Nuclear zinc transporter ZnT9 makes Zn available for transcription factors. Metallothionein is expressed in both the cytoplasm and the nucleus of the cells and may assist with Zn uptake in the nucleus. Whether this affects insulin gene transcription is not known. (3) Binding of Zn to proinsulin in the rough endoplasmic reticulum facilitates correct folding of proinsulin. Zn homeostasis in this organelle is regulated via influx and efflux via ZIP6 and ZIP7, respectively. (4) Proinsulin is transported to the Golgi for processing and incorporation into secretory granules. Zn transporters operating in the Golgi include ZIP7, ZIP13, ZnT6, and ZnT7. Zn is thought to assist in the formation of immature insulin hexameric complexes in the Golgi. (5) ZnT5 and ZnT8 are highly expressed in the secretory vesicles and are involved in bringing extra Zn from the cytoplasm to the insulin vesicles. Because of the many pieces of evidence that ZnT8 is important for β cell function, this ZnT8-dependent influx of Zn appears critical for mature insulin crystallization in the vesicles. ZnT5 may facilitate in this process. (6) ZIP8 is expressed in the lysosomes and could be involved in recycling. (7) Finally, there is the potential and intriguing role of presynaptic Zn, under the control of ZnT3, in neuroendocrine control of insulin secretion

Zn ions are reabsorbed by β cells is not clear, but they have been shown to exert an inhibitory action on glucagon secretion by neighboring α cells (Ravier and Rutter 2005). α cells also contain Zn but at lower levels than β cells, and most likely involved in the maturation, synthesis, and secretion of glucagon, although this is not yet proven (Egefjord et al. 2010). It has been reported that zinc inhibits

glucagon secretion by inhibiting ATP-sensitive potassium channels k + (K_{ATP}) and was independent of low- or high-glucose conditions. Franklin et al. (2005) suggested that zinc inhibits the electrical activity (hyperpolarization) by acting through the potassium channels, thereby leading to a decrease in the release of calcium and inhibiting glucagon secretion (Franklin et al. 2005). There are still controversies about zinc being the regulator of glucagon secretion in α cells. Almost nothing is known about zinc in the delta and polypeptide-producing pp cells.

Zinc and Type 2 Diabetes

Zn deficiency may play a role in type 2 diabetes in humans. It was reported 75 years ago that there was a significant reduction (~50 %) of Zn in the pancreata of type 2 diabetic patients compared to normal patients 1. Type 2 diabetic patients are also reported to have reduced plasma Zn and hyperzincuria compared to normal individuals (Heise et al. 1988). In rodents, in a leptin receptor mutant mouse model of type 2 diabetes, pancreatic Zn was significantly reduced compared to their wild-type litter mates and dietary Zn supplementation of these mice normalized their pancreatic Zn and reduced symptoms of diabetes such as hyperglycemia (Simon and Taylor 2001). However, in another rodent model of type 2 diabetes (Zucker diabetic fatty rat), there were no changes, at least at an ultrastructural level, to Zn and insulin in the islet β cells (Sondergaard et al. 2003).

TRPM3, PDLIM7, and Calcium Channels

Transient receptor potential channels (TRP) are cation influx channels, involved in vital roles in the body including metal homeostasis, tumorigenesis, sensory temperature, and pain sensation. TPRM3 has six transmembrane domains including a pore domain between the fifth and sixth transmembrane segments with both amino and carboxy termini located at the cytosolic side (Thiel et al. 2013). TPRM3 has been reported to be a transporter of both calcium and Zn into the β cells. The authors suggested that it is involved in taking up Zn under physiologic conditions through its ion pore and that insulin secretion occurs during depolarization of β cells when the blood glucose levels are elevated in the plasma (Wagner et al. 2010). Depolarization occurs in the nerve ending in the β cells where TPRM3 channels are activated leading to the activation of voltage-gated calcium channels leading to further influx of both calcium and Zn through these channels. Zn that has entered into the cytoplasm may be part of the Zn pools made available to organelle Zn transporter proteins that are involved in the maturation and synthesis of insulin (Wagner et al. 2010) (Fig. 2). However, this finding is still to be confirmed in other studies.

PDLIM7 proteins are scaffolding proteins that are involved in cellular migration, signal transduction, differentiation, heart development, and oncogenesis (Camarata

et al. 2010a; Camarata et al. 2010b). Found that ZnT8 interacts with PDLIM7 possibly involved in making zinc available for the insulin crystallization. These studies if confirmed reflect that TPRM3, calcium channels, and PDLIM7 are important proteins and transporters in the processes that Zn regulates insulin synthesis and secretion in β cells.

Zinc Binding Protein Metallothionein

Metallothioneins are cysteine-rich proteins of low molecular weight 6–10 kDa in size, which play an important role in Zn homeostasis (Suhy et al. 1999). Metallothionein proteins were first discovered as cadmium-binding proteins derived from horse kidney (Wu et al. 2007). In mammals, four subtypes are found (MT1–4); MT1–2 isoforms are ubiquitously expressed in many cell types, MT3 is expressed highly in the brain and in the male reproductive organs, and MT4 is expressed in the stratified tissues 2. MT1 and MT2 are known to be inducible by glucocorticoids, cytokines, reactive oxygen species, and metal ions such as cadmium and Zn, whereas MT3 and MT4 are not induced by these compounds (Kimura and Itoh 2008). Metallothioneins are soluble cytosolic proteins that both transport Zn intracellularly, including from cytoplasm to nucleus (Cousins and Lee-Ambrose 1992), and buffer cytosolic Zn as free zinc is toxic to cells (Maret and Krezel 2007) (Fig. 2). Transportation of Zn into the nucleus is known to be important in controlling transcription of many genes and also cell differentiation. Zinc-metallothionein complex is also an antioxidant, which scavenges reactive oxygen species such as nitric oxide, superoxide, and hydroxyl radicals (Ruttkay-Nedecky et al. 2013).

Metallothionein in Islets

Immunocytochemical staining shows that the expression of metallothionein corresponds to the levels of labile Zn (anti-metallothionein antibody) in the pancreatic islets of humans (Tomita 2000a). Metallothionein is involved in the zinc homeostasis and metabolism in pancreatic islets (Tomita 2000b). Metallothionein may also modulate pancreatic hormone secretion (Andrews and Geiser 1999), but this is not proven. Metallothionein subtypes MT1 and MT2 are abundant in the pancreatic islets. In rodent pancreatic β cell line (MIN6), MT2 expression is higher than MT1 expression. There was significant reduction of both MT1 and MT2 when β cells were treated with high glucose (Bellomo et al. 2011). Transgenic mice (overexpression of metallothionein) had significantly reduced hyperglycemia and also decreased islet cell death in response to treatment with STZ compared to control mice treated with STZ (Chen et al. 2001). Thus, metallothionein may play a protective role in islet cell function, protecting them from β cell death, as well as an important role in storing Zn.

Zinc Transporter Family Zip

The ZIP transporters were first identified in yeast (S. cerevisiae) and plant root Arabidopsis Irt1 protein (Claus and Chavarria-Krauser 2012). ZIP transporters are eight transmembrane proteins with the N-and C terminus in the cytoplasmic side of the cell membrane (Hill and Link 2009). In mammals, 14 homologues of zinc influx proteins have been studied (ZIP1–14 and are summarized in Table 1) (Taylor and Nicholson 2003). The functional role of these ZIP transporters was initially shown in A. thaliana root cells where iron deficiency increased the expression of these transporters (Vert et al. 2002). Some ZIP transporters have been shown to transport not only Zn but also iron, magnesium, cobalt, and cadmium. ZIP transporters primarily transport Zn from the extracellular space or the organelle lumen into the cytoplasm of the cell (Grass et al. 2005).

Both transcriptional and posttranslational expressions of some of these proteins are reported to be regulated by Zn availability, cytokines, and hormones (Myers et al. 2012). Some ZnT and ZIP transporters are ubiquitously expressed, whereas others are specific to certain tissues. They reside on either intracellular or plasma membranes. It is also known that the regulation of expression of the zinc transporters in human islets, mouse islets, rat islets, and α and β cell lines is different (Table 1). ZIP transporters have been implicated in many disease states, although the mechanism of how these proteins are altered is still not well understood.

In an earlier section, depolarization-regulated uptake of Zn into β cells was described. Zinc also enters into the β cells independent of electrical activity and via the ZIP transporters.

At this stage, there is no convincing evidence that ZIP1, ZIP2, and ZIP3 are present in β cells. ZIP1 is a ubiquitous plasma membrane transporter, involved in bringing zinc from the extracellular space to the cytoplasm of the cells (Khadeer et al. 2005). ZIP1 mRNA transcripts are expressed highly in the α cell line; however, no expression was seen in the MIN6 β cell line (El Muayed et al. 2012). ZIP1 protein expression is yet to be investigated in pancreatic islets. The expression of ZIP1 in pancreatic islets and specifically α cell lines indicates that ZIP1 may be involved in bringing Zn into the α cells of the pancreatic islets. ZIP2 is also known to be a plasma membrane transporter (Desouki et al. 2007). Human and mouse pancreatic islets and α and β cell lines did not show any expression of ZIP240. ZIP3 is expressed in the apical membrane of acinar cells (Kelleher et al. 2011). ZIP3 mRNA transcripts are expressed at the mRNA level in low abundance in human and mouse pancreatic islets and α and β cell lines, but the protein expression of ZIP3 is yet to be determined. ZIP3 could be involved in bringing Zn into the pancreatic acinar cells, assisting with digestive enzyme metabolism (El Muayed et al. 2012; Wijesekara et al. 2009).

ZIP9 is expressed in the Golgi apparatus possibly releasing Zn from Golgi to the cytosol (Taniguchi et al. 2013). It was expressed in low abundance in human and mouse pancreatic islets, α and β cells (El Muayed et al. 2012; Wijesekara et al. 2009). Protein expression of ZIP9 in β cells is still unknown in the pancreatic islets of human and mouse. ZIP9 may not have an important role compared to the

Table 1 Location and Expression of ZIP Transporters in the Endocrine Pancreas

		mRNA expression			Protein expression		
SLC39A	**Location**	**α**	**β**	**Acinar cells**	**α**	**β**	**Acinar cells**
ZIP1 SLC39A1	**Plasma membrane**	Expressed	Not expressed	Unknown	Unknown	Unknown	Unknown
ZIP2 SLC39A2	**Plasma membrane**	Not expressed	Not expressed	Unknown	Unknown	Unknown	Unknown
ZIP3 SLC39A3	**Plasma membrane**	Expressed	Expressed	Expressed	Unknown	Unknown	Unknown
ZIP4 SLC39A4	**Plasma membrane**	Expressed	Expressed	Expressed	Unknown	Expressed	Unknown
ZIP5 SLC39A5	**Basolateral membrane of acinar cells**	Expressed	Expressed	Expressed	Unknown	Unknown	Expressed
ZIP6 SLC39A6	**Plasma membrane: endoplasmic reticulum**	Expressed	Expressed	Unknown	Unknown	Expressed	Unknown
ZIP7 SLC39A7	**Plasma membrane: endoplasmic reticulum Golgi apparatus**	Expressed	Expressed	Unknown	Unknown	Expressed	Unknown
ZIP8 SLC39A8	**Lysosomes**	Expressed	Expressed	Unknown	Unknown	Expressed	Unknown
ZIP9 SLC39A9	**Plasma membrane**	Expressed	Expressed	Unknown	Unknown	Unknown	Unknown
ZIP10 SLC39A10	**Plasma membrane**	Not expressed	Not expressed	Not expressed	Unknown	Unknown	Unknown
ZIP11 SLC39A11	**Plasma membrane**	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
ZIP12 SLC39A12	**Plasma membrane**	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
ZIP13 SLC39A13	**Golgi apparatus**	Expressed	Expressed	Unknown	Unknown	Unknown	Unknown
ZIP14 SLC39A14	**Plasma membrane**	Expressed	Expressed	Expressed	Unknown	Unknown	Unknown

other ZIPs in the β cell. ZIP10, ZIP11, and ZIP12 are not expressed in the pancreatic islets of human and mouse or the islet cell lines (El Muayed et al. 2012; Wijesekara et al. 2009).

The following ZIP transporters are likely involved in islet zinc homeostasis. ZIP4 is a plasma membrane transporter which is expressed predominantly in β cells (Dufner-Beattie et al. 2004a). It was reported that human pancreatic islets have a higher ZIP4 expression compared to mouse islets, α and β cells (El Muayed et al. 2012; Wijesekara et al. 2009; Dufner-Beattie et al. 2004b). Protein expression data showed high expression of ZIP4 in mouse pancreatic islets. It is also expressed in the pancreatic acinar tissue. The expression pattern of ZIP4 in the β cells of the

pancreatic islets indicates that ZIP4 may assist in bringing Zn into the β cells and thereby contribute to insulin synthesis and digestive enzyme metabolism, respectively (Dufner-Beattie et al. 2004b). ZIP5 is expressed in the basolateral membrane of pancreatic acinar cells (Kelleher et al. 2011). ZIP5 mRNA expression is highly expressed in human pancreatic islets compared to mouse islets, α and β cells (El Muayed et al. 2012; Wijesekara et al. 2009).

Protein expression of ZIP5 is expressed mainly in pancreatic acinar cells with low expression in the pancreatic islets (Figs. 1 and 2) (Weaver et al. 2007). ZIP5 protein is rapidly degraded during periods of Zn deficiency in the pancreas and rapidly induced upon Zn diet supplementation. Protein change occurs without a concomitant change in mRNA level (Weaver et al. 2007). ZIP5 is possibly involved in bringing Zn from the extracellular space to the cytoplasm of the β cell, although its major function seems to be in the exocrine tissue. ZIP6 is expressed in the endoplasmic reticulum of cells (Taylor and Nicholson 2003). ZIP6 mRNA was expressed predominantly compared to the other ZIPs in human and mouse pancreatic islets and β cells. There was low expression of ZIP6 in the α cell line (El Muayed et al. 2012; Wijesekara et al. 2009). Western blot analysis showed that ZIP6 is expressed lowly in β cells and induced when stimulated by high glucose (Bellomo et al. 2011).

The localization of ZIP6 in the pancreatic islets is still yet to be investigated. ZIP6 could possibly be involved in bringing Zn into the rough endoplasmic reticulum. ZIP7 is localized in the membranes of the endoplasmic reticulum and Golgi apparatus of cells (Yan et al. 2012). ZIP7 mRNA was reported to be also one of the abundantly expressed ZIP transporters in human and mouse pancreatic islets. mRNA transcripts of ZIP7 were higher in α cells compared to β cells (El Muayed et al. 2012; Wijesekara et al. 2009). Western blot analysis confirmed the mRNA results that ZIP7 is expressed in β cells and was not induced by high glucose (Bellomo et al. 2011). ZIP7 could be involved in importing Zn into the endoplasmic reticulum and Golgi involved in both insulin and glucagon metabolism. ZIP14 is a plasma membrane transporter (Jenkitkasemwong et al. 2012). mRNA data on ZIP14 revealed that it is highly expressed in human pancreatic islets and α cells compared to mouse islets and β cells (El Muayed et al. 2012; Wijesekara et al. 2009). ZIP14 protein expression is still yet to be investigated. Recent studies reported that ZIP14 knockout mice showed significant upregulation of ZnT8 protein in the pancreatic islets (Beker Aydemir et al. 2012). This could indicate that ZIP14 may play a very important role in bringing Zn into α and β cells of the pancreatic islets, possibly involved in both insulin and glucagon metabolism.

The following ZIP transporters may play a role in zinc homeostasis in islet cells, but more information is required. ZIP8 transporter is expressed on lysosomes of T cells (Aydemir et al. 2009). mRNA transcripts of ZIP8 were found in low abundance in human and mouse islets and α and β cell lines. ZIP8 protein expression is still yet to be determined. ZIP8 expressed in the lysosomes could be involved in Zn incorporation into lysosomes and processes involved in lysosomal function. ZIP13 is located on the Golgi apparatus involved in efflux of Zn to the cytoplasm

(Fukada et al. 2008). mRNA transcripts of ZIP13 are expressed in pancreatic islets of both human and mouse. α cells appear to contain more ZIP13 mRNA expressions than β cells (El Muayed et al. 2012; Wijesekara et al. 2009). ZIP13 protein expression in islet cells is still not determined in pancreatic islets. mRNA expression of ZIP13 in the pancreatic islets suggests that it is involved in maintaining Zn distribution in the Golgi in both α and β cells.

These studies suggest that some members of the ZIP family are important in islet Zn homeostasis. However, the role and importance of these transporters in insulin synthesis and secretion are not known. It is only recently known that ZIP transport activity is modulated posttranslationally by kinase-mediated phosphorylation (Taylor et al. 2012). Future studies looking at the role of phosphorylation and other posttranslational modifications of ZIP transporters in normal and diseased islets may prove informative.

Zinc Transporter Family ZnT

Unlike the ZIP transporters, the ZnT transporters have been extensively studied especially the structure and the alterations that occur during disease pathogenesis. In mammals, ten homologues of Zn export proteins are expressed (ZnT1–10 and are summarized in Table 2) (Sekler et al. 2007). ZnT transporters have six transmembrane domains with N and C terminus localized to the cytoplasmic side of the cell membrane (Palmiter and Huang 2004). They may also transport metals besides Zn including cobalt, cadmium, nickel, copper, and mercuric ions (Kambe 2012). Not all ZnT transporters efflux Zn from cytoplasm across the plasma membrane to the extracellular space of the cell; they also take up metals from the cytoplasm into organelles (ZnT2–7), including the Golgi apparatus (Lichten and Cousins 2009).

The mechanism of how these transporters transport metals was studied using E. coli zinc transporter (ZnT) YiiP (Chao and Fu 2004). The x-ray structure of YiiP was studied by Lu and Fu in 2007. This transporter is a homodimer, held together in a parallel orientation with four Zn ions at the interface of the cytoplasmic domains. It consists of two transmembrane domains which appear like a Y-shaped structure. The transmembrane domain consists of six transmembrane helices and a tetrahedral zinc binding site that is located in the cavity that is open to both the outer membrane and the periplasm (Lu and Fu 2007).

Following this study in 2013, Coudray et al. investigated the molecular dynamics of YiiP transporter within a lipid bilayer using Shewanella oneidensis in bacteria. In the presence of Zn, there is a conformational change which involves the pivoting of the six transmembrane helices from outward-facing state to an inward-facing state (Coudray et al. 2013). Knowing the structure and function of the ZnT gave important clues on which site may induce and inhibit function of the protein. It was only after knowing this information the structure of ZnT8 was extensively studied and is now targeted for drug therapy.

ZnT1 is an efflux transporter expressed in the plasma membrane of the cells where it transports Zn from the cytoplasm of the cell to the extracellular fluid

Table 2 Location and Expression of ZnT Transporters in the Endocrine Pancreas

		mRNA expression			Protein expression		
SLC30A	**Location**	**α**	**β**	**Acinar cells**	**α**	**β**	**Acinar cells**
ZnT1 SLC30A1	**Plasma membrane**	Expressed	Expressed	Expressed	Unknown	Unknown	Expressed
ZnT2 SLC30A2	**Endosomes of zymogen granules**	Not expressed	Not expressed	Expressed	Expressed	Not expressed	Expressed
ZnT3 SLC30A3	**Synaptic vesicles (nerves)**	Not expressed	Not expressed	Unknown	Unknown	Unknown	Unknown
ZnT4 SLC30A4	**Cytoplasm plasma membrane vesicles**	Expressed	Expressed	Unknown	Unknown	Unknown	Unknown
ZnT5 SLC30A5	**Secretory vesicles/ granules**	Expressed	Expressed	Unknown	Unknown	Unknown	Unknown
ZnT6 SLC30A6	**Peripheral region of the Golgi apparatus**	Expressed	Expressed	Unknown	Unknown	Unknown	Unknown
ZnT7 SLC30A7	**Golgi apparatus**	Expressed	Expressed	Unknown	Unknown	Expressed	Unknown
ZnT8 SLC30A8	**Secretory granular vesicles**	Expressed	Expressed	Not expressed	Not expressed	Expressed	Not expressed
ZnT9 SLC30A9	**Cytoplasm (nuclear fraction) nucleus**	Expressed	Expressed	Unknown	Unknown	Unknown	Unknown
ZnT10 SLC30A10	**Golgi apparatus**	Not expressed	Not expressed	Unknown	Unknown	Unknown	Unknown

(Devergnas et al. 2004). ZnT1 mRNA transcripts were abundantly expressed in pancreatic acinar cells and reduced during Zn depletion (Dufner-Beattie et al. 2004a). There is moderate expression of ZnT1 mRNA in human and mouse islets. Liuzzi et al. also confirmed the absence of protein expression of ZnT1 in pancreatic islets in mice (Liuzzi et al. 2004); protein expression of ZnT1 in human pancreatic islets and α and β cell lines was not determined. ZnT1 could be possibly one of the main efflux transporters expressed in the pancreatic exocrine tissue, but as yet, there is no evidence of a role in islet cell function. ZnT2 is mainly localized in the endosomes of zymogen granules in the acinar cell and may facilitate with provision of Zn to the metalloenzymes (Liuzzi et al. 2004; Guo et al. 2010). By contrast, ZnT2 mRNA transcripts were lowly expressed in mouse pancreatic islets (Liuzzi et al. 2004). ZnT3 is located in presynaptic vesicles of neurons and is responsible for the transportation of Zn into synaptic vesicles involved in nerve transmission (Palmiter et al. 1996). ZnT3 transcripts are expressed lowly in human and mouse islets and β cell lines (El Muayed et al. 2012; Wijesekara et al. 2009), indicating that ZnT3 may possibly be involved in

neuroendocrine control of insulin secretion. ZnT4 is ubiquitously expressed in both cytoplasm and plasma membrane of cells with similar functions to ZnT2. ZnT4 transcripts were the third most abundant in β cells compared to α cells and human and mouse pancreatic islets (El Muayed et al. 2012; Wijesekara et al. 2009). ZnT4 is critical for providing Zn to the trans Golgi (McCormick and Kelleher 2012), indicating that ZnT4 may be making Zn available for the formation of insulin hexamers. However, the protein expression of ZnT4 in β cells is yet to be studied. ZnT5 is the second most abundant transcript and protein expressed in both human and mouse pancreatic islets and β cells (El Muayed et al. 2012; Wijesekara et al. 2009).

ZnT5 is located in the secretory granules of insulin-producing β cells (Sheline et al. 2012). Another study reported that ZnT5 is also expressed in the Golgi apparatus of the cell. To what extent ZnT5 is involved in Zn homeostasis in early and late stages of insulin processing is not yet clear. Nor is it clear whether ZnT5 and ZnT8 play similar roles in Zn-promoted crystalline structures of insulin.

ZnT6 is located on the membrane of the trans Golgi network, where it regulates zinc within vesicular compartments (Huang et al. 2002). ZnT6 mRNA transcripts are abundantly expressed in β cell lines compared to human and mouse islets (El Muayed et al. 2012; Wijesekara et al. 2009). The protein expression of ZnT6 is still not determined.

It was speculated that ZnT6 expressed in the Golgi might assist with insulin hexamer formation. ZnT7 is also located in the Golgi apparatus (Ishihara et al. 2006; Kirschke and Huang 2003) where it mediates incorporation of Zn into newly synthesized zinc transporter proteins. There were higher transcript levels of ZnT7 in β cell line compared to α cells, human and mouse pancreatic islets (El Muayed et al. 2012; Wijesekara et al. 2009). Huang and colleagues showed that ZnT7 protein is expressed in pancreatic islets of mice specifically the β cells (when co-stained with an insulin antibody) (Huang et al. 2012). Overexpression of ZnT7 in β cells significantly increased total insulin content and basal insulin secretion (Huang et al. 2010), indicating that ZnT7 may be involved in insulin processing.

ZnT8 is located in the membrane of the insulin secretory vesicles (Nicolson et al. 2009). It is the most abundantly expressed gene or protein compared to all of the ZnTs in the pancreatic islets (Fig. 1). ZnT8 transcripts were much higher in human and mouse islets and β cell lines compared to α cells (Chimienti et al. 2006; Wijesekara et al. 2010). It was also expressed at the protein level in both rat insulinoma β cell line (INS-1) and mouse α and β cells, as well as mouse and human pancreatic islets. Chimienti and colleagues were the first to report that overexpression of ZnT8 in β cells increased insulin content and also increased insulin secretion to glucose stimulus (Chimienti et al. 2006). When ZnT8 was knocked down, this capacity was reduced. Initially, it was reported that global knockdown of ZnT8 in mice reduced granule Zn content and led to age-dependent changes in granule morphology, with markedly fewer dense cores but more rodlike crystals. However, glucose-stimulated insulin secretion appeared normal (Nicolson et al. 2009). However, subsequent studies have suggested that insulin secretion is abnormal. Using mice with knockdown of ZnT8 targeted to β cells only, it was reported that these mice were glucose intolerant and had reduced β cell Zn

accumulation and atypical insulin granules. They also had alterations in glucose-stimulated insulin secretion and increased levels of proinsulin, perhaps suggesting a defect in insulin processing (Wijesekara et al. 2010). Knockdown of ZnT8 in α cells did not affect the insulin content or glucose metabolism. Knockdown of ZnT8 in β cells may decrease the availability of Zn into the insulin vesicles to form insulin hexamers (Wijesekara et al. 2010; Petersen et al. 2010). When $ZnT8^{-/-}$ mice were fed a control diet, glucose tolerance and insulin sensitivity were normal.

However, after feeding a high-fat diet, glucose tolerance and diabetes were seen in the $ZnT8^{-/-}$ mice and their islets became less responsive to glucose (Lemaire et al. 2009). The authors concluded that ZnT8 is essential for the formation of mature insulin crystals in β cells and there is an intriguing interaction between the $ZnT8^{-/-}$ genotype and diet in the mice resulting in a diabetic phenotype. If correct, this has important implications for obesity-induced type 2 diabetes in humans.

ZnT9 transcripts were expressed highly in α and β cells compared to human and mouse pancreatic islets (El Muayed et al. 2012; Wijesekara et al. 2009). The location and the role of ZnT9 are unknown; however, it has been reported to be expressed in the cytoplasm and nuclear fraction of human embryonic lung cells (MRC-S) (Lichten and Cousins 2009). There is no evidence yet of ZnT9 protein expression in pancreatic islets. ZnT9 could possibly be expressed in the nucleus and the cytoplasm of both α and β cells, making Zn available for transcription and translation of insulin and other Zn binding or transporter proteins.

ZnT10 is expressed in the Golgi apparatus of the cell, involved in regulating Zn homeostasis (Bosomworth et al. 2013).

ZnT10 transcripts were not expressed in both human and mouse islets and α and β cells (El Muayed et al. 2012; Wijesekara et al. 2009), and the protein expression of ZnT10 in pancreatic tissue is yet to be determined. This could mean that ZnT10 may not play an important role in the β cells of the pancreatic islets and it is possible that they are highly tissue specific.

The above studies suggest that some members of the ZnT family play an important role in the synthesis and maturation of insulin. The regulation and mechanism of ZnT transporters function are still unknown. Future studies need to elucidate the mechanisms involved and their functional significance for both normal and diabetic islets.

Conclusion

Molecular techniques have begun to unravel the intricacies of how zinc is handled in the pancreas. Zinc transporters, which are crucial in maintaining normal zinc levels, have been implicated as targets for autoimmune attack in T1D. The demonstration that a single-nucleotide polymorphism in ZnT8 gene, substituting arginine for tryptophan at position 325 in the encoded protein in a region believed to be located at the interface between ZnT8 monomers, results in lower apparent Zn ion transport activity of ZnT8 by fluorescence-based assay (Nicolson et al. 2009) and an increased risk of type 2 diabetes in humans 5 is intriguing and

may suggest that ZnT8 functions as a Zn ion channel when dimeric or in higher multimeric forms. Understanding how zinc transporters can be changed or manipulated may provide new option for therapies.

Cross-References

- ▶ Exocytosis in Islet β-Cells
- ▶ Microscopic Anatomy of the Human Islet of Langerhans
- ▶ Physiological and Pathophysiological Control of Glucagon Secretion by Pancreatic α-Cells
- ▶ The β-Cell in Human Type 2 Diabetes

References

Achenbach P, Lampasona V, Landherr U et al (2009) Autoantibodies to zinc transporter 8 and SLC30A8 genotype stratify type 1 diabetes risk. Diabetologia 52:1881–1888

Andrews GK, Geiser J (1999) Expression of the mouse metallothionein-I and -II genes provides a reproductive advantage during maternal dietary zinc deficiency. J Nutr 129:1643–1648

Aydemir TB, Liuzzi JP, McClellan S, Cousins RJ (2009) Zinc transporter ZIP8 (SLC39A8) and zinc influence IFN-gamma expression in activated human T cells. J Leukoc Biol 86:337–348

Beker Aydemir T, Chang SM, Guthrie GJ et al (2012) Zinc transporter ZIP14 functions in hepatic zinc, iron and glucose homeostasis during the innate immune response (endotoxemia). PLoS One 7:e48679

Bellomo EA, Meur G, Rutter GA (2011) Glucose regulates free cytosolic Zn^{2+} concentration, Slc39 (ZiP), and metallothionein gene expression in primary pancreatic islet β-cells. J Biol Chem 286:25778–25789

Bosomworth HJ, Adlard PA, Ford D, Valentine RA (2013) Altered expression of ZnT10 in Alzheimer's disease brain. PLoS One 8:e65475

Camarata T, Krcmery J, Snyder D, Park S, Topczewski J, Simon HG (2010a) Pdlim7 (LMP4) regulation of Tbx5 specifies zebrafish heart atrio-ventricular boundary and valve formation. Dev Biol 337:233–245

Camarata T, Snyder D, Schwend T, Klosowiak J, Holtrup B, Simon HG (2010b) Pdlim7 is required for maintenance of the mesenchymal/epidermal Fgf signaling feedback loop during zebrafish pectoral fin development. BMC Dev Biol 10:104

Chao Y, Fu D (2004) Thermodynamic studies of the mechanism of metal binding to the Escherichia coli zinc transporter YiiP. J Biol Chem 279:17173–17180

Chen H, Carlson EC, Pellet L, Moritz JT, Epstein PN (2001) Overexpression of metallothionein in pancreatic β-cells reduces streptozotocin-induced DNA damage and diabetes. Diabetes 50:2040–2046

Chimienti F, Devergnas S, Pattou F et al (2006) In vivo expression and functional characterization of the zinc transporter ZnT8 in glucose-induced insulin secretion. J Cell Sci 119:4199–4206

Claus J, Chavarria-Krauser A (2012) Modeling regulation of zinc uptake via ZIP transporters in yeast and plant roots. PLoS One 7:e37193

Coudray N, Valvo S, Hu M et al (2013) Inward-facing conformation of the zinc transporter YiiP revealed by cryoelectron microscopy. Proc Natl Acad Sci U S A 110:2140–2145

Cousins RJ, Lee-Ambrose LM (1992) Nuclear zinc uptake and interactions and metallothionein gene expression are influenced by dietary zinc in rats. J Nutr 122:56–64

Cousins RJ, Liuzzi JP, Lichten LA (2006) Mammalian zinc transport, trafficking, and signals. J Biol Chem 281:24085–24089

Desouki MM, Geradts J, Milon B, Franklin RB, Costello LC (2007) hZip2 and hZip3 zinc transporters are down regulated in human prostate adenocarcinomatous glands. Mol Cancer 6:37

Devergnas S, Chimienti F, Naud N et al (2004) Differential regulation of zinc efflux transporters ZnT-1, ZnT-5 and ZnT-7 gene expression by zinc levels: a real-time RT-PCR study. Biochem Pharmacol 68:699–709

Dufner-Beattie J, Kuo YM, Gitschier J, Andrews GK (2004) The adaptive response to dietary zinc in mice involves the differential cellular localization and zinc regulation of the zinc transporters ZIP4 and ZIP5. J Biol Chem 279:49082–49090

Egefjord L, Petersen AB, Rungby J (2010) Zinc, α cells and glucagon secretion. Curr Diabetes Rev 6:52–57

El Muayed M, Raja MR, Zhang X et al (2012) Accumulation of cadmium in insulin-producing β cells. Islets 4:405–416

Emdin SO, Dodson GG, Cutfield JM, Cutfield SM (1980) Role of zinc in insulin biosynthesis. Diabetologia 19:174–182

Foster MC, Leapman RD, Li MX, Atwater I (1993) Elemental composition of secretory granules in pancreatic islets of Langerhans. Biophys J 64:525–532

Franklin I, Gromada J, Gjinovci A, Theander S, Wollheim CB (2005) β-cell secretory products activate α-cell ATP-dependent potassium channels to inhibit glucagon release. Diabetes 54:1808–1815

Fukada T, Civic N, Furuichi T et al (2008) The zinc transporter SLC39A13/ZIP13 is required for connective tissue development; its involvement in BMP/TGF-? Signaling pathways. PLoS One 3:e3642

Grass G, Franke S, Taudte N et al (2005) The metal permease ZupT from escherichia coli is a transporter with a broad substrate spectrum. J Bacteriol 187:1604–1611

Guo L, Lichten LA, Ryu M-S, Liuzzi JP, Wang F, Cousins RJ (2010) STAT5-glucocorticoid receptor interaction and MTF-1 regulate the expression of ZnT2 (Slc30a2) in pancreatic acinar cells. Proc Natl Acad Sci 107:2818–2823

Heise CC, King JC, Costa FM, Kitzmiller JL (1988) Hyperzincuria in IDDM women. Relationship to measures of glycemic control, renal function, and tissue catabolism. Diabetes Care 11:780–786

Hijova E (2004) Metallothioneins and zinc: their functions and interactions. Bratisl Lek Listy 105:230–234

Hill GM, Link JE (2009) Transporters in the absorption and utilization of zinc and copper. J Anim Sci 87:E85–E89

Huang L, Kirschke CP, Gitschier J (2002) Functional characterization of a novel mammalian zinc transporter, ZnT6. J Biol Chem 277:26389–26395

Huang L, Yan M, Kirschke CP (2010) Over-expression of ZnT7 increases insulin synthesis and secretion in pancreatic β-cells by promoting insulin gene transcription. Exp Cell Res 316:2630–2643

Huang L, Kirschke CP, Lay YA, Levy LB, Lamirande DE, Zhang PH (2012) Znt7-null mice are more susceptible to diet-induced glucose intolerance and insulin resistance. J Biol Chem 287:33883–33896

Ishihara K, Yamazaki T, Ishida Y et al (2006) Zinc transport complexes contribute to the homeostatic maintenance of secretory pathway function in vertebrate cells. J Biol Chem 281:17743–17750

Jenkitkasemwong S, Wang CY, Mackenzie B, Knutson MD (2012) Physiologic implications of metal-ion transport by ZIP14 and ZIP8. Biometals 25:643–655

Kambe T (2012) Molecular architecture and function of ZnT transporters. Curr Top Membr 69:199–220

Kelleher SL, McCormick NH, Velasquez V, Lopez V (2011) Zinc in specialized secretory tissues: roles in the pancreas, prostate, and mammary gland. Adv Nutr 2:101–111

Khadeer MA, Sahu SN, Bai G, Abdulla S, Gupta A (2005) Expression of the zinc transporter ZIP1 in osteoclasts. Bone 37:296–304

Kimura T, Itoh N (2008) Function of metallothionein in gene expression and signal transduction: newly found protective role of metallothionein. J Health Sci 54:251–260

Kirschke CP, Huang L (2003) ZnT7, a novel Mammalian zinc transporter, accumulates zinc in the Golgi apparatus. J Biol Chem 278:4096–4102

Lemaire K, Ravier MA, Schraenen A et al (2009) Insulin crystallization depends on zinc transporter ZnT8 expression, but is not required for normal glucose homeostasis in mice. Proc Natl Acad Sci 106:14872–14877

Lichten LA, Cousins RJ (2009) Mammalian zinc transporters: nutritional and physiologic regulation. Annu Rev Nutr 29:153–176

Liuzzi JP, Bobo JA, Lichten LA, Samuelson DA, Cousins RJ (2004) Responsive transporter genes within the murine intestinal-pancreatic axis form a basis of zinc homeostasis. Proc Natl Acad Sci USA 101:14355–14360

Lu M, Fu D (2007) Structure of the zinc transporter YiiP. Science 317:1746–1748

Maret W, Krezel A (2007) Cellular zinc and redox buffering capacity of metallothionein/thionein in health and disease. Mol Med 13:371–375

McCormick NH, Kelleher SL (2012) ZnT4 provides zinc to zinc-dependent proteins in the trans-Golgi network critical for cell function and Zn export in mammary epithelial cells. Am J Physiol Cell Physiol 303:C291–C297

Myers SA, Nield A, Myers M (2012) Zinc transporters, mechanisms of action and therapeutic utility: implications for type 2 diabetes mellitus. J Nutr Metab 2012:173712

Nicolson TJ, Bellomo EA, Wijesekara N et al (2009) Insulin storage and glucose homeostasis in mice null for the granule zinc transporter ZnT8 and studies of the type 2 diabetes-associated variants. Diabetes 58:2070–2083

Palmiter RD, Huang L (2004) Efflux and compartmentalization of zinc by members of the SLC30 family of solute carriers. Pflugers Arch 447:744–751

Palmiter RD, Cole TB, Quaife CJ, Findley SD (1996) ZnT-3, a putative transporter of zinc into synaptic vesicles. Proc Natl Acad Sci U S A 93:14934–14939

Petersen AB, Smidt K, Magnusson NE, Moore F, Egefjord L, Rungby J (2010) siRNA-mediated knock-down of ZnT3 and ZnT8 affects production and secretion of insulin and apoptosis in INS-1E cells. APMIS 119:93–102

Ravier MA, Rutter GA (2005) Glucose or insulin, but not zinc ions, inhibit glucagon secretion from mouse pancreatic α-cells. Diabetes 54:1789–1797

Ruttkay-Nedecky B, Nejdl L, Gumulec J et al (2013) The role of metallothionein in oxidative stress. Int J Mol Sci 14:6044–6066

Scott DA, Fisher AM (1938) The insulin and the zinc content of normal and diabetic pancreas. J Clin Invest 17:725–728

Sekler I, Sensi SL, Hershfinkel M, Silverman WF (2007) Mechanism and regulation of cellular zinc transport. Mol Med 13:337–343

Sheline CT, Shi C, Takata T et al (2012) Dietary zinc reduction, pyruvate supplementation, or zinc transporter 5 knockout attenuates β-cell death in nonobese diabetic mice, islets, and insulinoma cells. J Nutr 142:2119–2127

Simon SF, Taylor CG (2001) Dietary zinc supplementation attenuates hyperglycemia in db/db mice. Exp Biol Med (Maywood) 226:43–51

Skelin M, Rupnik M, Cencic A (2010) Pancreatic β cell lines and their applications in diabetes mellitus research. Altex 27:105–113

Sondergaard LG, Stoltenberg M, Flyvbjerg A et al (2003) Zinc ions in β-cells of obese, insulin-resistant, and type 2 diabetic rats traced by autometallography. APMIS 111:1147–1154

Suhy DA, Simon KD, Linzer DIH, O'Halloran TV (1999) Metallothionein is part of a zinc-scavenging mechanism for cell survival under conditions of extreme zinc deprivation. J Biol Chem 274:9183–9192

Taniguchi M, Fukunaka A, Hagihara M et al (2013) Essential role of the zinc transporter ZIP9/SLC39A9 in regulating the activations of Akt and Erk in β-cell receptor signaling pathway in DT40 cells. PLoS One 8:e58022

Taylor KM, Nicholson RI (2003) The LZT proteins; the LIV-1 subfamily of zinc transporters. Biochim Biophys Acta 1611:16–30

Taylor KM, Hiscox S, Nicholson RI, Hogstrand C, Kille P (2012) Protein kinase CK2 triggers cytosolic zinc signaling pathways by phosphorylation of zinc channel ZIP7. Sci Signal 5:ra11

Thiel G, Muller I, Rossler OG (2013) Signal transduction via TRPM3 channels in pancreatic β-cells. J Mol Endocrinol 50:R75–R83

Tomita T (2000) Metallothionein in pancreatic endocrine neoplasms. Mod Pathol 13:389–395

Truong-Tran AQ, Ho LH, Chai F, Zalewski PD (2000) Cellular zinc fluxes and the regulation of apoptosis/gene-directed cell death. J Nutr 130:1459S–1466S

Vert G, Grotz N, Dedaldechamp F et al (2002) IRT1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. Plant Cell 14:1223–1233

Wagner TJ, Drews A, Loch S et al (2010) TRPM3 channels provide a regulated influx pathway for zinc in pancreatic β cells. Pflugers Arch Eur J Physiol 460:755–765

Weaver BP, Dufner-Beattie J, Kambe T, Andrews GK (2007) Novel zinc-responsive post-transcriptional mechanisms reciprocally regulate expression of the mouse Slc39a4 and Slc39a5 zinc transporters (Zip4 and Zip5). Biol Chem 388:1301–1312

Weijers RN (2010) Three-dimensional structure of β-cell-specific zinc transporter, ZnT-8, predicted from the type 2 diabetes-associated gene variant SLC30A8 R325W. Diabetol Metab Syndr 2:33

Wijesekara N, Chimienti F, Wheeler MB (2009) Zinc, a regulator of islet function and glucose homeostasis. Diabetes Obes Metab 11(Suppl 4):202–214

Wijesekara N, Dai F, Hardy A et al (2010) β cell-specific Znt8 deletion in mice causes marked defects in insulin processing, crystallisation and secretion. Diabetologia 53:1656–68

Wu JP, Ma BY, Ren HW, Zhang LP, Xiang Y, Brown MA (2007) Characterization of metallothioneins (MT-I and MT-II) in the yak. J Anim Sci 85:1357–1362

Yan G, Zhang Y, Yu J et al (2012) Slc39a7/zip7 plays a critical role in development and zinc homeostasis in zebrafish. PLoS One 7:e42939

Zalewski P, Millard S, Forbes I et al (1994) Video image analysis of labile zinc in viable pancreatic islet cells using a specific fluorescent probe for zinc. J Histochem Cytochem 42:877–884

Zalewski PD, Truong-Tran A, Lincoln SF et al (2006) Use of a zinc fluorophore to measure labile pools of zinc in body fluids and cell-conditioned media. Biotechniques 40:509–520

High-Fat Programming of β-Cell Dysfunction

18

Marlon E. Cerf

Contents

Abstract

High saturated fat intake contributes to insulin resistance, β-cell dysfunction, and type 2 diabetes. Developmental programming refers to a stimulus or insult during critical periods of life which includes fetal and subsequent early neonatal life. Programming alters progeny physiology and metabolism with immediate, transient, and durable effects. Maternal nutrition and metabolic state in gestation and lactation shapes progeny development and health. However, paternal nutrition and metabolic state also shapes progeny outcomes, albeit to a lesser extent. A high saturated fat diet ingested by mothers during gestation and/or lactation presents a nutritional insult that induces diabetogenic changes in progeny physiology and metabolism. High-fat programming is induced by maternal high saturated fat intake during defined periods of gestation and/or lactation and programs the physiology and metabolism of the progeny throughout life. This more recently adopted form of developmental programming reflects society in both affluent and developing countries. High-fat programming induces adverse changes in β-cell development and function in neonatal, weanling, and

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_3,

adolescent progeny. These changes are characterized by compromised β-cell development and function, evident by altered expression of key factors that maintain the β-cell phenotype. High-fat programming likely prompts β-cell dysfunction and eventual type 2 diabetes. Dietary consumption, limited in high saturated fat content, particularly in fetal and early postnatal life should be adopted in progeny. Healthy parental nutrition and metabolism pre-, during, and postconception, particularly maternal, should be maintained to enhance progeny health outcomes. These early intervention strategies may prevent the onset of metabolic disease.

Keywords
Glucolipotoxicity • Nutrition • Pancreas • Type 2 diabetes

Introduction

Changes in lifestyle, such as the consumption of high-calorie diets and physical inactivity, have increased the global prevalence of obesity and diabetes (Kasuga 2006). Modern lifestyles with abundant nutrient supply and reduced physical activity have resulted in the increased prevalence of metabolic disease (Ferreira et al. 2010). Between 60 % and 90 % of type 2 diabetes cases appear to be related to obesity (Anderson et al. 2003) with a strong correlation between obesity and insulin resistance in both diabetic and nondiabetic subjects (Ludvik et al. 1995). Excessive high-fat consumption promotes obesity and insulin resistance. High-fat-fed rodents develop glucose intolerance, suggesting that eventually, compensation for fat-induced insulin resistance becomes inadequate due to defects in β-cell function or mass (Giacca et al. 2011). Developmental programming is defined as a stimulus or insult in utero or in early postnatal life (during suckling) that induces immediate, transient, and durable effects in progeny physiology and metabolism. High-fat programming is induced by maternal high saturated fat intake during defined periods of gestation and/or lactation and programs the physiology and metabolism of the progeny. This contrasts from previous studies focusing on protein deficiency in utero. Instead of mimicking famine as in protein restriction and undernutrition, which still exists in several parts of the world, high-fat programming reflects current society in both affluent and developing nations. In affluent societies, there is an overabundance of food available. With economic improvement and migration in the developing world, there is a transition from the traditional way of life, where the diet comprises whole food and exercise is a regular routine in daily life, to a more Westernized lifestyle – characterized by the consumption of convenient processed and fast foods with high saturated fat content and a sedentary lifestyle due to a greater reliance on transport which reduces the need for exercise. With migration and improving economic status, people often switch diets from whole food or a state of undernutrition to diets that include high contents of saturated fat and overnutrition. Disease risk is amplified by a greater mismatch between the

prenatally predicted and actual adult environments (Godfrey et al. 2007). As a result, societies in rapid economic transition are particularly vulnerable (Popkin 2001; Gluckman and Hanson 2004a; Prentice and Moore 2005; Bhargava et al. 2004). Long-term consumption of a high-fat diet (HFD) concomitant with physical inactivity leads to obesity which is a major risk factor for inducing β-cell dysfunction and insulin resistance and contributes to the increase in incidence of type 2 diabetes.

Critical Programming Windows

Both the intrauterine and lactational environments represent critical developmental periods that provide a platform for programming. The intrauterine environment shapes fetal health. The developing fetus is highly sensitive to its environment, and nutrition is a key determinant of fetal growth and maturation (Gluckman and Hanson 2004b). The fetus makes adaptations that anticipate the postnatal nutrition which impacts its future metabolic status. A mechanism by which diet influences fetal growth is by altering circulating concentrations of key maternal metabolic hormones that regulate placental nutrient transport and therefore fetal growth (Jansson et al. 2008). Lactation is a critical developmental stage for metabolic programming of later disease and modifying the impact of prenatal challenges (Heywood et al. 2004; Siebel et al. 2008). Fluctuations in glycemic and saturated fatty acid concentrations have adverse effects on progeny. The level of nutrition available during pregnancy and lactation plays a major role in determining progeny metabolic phenotype (Zambrano et al. 2006). Progeny adopt the nutrition experienced during these critical developmental periods, i.e., the diet exposed to during fetal and early postnatal life. A high saturated fat diet is strongly associated with the pathogenesis of β-cell dysfunction, insulin resistance, and type 2 diabetes (Cerf 2007). In pregnant mothers, maintenance on a HFD results in the exposure of progeny to an insult during the critical period of fetal life. These events, concomitant with the altered metabolic state of insulin resistance in the pregnant mother (typical in healthy mothers during late pregnancy), likely increase the risk of progeny to develop metabolic disease. However, there is also a paternal role in shaping progeny health outcomes. Paternal chronic HFD consumption induced increased body weight, adiposity, impaired glucose tolerance, and insulin insensitivity in their progeny (Ng et al. 2010). However, the metabolic changes in progeny of paternal diabetes were milder relative to maternal diabetes (Grasemann et al. 2012). This may be attributed to maternal hyperglycemia inducing direct programming of metabolic traits in developing fetuses, whereas the influence of paternal diabetes on progeny is indirect, potentially through transgenerationally transmitted marks of fetal programming from the fathers (Grasemann et al. 2012). However, both parents' diets and genes contribute to progeny health outcomes. With parental high-fat programming (parents maintained on a high saturated fat diet pre- and during conception), progeny face challenges in β-cell development in utero and in early neonatal life which diminishes β-cell turnover and function later in life

(Cerf 2011). Therefore, parental nutrition and metabolic state, pre-, during, and postconception, influences progeny development, growth, maturation, and health. Fetal life and maternal health are the most critical determinants of progeny health.

High-Fat Programming Concept

High-fat programming can be defined as maternal high saturated fat intake during defined periods of gestation and/or lactation that programs progeny physiology and metabolism (Cerf and Louw 2008) at birth, during early life, and in adulthood. Progeny can be studied at various stages of life: as fetuses, neonates, weanlings, adolescents, adults, and aged. In terms of the experimental design, high saturated fat diets were administered to pregnant and/or lactating Wistar rats, thereby exposing progeny to this dietary insult. Specifically, mothers and their progeny were maintained on a HFD throughout gestation only (fetal high-fat programming = HFG), throughout lactation only (lactational high-fat programming = HFL), or throughout both gestation and lactation (fetal and lactational high-fat programming = HFGL; Fig. 1). These progeny are therefore programmed as high-fat exposure during these critical developmental periods influences their metabolic state. The HFD administered contained 40% energy as fat compared to 10% energy as fat in the standard laboratory diet. Similar protein levels were maintained in both diets to avoid the adverse effects of protein deficiency on β-cell morphology and function.

Further, high-fat programming studies can be extended to include different time period interventions (Fig. 2). In these studies, the nutritional trajectory of pregnant mothers was maintenance on a HFD for either the first, second, or third week of gestation (to mimic human trimesters) and throughout gestation (Cerf et al. 2005). Neonatal progeny, maintained on the HFD for those specified periods of fetal life, were studied. These neonatal groups were also studied at weaning (3 weeks of age) (Cerf et al. 2007). During lactation, these progeny were maintained on a standard laboratory (low-fat) diet.

Further extension of the weanling groups included those maintained on a fetal HFD and either the first (HFGL1), or the second (HFGL2), or the final (HFGL3; Fig. 2) week of lactation (Cerf et al. 2006). When the neonatal and weanling progeny were not maintained on a HFD, they were instead maintained on a standard laboratory diet.

These studies demonstrated that high-fat consumption increased food intake in pregnant mothers which subsequently increased their body weights and induced hyperglycemia (Cerf et al. 2005). At birth, the β-cell mass is largely determined by the recruitment of undifferentiated precursors, as well as the replication of differentiated cells and apoptosis of the β-cells (Reusens et al. 2011). In terms of high-fat programming, neonates displayed alterations in glycemia and β-cell development (Cerf et al. 2005) that persisted in weanlings (Cerf et al. 2007). High-fat programming over different time periods of gestation and lactation also altered expression of key β-cell factors, including GLUT-2, glucokinase (GCK), and, to a lesser extent, Pdx-1 (Cerf et al. 2006). A gestational HFD induced maternal hyperglycemia and

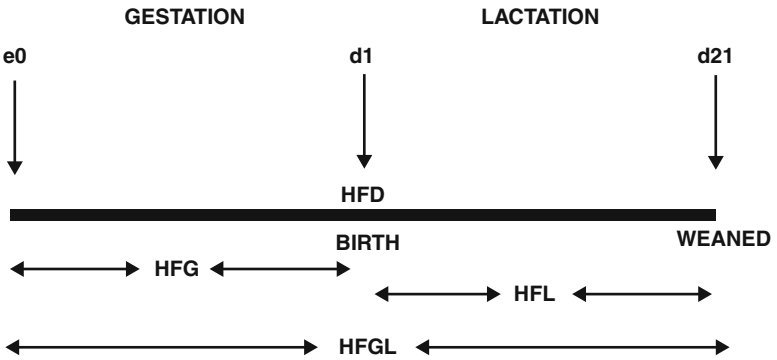

Fig. 1 High-fat programming: basic experimental design. *e* fetal (gestational) day, *d* postnatal (lactational) day, *HFD* high-fat diet, *HFG* fetal high-fat diet, *HFL* lactational high-fat diet, *HFGL* fetal and lactational high-fat diet; *downward arrows* denote time points; *horizontal arrows* denote period of high-fat diet maintenance

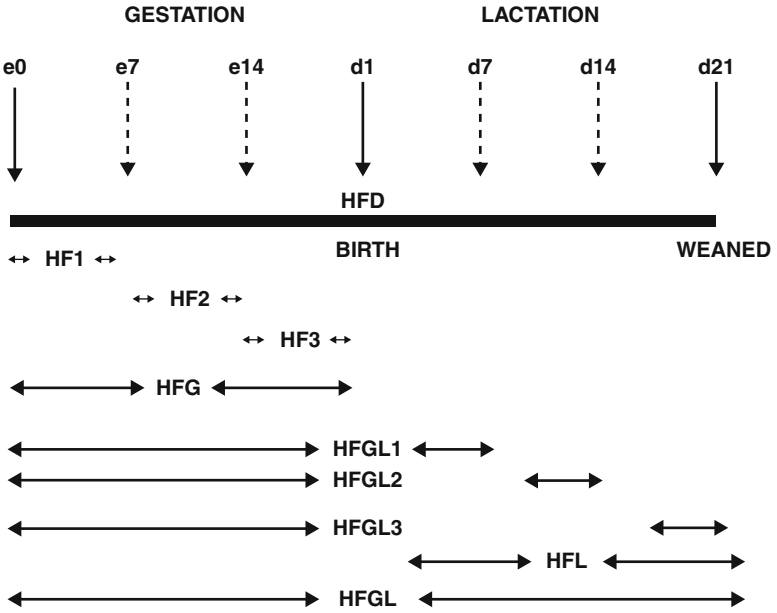

Fig. 2 High-fat programming: extended experimental design. *e* fetal (gestational) day, *d* postnatal (lactational) day. Numerals refer to the specific day of fetal life (gestation) (*e*) or postnatal life (lactation) (*d*). *HFD* high-fat diet, *HF1* high-fat diet for the first week of fetal life, *HF2* high-fat diet for the second week of fetal life, *HF3* high-fat diet for the third week of fetal life. *HFG* fetal high-fat diet, *HFGL1* high-fat diet throughout fetal life and for the first week of lactation, *HFGL2* high-fat diet throughout fetal life and for the second week of lactation, *HFGL3* high-fat diet throughout fetal life and for the third week of lactation, *HFL* lactational high-fat diet, *HFGL* fetal and lactational high-fat diet; *downward arrows* denote time points; *horizontal arrows* denote period of high-fat diet maintenance

the programming effects resulted in neonates with hyperglycemia (Cerf et al. 2005). Both the mothers and neonates displayed no changes in insulinemia. Different outcomes in β-cell development and function were evident in both neonatal and weanling progeny from mothers maintained on a HFD during specified periods of gestation and/or lactation (Cerf et al. 2005, 2006, 2007). These alterations appear to be dependent on the specific period of exposure to the HFD.

Recently high-fat programming was studied in 3-month-old progeny. Postnatal high-fat programming, from birth to 3 months of age, induced the most diabetogenic phenotype with high-fat maintenance throughout fetal and postnatal life resulting in a severely obese phenotype (Cerf et al. 2012). In contrast, fetal high-fat programming induced no changes in either metabolism or islet architecture presenting a normal phenotype with the absence of a diabetogenic and/or obese phenotype in both male and female progeny (Cerf et al. 2012). These findings emphasized the importance of maternal gestational and lactational nutrition in shaping progeny health and disease outcomes and confirmed the durable effects of high-fat programming (Cerf et al. 2012). Further, the nutrition adopted by progeny from birth to adulthood is also a critical determinant of their health. This was evident in adolescent male progeny maintained on a HFD throughout postnatal life displaying a diabetogenic phenotype characterized by hyperglycemia, β-cell hyperplasia, and hypertrophy (Cerf et al. 2012) reflecting a β-cell compensatory response to hyperglycemia.

β-Cell Regulation

The ability of an organism to maintain its β-cell mass during adulthood is critical for maintaining glucose homeostasis and preventing diabetes (Ackermann and Gannon 2007). β-cell mass is enhanced by proliferation (replication of β-cells), neogenesis (differentiation from non-β-cells), hyperplasia (increased β-cell number), and hypertrophy (increased β-cell size) and is decreased by β-cell death, through apoptosis, necrosis, autophagy, and potentially ferroptosis and hypoplasia (decreased β-cell number) and hypotrophy (decreased β-cell size). The expansion and demise of β-cell mass through stimulants and insults, respectively, are likely triggered through one or more of these processes of β-cell replenishment (β-cell expansion) and death (β-cell demise). The balance between pro-apoptotic and antiapoptotic (protective) processes determines the fate of β-cells (Li et al. 2008). ATF3, a stress-inducible pro-apoptotic gene, represses the expression of IRS2, a pro-survival gene, thus providing a direct link between the stress response and a potent pro-survival pathway (Li et al. 2008). Because ATF3 is induced by a variety of stress signals, it can function as a conduit for stress signals to dampen a potent pro-survival pathway in β-cells (Li et al. 2008). An early loss of β-cell mass may subsequently favor dysfunction of the residual β-cells, possibly due to overstimulation or toxic effects of even mild chronic hyperglycemia and/or hyperlipidemia (Donath and Halban 2004). Partial recovery of the lost β-cell mass may result from post-insult induction of β-cell regeneration and/or neogenesis and

eventually with treatment with growth-stimulating pharmacological agents (Masiello 2006). Hypertrophy and increased insulin responsiveness to glucose and free fatty acids (FFA) also occur in residual β-cells (Masiello 2006). The adaptive response may be insufficient or temporary because of incomplete differentiation of newly formed β-cells and/or acquired dysfunction of residual β-cells chronically exposed to a metabolically altered environment (Masiello 2006). An increased frequency of apoptosis due to prolonged overstimulation of residual β-cells, chronic hyperglycemia, and/or hyperlipidemia may accelerate decomposition (Masiello 2006).

An increase in β-cell mass usually occurs over a period of time for the endocrine pancreas to maintain glucose homeostasis when challenged by diabetogenic states such as obesity and insulin resistance. This allows the pancreas to cope, for a defined time, with the maintenance of glucose homeostasis. However, after a threshold is reached, the β-cells become exhausted and hyperglycemia is manifested usually along with an altered insulinemic profile. Initially hyperinsulinemia is exhibited, i.e., the β-cells secrete more insulin to restore glucose homeostasis, but eventually, the β-cells fail to secrete sufficient insulin resulting in hypoinsulinemia which often progresses to type 2 diabetes. Hypoinsulinemia is indicative of the more severe type 1 diabetes, which reflects how type 2 diabetes evolves to closely mimic type 1 diabetes, as it progresses to deteriorate to a state where exogenous insulin is required to counteract the persistently elevated circulating glucose concentrations. Molecules, signaling pathways, and cellular machinery involved in the demise of β-cells under seemingly divergent pathophysiological conditions, i.e., type 1 and 2 diabetes, are therefore similar to a large extent (Li et al. 2008). Chronic high saturated fat consumption and persistent hyperglycemia contribute significantly to reducing β-cell mass thereby impairing β-cell function.

High-Fat Programming May Induce β-Cell Dysfunction via Glucolipotoxicity

Glucotoxicity is the slow and progressively irreversible effects of chronic hyperglycemia on β-cell function (Poitout and Robertson 2002). Chronic hyperglycemia decreases β-cell mass by inducing apoptosis (Pick et al. 1998; Donath et al. 1999) and adversely affects insulin secretion (Poitout and Robertson 2002). Lipotoxicity, characterized by chronic exposure to elevated FFA concentrations, impairs insulin secretion leading to hyperglycemia and β-cell dysfunction (Skelly et al. 1998). Hyperglycemia is proposed to be a prerequisite for lipotoxicity to occur (Poitout and Robertson 2008). Glucolipotoxicity, the simultaneous elevation of glucose and lipids, results in the intracellular accumulation of lipids and lipid metabolites that are ultimately detrimental to β-cell function and survival (Ruderman and Prentki 2004). High-fat programming may induce adverse effects on metabolism and physiology by elevating both circulating glucose and FFA concentrations in progeny. This impairs β-cell development and function resulting in loss of glucose homeostasis.

Chronic exposure of islets to elevated levels of nutrients induces β-cell dysfunction and triggers β-cell death. Exposure of isolated rodent islets to hyperglycemia for several days raises basal insulin secretion but impairs insulin secretion in response to stimulatory glucose concentrations (Chen et al. 1994; Khaldi et al. 2004). Similarly, exposure of islets to elevated levels of FFA does not impair glucose-stimulated insulin secretion (GSIS) unless the islets are cultured at or above a threshold concentration of glucose (about 8 mmol/l) (Poitout and Robertson 2002; Prentki et al. 2002). This suggests that β-cell dysfunction is likely a consequence of glucolipotoxicity as opposed to either gluco- or lipotoxicity as separate entities (Poitout and Robertson 2002; Prentki et al. 2002). High-fat programming may induce β-cell dysfunction by glucolipotoxicity due to potential exposure of progeny to hyperglycemic and hyperlipidemic intrauterine environments. In addition, the milk of hyperglycemic mothers may also contain elevated FFA concentrations that have a glucolipotoxic effect on the suckling progeny. One theory is that β-cells become sensitized to FFA and preferentially metabolize FFA rather than glucose as fuel, which may explain the reduced glucose-stimulated insulin release (GSIR) typically observed following prolonged exposure to FFA in vitro (Poitout and Robertson 2008). In high-fat programming, it is therefore likely that preferential metabolism of FFA over glucose will further exacerbate hyperglycemia.

Elevated levels of glucose and saturated FFA can independently and potentially synergistically induce β-cell dysfunction. However, the presence of both gluco- and lipotoxicity, i.e., glucolipotoxicity, will accelerate the impairment of β-cell function. This simultaneous dual insult will more rapidly increase the β-cell metabolic overload, inhibiting β-cell compensation, and thus increase susceptibility to β-cell dysfunction. In obese type 2 diabetic patients, their hyperglycemic state concomitant with the readily available fat stores for release of FFA infers their glucolipotoxic state. Obese type 2 diabetic individuals who continuously ingest a high intake of harmful saturated FA will further exacerbate their hyperglycemia, obesity, insulin resistance, and β-cell dysfunction which will increase the severity of diabetes.

β-cells initially compensate for the insulin resistance associated with obesity by upregulating insulin secretion (Kasuga 2006). During β-cell compensation, β-cells are exposed to metabolic changes associated with obesity; therefore, factors commonly associated with obesity such as insulin resistance, adipokines, FFA, reactive oxygen species (ROS), and ER-associated stress are inducers of β-cell dysfunction (Kasuga 2006). β-cell dysfunction in type 2 diabetes manifests when islets are unable to sustain β-cell compensation for insulin resistance (Prentki and Nolan 2006). β-cell dysfunction is progressive, particularly after hyperglycemia is established, which leads to poorly functioning, dedifferentiated β-cells and loss of β-cell mass from apoptosis (Prentki and Nolan 2006) and other β-cell death processes. β-cell destruction in various pathophysiological conditions signals a stress response (Li et al. 2008). The potential mechanisms of early β-cell dysfunction include mitochondrial dysfunction, ROS, ER stress, dysfunctional triglyceride/FFA (TG/FFA) cycling, and glucolipotoxicity (Prentki and Nolan 2006). Further,

β-cell dysfunction is likely induced by a combination of chronic hyperglycemia, hyperlipidemia, and/or certain cytokines that interfere with the signaling pathways that maintain normal β-cell growth and survival (Rhodes 2005). This results in a reduction in functional β-cell mass in diabetic states (Rhodes 2005). Other underlying mechanisms in β-cell dysfunction include genetic susceptibility, β-cell metabolic overload, and amyloid fibrils (Muoio and Newgard 2008). Once hyperglycemia has manifested, which occurs in specific instances of high-fat programming dependent on the period of exposure, additional processes linked to glucotoxicity and the diabetic milieu, such as islet inflammation, O-linked glycosylation, and amyloid deposition, accelerate β-cell dysfunction, resulting in severe β-cell phenotypic alterations and loss of β-cell mass by apoptosis (Prentki and Nolan 2006).

Mice fed a diet rich in saturated fat develop overt diabetes characterized by hyperinsulinemia associated with hyperglycemia (Gniuli et al. 2008). In progeny where the HFD was only administered during fetal life (similar to HFG rats) and during both fetal and neonatal life (similar to HFGL rats), the β-cell insult was severe evident by sustained hyperglycemia during adulthood (Gniuli et al. 2008). Thus it seems feasible that the programming effects in both HFG and HFGL, and likely also in HFL weanlings, will further exacerbate as they reach adulthood. At 3 months of age, HFG (maintained on a HFD throughout fetal life) male and female progeny were normoglycemic, HFGP (maintained on a HFD throughout life) male progeny were hyperglycemic, and HFP (maintained on a HFD throughout postnatal life) male and female progeny were hyperglycemic (Cerf et al. 2012). Hyperinsulinemia was also evident in 3-month-old male HFGP progeny (Cerf et al. 2012). This demonstrated that high-fat programming induces variable and durable effects in adolescent progeny, which is dependent on the specific period of exposure. In a recent study, 3-month-old progeny (mice) from mothers fed a HFD during gestation had increased β-cell mass, hyperglycemia, and hyperinsulinemia, reflecting insulin resistance which precedes type 2 diabetes (Gregorio et al. 2013). This contrasted to the findings of HFG progeny who were normoglycemic and normoinsulinemic without insulin resistance or changes in β-cell morphology (Cerf et al. 2012). However, aging is an independent risk factor for β-cell dysfunction (Utzschneider et al. 2004), and it is therefore expected that a diabetogenic phenotype will present in these HFG progeny as they age and the β-cells fail to compensate adequately in response to metabolic demand for insulin.

Obesity often leads to insulin resistance but only a subset of obese insulin-resistant individuals progress to type 2 diabetes (Muoio and Newgard 2008), which may be due to genetic predisposition, poor dietary control, and physical inactivity. In humans and animals, the triggering factor is β-cell dysfunction, which involves a decrease in β-cell mass and more critically the loss of key β-cell functions like GSIS (Muoio and Newgard 2008). The severity of high-fat programming in inducing β-cell dysfunction may be dependent on the stage of programming (e.g., fetal and/or lactational), the metabolic status of the mother and, to a lesser extent, the father, and the duration of the insult. It appears that limited exposure to programming, such as maintenance on a HFD for only a single gestational week, has a reduced impact on

adversely affecting β-cell function. This however renders the progeny susceptible to the predictive adaptive response whereby they cannot accurately anticipate future nutrition as it differs from the nutrition experienced in utero which is hypothesized to have adverse health consequences. Maintenance on a HFD throughout fetal life and lactation represents extreme high-fat programming. It is hypothesized that if these progeny are continuously maintained on a HFD, with time β-cell dysfunction will ensue. Initially the β-cells may undergo compensation to cope with the maintenance of glucose homeostasis. However, high-fat programming coupled to chronic high-fat feeding is likely to increase glucolipotoxicity resulting in eventual β-cell dysfunction.

Chronic hyperglycemia can increase the rate of development of the early diabetic state by affecting the secretory capacity of pancreatic cells, which in turn increases blood glucose concentrations (Brunner et al. 2009) and ultimately leads to the total incapacity of β-cells to secrete insulin (LeRoith 2002; Dubois et al. 2007). High-fat programming induces hyperglycemia and hypoinsulinemia, which is characterized by reduced β-cell volume, number, and size with reduced expression of key β-cell factors like Pdx-1 and GCK. Collectively, these adverse metabolic effects of programming would predispose these progeny to β-cell dysfunction. Also, high-fat programming may increase circulating FFA concentrations due to metabolism of the HFD which releases excess FFA. This may lead to reduced insulin secretion, which induces hyperglycemia resulting in β-cell dysfunction. High-fat programming may follow a glucolipotoxic mechanism to gradually but steadily impair β-cell function ultimately leading to β-cell dysfunction.

High-Fat Programming Diminishes β-Cell Integrity

A HFD is known to compromise glucose sensing and insulin signaling, evident by reduced expression of insulin, Pdx-1, GLUT-2, and GCK after high-fat feeding or exposure to FFA (Kim et al. 1995; Jorns et al. 2002; Reimer and Ahren 2002; Gremlich et al. 1997). The percentage of L-type calcium channels that are considered most important for insulin secretion is reduced in neonatal rats, concomitant with reduced expression of GLUT-2 (Navarro-Tableros et al. 2007). HFG neonates released reduced insulin at stimulatory 13 and 22 mmol/l glucose concentrations concomitant with reduced Pdx-1 and GCK immunoreactivity (Cerf et al. 2009). Chronic hyperglycemia adversely affects insulin secretion (Poitout and Robertson 2002) and decreases β-cell mass by inducing apoptosis (Pick et al. 1998; Donath et al. 1999). As HFG neonates displayed reduced β-cell volume and number (Cerf et al. 2005) and both Pdx-1 and GCK immunoreactivity were reduced (Cerf et al. 2009), the functional capacity of their β-cells was impaired. These effects, concomitant with the reduced insulin release from islets of HFG neonates at stimulatory glucose concentrations, confirm that high-fat programming during fetal life impairs β-cell function in neonates.

An altered metabolic milieu decreases Pdx-1 transcription by mediating a cascade of epigenetic modifications which silences Pdx-1 (Park et al. 2008).

In intrauterine growth-retarded (IUGR) rats, Pdx-1 expression was permanently reduced in β-cells (Park et al. 2008). Fetal high-fat programming reduced Pdx-1 immunoreactivity in the neonates which could adversely affect insulin gene expression and, in addition, appears to have contributed to the reduction in β-cell volume and number in HFG neonates.

Haploinsufficiency of β-cell-specific GCK ($GCK^{+/-}$) in mice results in mild diabetes with impaired insulin secretion in response to glucose (Terauchi et al. 1995). Wild-type mice fed a HFD showed marked β-cell hyperplasia, whereas $GCK^{+/-}$ mice displayed insufficient β-cell hyperplasia despite a similar degree of insulin resistance (Terauchi et al. 2007). Permanent exposure of weanlings to a HFD, i.e., during the entire duration of both fetal and suckling life, resulted in reduced GLUT-2 and GCK mRNA expression (Cerf et al. 2006). These HFGL weanlings displayed hypoinsulinemia suggesting impaired insulin secretion attributed partly to the reduced GCK expression both at gene and protein level (Cerf et al. 2006). HFL, HFGL, and HFG weanlings display glucose intolerance in descending order of severity (Cerf and Louw 2010). Thus HFGL weanlings display impaired β-cell function which may predispose them to β-cell dysfunction. Both HFG and HFGL weanlings were normoglycemic and hypoinsulinemic (Cerf et al. 2006), but both were glucose intolerant, an effect that was more marked in HFGL weanlings. As the HFG and HFGL weanlings were normoglycemic yet glucose intolerant, it is thus important to assess β-cell function in the absence of hyperglycemia. In contrast, HFL weanlings were hyperglycemic and normoinsulinemic (Cerf et al. 2006) and displayed the greatest severity in glucose intolerance. Glucose intolerance may represent an early event in β-cell dysfunction and is exacerbated by hyperglycemia.

High-Fat Programming: Potential Mechanism of Induction of Type 2 Diabetes

Type 2 diabetes is associated with genetic and environmental factors (Fig. 3). Genetic factors include candidate genes (several are currently investigated including *tcf7l2*, *kcnq1*, *kcnj11*, *gck*, *hnf1α*, and *hnf1β*), which result in progeny inheriting the disease from their parents. The maternal influence supersedes the paternal effects on progeny health outcomes. Environmental factors are more broadly defined as they include nutrition (e.g., malnutrition and overnutrition), level of physical activity, developmental programming, oxidative stress, ER stress, cytokines, inflammation, and glucolipotoxicity. High saturated fat diets, sedentary lifestyles, high oxidative stress levels, high ER stress levels, pro-inflammatory cytokines, and inflammation all play roles in the pathogenesis of diabetes via different mechanisms. Glucolipotoxicity, however, appears to be strongly associated with high-fat programming-induced β-cell dysfunction.

Developmental programming by feeding pregnant mothers a HFD is an environmental insult that induces adverse changes in β-cell development and function in

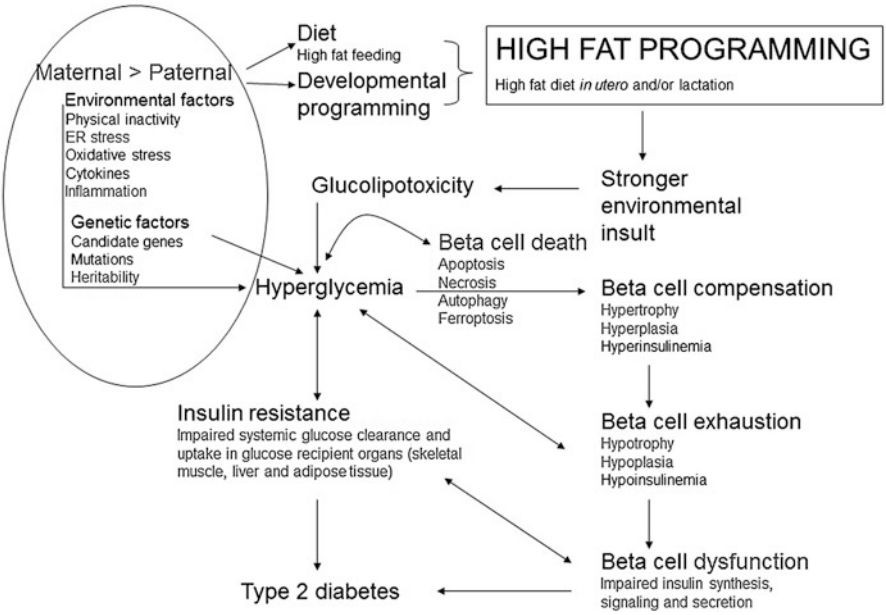

Fig. 3 High-fat programming of type 2 diabetes. Apart from other environmental and genetic factors, high-fat programming can induce hyperglycemia as a stronger environmental insult as the high-fat diet is administered during a critical developmental period. Glucolipotoxicity appears to be the mechanism whereby high-fat programming induces hyperglycemia and subsequent β-cell dysfunction. Hyperglycemia is the clinical hallmark of type 2 diabetes and reciprocally promotes β-cell death (apoptosis, necrosis, autophagy, and potentially ferroptosis). In response to hyperglycemia induced by high-fat programming, β-cells initially compensate through hypertrophy, hyperplasia, and subsequent hypersecretion of insulin. Glucose homeostasis is restored and maintained by these β-cell compensatory mechanisms. However, with prolonged exposure to hyperglycemia, β-cell exhaustion manifests characterized by β-cell hypotrophy, hypoplasia, and subsequent hyposecretion of insulin that may lead to impaired glucose tolerance. β-cell exhaustion reciprocally exacerbates hyperglycemia. Eventually β-cell exhaustion leads to β-cell dysfunction characterized by impaired insulin synthesis, signaling, and secretion that may progress to overt type 2 diabetes. Skeletal muscle, liver, and adipose tissue are the major sites of glucose uptake. Hyperglycemia, which is further exacerbated by hypoinsulinemia, results in systemic and organ-specific insulin resistance due to impaired glucose clearance and uptake, respectively. Insulin resistance reciprocally exacerbates hyperglycemia. Like β-cell dysfunction, insulin resistance precedes overt type 2 diabetes. β-cell dysfunction reciprocally exacerbates insulin resistance. The coexistence of both β-cell dysfunction and insulin resistance presents a severe diabetic state

young progeny. High-fat programming is a more robust environmental insult as high saturated fat intake (independently) and developmental programming (independently) reinforce and potentially synergize their detrimental environmental influence on β-cells.

Glucolipotoxicity proposes that the simultaneous and persistent elevation of circulating glucose and FFA concentrations induces β-cell dysfunction. β-cells exposed to high glucose and FFA concentrations display altered gene expression, function, survival, and growth that slowly diminishes the functional β-cell mass in

type 2 diabetes (Kim and Yoon 2011). These glucolipotoxic effects may result from various β-cell stressors, including oxidative stress, ER stress, cytokine-induced apoptosis (Kim and Yoon 2011), inflammatory macrophage infiltration (Ehses et al. 2007), and islet inflammation (Van Raalte and Diamant 2011).

High-fat programming induces hyperglycemia, and metabolism of the HFD may result in increased circulating FFA concentrations, particularly saturated FA that adversely affect β-cells. High-fat programming may therefore, via glucolipotoxic effects, induce β-cell dysfunction.

Chronic hyperglycemia adversely affects insulin secretion (Poitout and Robertson 2002) and decreases β-cell mass by inducing β-cell death by apoptosis (Pick et al. 1998; Donath et al. 1999), necrosis, autophagy, and potentially ferroptosis. Further, chronic hyperglycemia leads to progressive loss of β-cell mass with a prolonged increase in the rate of β-cell apoptosis without a compensatory increase in β-cell growth (Sone and Kagawa 2005). Hyperglycemia burdens β-cells disrupting their integrity thereby increasing susceptibility to diabetes and metabolic disease (Cerf 2012) whereas maternal hyperglycemia per se increases the probability of adolescent obesity and type 2 diabetes (Portha et al. 2011). Hyperglycemia is central to type 2 diabetes and can be induced by environmental and genetic factors. β-cell death processes reduce β-cell mass thereby further aggravating hyperglycemia. With high-fat programming, progeny are compromised at an early age, as normal β-cell development is impaired and they display reduced β-cell function. β-cell populations (i.e., the β-cell numbers that constitute β-cell mass) are balanced, to a large extent, by β-cell replenishment and death. In the face of hyperglycemia, β-cell compensation by hypertrophy, hyperplasia, and subsequent hyperinsulinemia occurs to restore normoglycemia and temporarily maintain glucose homeostasis. Normal glucose transport into β-cells, normal β-cell function, and the preservation of β-cell insulin signaling (to promote β-cell mass expansion) are required to maintain adaptive hyperinsulinemia during worsening insulin sensitivity (Kiraly et al. 2008). IRS-2 appears to be a positive regulator of β-cell compensation, whereas IRS-1 predominantly regulates insulin secretion (Kulkarni et al. 2012). When β-cell compensation is inadequate for the degree of insulin resistance, progression to diabetes eventually manifests (Weyer et al. 1999; Withers et al. 1998). However, if hyperglycemia recurs and perseveres, β-cell exhaustion characterized by hypotrophy, hypoplasia, and subsequent hypoinsulinemia may ensue, resulting in β-cell dysfunction. A reduction in β-cell mass results from a concomitant downregulation of β-cell proliferation and upregulation of β-cell death (Cai et al. 2012). Hyperglycemia reciprocally exacerbates β-cell death, β-cell exhaustion, and insulin resistance. β-cell dysfunction characterized by impaired insulin synthesis, signaling, and secretion and insulin resistance characterized by impaired systemic glucose clearance and uptake in glucose recipient tissues are key pathological events that precede the pathogenesis of type 2 diabetes. β-cell dysfunction reciprocally promotes insulin resistance. However, β-cell dysfunction is the critical determinant for type 2 diabetes (Ashcroft and Rorsman 2012) and is compounded by insulin resistance. Insulin resistance precedes the pathogenesis for several modern diseases (Samuel and Shulman 2012), whereas β-cell dysfunction

signals an advanced state of diabetes as insufficient insulin is secreted to meet demand. High-fat programming potentially accelerates the onset of overt type 2 diabetes by primarily inducing β-cell dysfunction. Further studies are required to elaborate on this potential mechanism of high-fat programming of β-cell dysfunction and to determine the effects of high-fat programming in the potential induction of insulin resistance in glucose recipient organs.

Perspectives

High-fat programming diminishes β-cell integrity by impairing both β-cell development and function, therefore compromising future progeny health by predisposing them to metabolic disease. There appears to be a link between high-fat programming and glucolipotoxicity. Nutrition during critical developmental periods shapes progeny health. The intrauterine milieu and the lactation period greatly influence the health of the progeny. Dietary intervention to ensure adequate nutrition with the correct macronutrient balance, concomitant with sufficient levels of micronutrients and the optimum ratios of fatty acids, is a strategy to optimize the growth and health of the fetus and neonate. During fetal and early postnatal life, maintenance on an unhealthy diet, such as a high saturated fat diet, is likely to induce adverse changes in progeny physiology and metabolism. Specifically high-fat programming has been demonstrated to adversely affect β-cell function, thus predisposing progeny to β-cell dysfunction. Hyperglycemia reciprocally exacerbates β-cell death, β-cell exhaustion, and insulin resistance, whereas β-cell dysfunction reciprocally exacerbates insulin resistance. The key for future research is to clearly elucidate the mechanisms such as glucolipotoxicity, followed by manipulation and correction of these changes to maintain healthy β-cells that can cope with fluctuating metabolic insulin demand and improve outcomes for β-cell survival. Early intervention by applying programming as a positive window of opportunity to equip organisms and protect against metabolic disease will greatly improve health outcomes and subsequently reduce the burden of metabolic disease (Cerf 2012).

References

Ackermann AM, Gannon M (2007) Molecular regulation of pancreatic β-cell mass development, maintenance, and expansion. J Mol Endocrinol 38:193–206

Anderson JW, Kendall CW, Jenkins DJ (2003) Importance of weight management in type 2 diabetes: review with meta-analysis of clinical studies. J Am Coll Nutr 22:331–339

Ashcroft FM, Rorsman P (2012) Diabetes mellitus and the β cell: the last ten years. Cell 148:1160–1171

Bhargava SK, Sachdev HS, Fall CH, Osmond C, Lakshmy R, Barker DJ, Biswas SK, Ramji S, Prabhakaran D, Reddy KS (2004) Relation of serial changes in childhood body mass index to impaired glucose tolerance in young adulthood. N Engl J Med 350:865–875

Brunner Y, Schvartz D, Priego-Capote F, Coute Y, Sanchez JC (2009) Glucotoxicity and pancreatic proteomics. J Proteomics 71:576–591

Cai EP, Casimir M, Schroer SA, Luk CT, Shi SY, Choi D, Dai XQ, Hajmrle C, Spigelman AF, Zhu D, Gaisano HY, MacDonald PE, Woo M (2012) In vivo role of focal adhesion kinase in regulating pancreatic β-cell mass and function through insulin signaling, actin dynamics, and granule trafficking. Diabetes 61:1708–1718

Cerf ME (2007) High fat diet modulation of glucose sensing in the β-cell. Med Sci Monit 13:RA12–RA17

Cerf ME (2011) Parental high-fat programming of offspring development, health and β-cells. Islets 3:118–120

Cerf ME (2012) Developmental programming, hyperglycemia and metabolic outcomes. Nova Science, Hauppauge, pp 73–84

Cerf ME, Louw J (2008) High fat-induced programming of β-cell development and function in neonatal and weanling offspring. Transworld Research Network, Kerala, pp 133–158

Cerf ME, Louw J (2010) High fat programming induces glucose intolerance in weanling Wistar rats. Horm Metab Res 42:307–310

Cerf ME, Williams K, Nkomo XI, Muller CJ, Du Toit DF, Louw J, Wolfe-Coote SA (2005) Islet cell response in the neonatal rat after exposure to a high-fat diet during pregnancy. Am J Physiol Regul Integr Comp Physiol 288:R1122–R1128

Cerf ME, Muller CJ, Du Toit DF, Louw J, Wolfe-Coote SA (2006) Hyperglycaemia and reduced glucokinase expression in weanling offspring from dams maintained on a high fat diet. Br J Nutr 95:391–396

Cerf ME, Williams K, Chapman CS, Louw J (2007) Compromised β-cell development and β-cell dysfunction in weanling offspring from dams maintained on a high-fat diet during gestation. Pancreas 34:347–353

Cerf ME, Chapman CS, Muller CJ, Louw J (2009) Gestational high-fat programming impairs insulin release and reduces Pdx-1 and glucokinase immunoreactivity in neonatal wistar rats. Metabolism 58:1787–1792

Cerf ME, Chapman CS, Louw J (2012) High-fat programming of hyperglycemia, hyperinsulinemia, insulin resistance, hyperleptinemia, and altered islet architecture in 3-month-old wistar rats. ISRN Endocrinol 2012:627270

Chen C, Hosokawa H, Bumbalo LM, Leahy JL (1994) Regulatory effects of glucose on the catalytic activity and cellular content of glucokinase in the pancreatic β cell. Study using cultured rat islets. J Clin Invest 94:1616–1620

Donath MY, Halban PA (2004) Decreased β-cell mass in diabetes: significance, mechanisms and therapeutic implications. Diabetologia 47:581–589

Donath MY, Gross DJ, Cerasi E, Kaiser N (1999) Hyperglycemia-induced β-cell apoptosis in pancreatic islets of Psammomys obesus during development of diabetes. Diabetes 48:738–744

Dubois M, Vacher P, Roger B, Huyghe D, Vandewalle B, Kerr-Conte J, Pattou F, Moustaid-Moussa N, Lang J (2007) Glucotoxicity inhibits late steps of insulin exocytosis. Endocrinology 148:1605–1614

Ehses JA, Perren A, Eppler E, Ribaux P, Pospisilik JA, Maor-Cahn R, Gueripel X, Ellingsgaard H, Schneider MK, Biollaz G, Fontana A, Reinecke M, Homo-Delarche F, Donath MY (2007) Increased number of islet-associated macrophages in type 2 diabetes. Diabetes 56:2356–2370

Ferreira MR, Lombardo YB, Chicco A (2010) β-cell adaptation/dysfunction in an animal model of dyslipidemia and insulin resistance induced by the chronic administration of a sucrose-rich diet. Islets 2:367–373

Giacca A, Xiao C, Oprescu AI, Carpentier AC, Lewis GF (2011) Lipid-induced pancreatic β-cell dysfunction: focus on in vivo studies. Am J Physiol Endocrinol Metab 300:E255–E262

Gluckman PD, Hanson MA (2004a) The developmental origins of the metabolic syndrome. Trends Endocrinol Metab 15:183–187

Gluckman PD, Hanson MA (2004b) Developmental origins of disease paradigm: a mechanistic and evolutionary perspective. Pediatr Res 56:311–317

Gniuli D, Calcagno A, Caristo ME, Mancuso A, Macchi V, Mingrone G, Vettor R (2008) Effects of high-fat diet exposure during fetal life on type 2 diabetes development in the progeny. J Lipid Res 49:1936–1945

Godfrey KM, Lillycrop KA, Burdge GC, Gluckman PD, Hanson MA (2007) Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease. Pediatr Res 61:5R–10R

Grasemann C, Devlin MJ, Rzeczkowska PA, Herrmann R, Horsthemke B, Hauffa BP, Grynpas M, Alm C, Bouxsein ML, Palmert MR (2012) Parental diabetes: the Akita mouse as a model of the effects of maternal and paternal hyperglycemia in wildtype offspring. PLoS One 7:e50210

Gregorio BM, Souza-Mello V, Mandarim-de-Lacerda CA, Aguila MB (2013) Maternal high-fat diet is associated with altered pancreatic remodelling in mice offspring. Eur J Nutr 52:759–769

Gremlich S, Bonny C, Waeber G, Thorens B (1997) Fatty acids decrease IDX-1 expression in rat pancreatic islets and reduce GLUT2, glucokinase, insulin, and somatostatin levels. J Biol Chem 272:30261–30269

Heywood WE, Mian N, Milla PJ, Lindley KJ (2004) Programming of defective rat pancreatic β-cell function in offspring from mothers fed a low-protein diet during gestation and the suckling periods. Clin Sci (Lond) 107:37–45

Jansson N, Nilsfelt A, Gellerstedt M, Wennergren M, Rossander-Hulthen L, Powell TL, Jansson T (2008) Maternal hormones linking maternal body mass index and dietary intake to birth weight. Am J Clin Nutr 87:1743–1749

Jorns A, Tiedge M, Ziv E, Shafrir E, Lenzen S (2002) Gradual loss of pancreatic β-cell insulin, glucokinase and GLUT2 glucose transporter immunoreactivities during the time course of nutritionally induced type-2 diabetes in Psammomys obesus (sand rat). Virchows Arch 440:63–69

Kasuga M (2006) Insulin resistance and pancreatic β cell failure. J Clin Invest 116:1756–1760

Khaldi MZ, Guiot Y, Gilon P, Henquin JC, Jonas JC (2004) Increased glucose sensitivity of both triggering and amplifying pathways of insulin secretion in rat islets cultured for 1 wk in high glucose. Am J Physiol Endocrinol Metab 287:E207–E217

Kim JW, Yoon KH (2011) Glucolipotoxicity in pancreatic β-cells. Diabetes Metab J 35:444–450

Kim Y, Iwashita S, Tamura T, Tokuyama K, Suzuki M (1995) Effect of high-fat diet on the gene expression of pancreatic GLUT2 and glucokinase in rats. Biochem Biophys Res Commun 208:1092–1098

Kiraly MA, Bates HE, Kaniuk NA, Yue JT, Brumell JH, Matthews SG, Riddell MC, Vranic M (2008) Swim training prevents hyperglycemia in ZDF rats: mechanisms involved in the partial maintenance of β-cell function. Am J Physiol Endocrinol Metab 294:E271–E283

Kulkarni RN, Mizrachi EB, Ocana AG, Stewart AF (2012) Human β-cell proliferation and intracellular signaling: driving in the dark without a road map. Diabetes 61:2205–2213

LeRoith D (2002) β-cell dysfunction and insulin resistance in type 2 diabetes: role of metabolic and genetic abnormalities. Am J Med 113:3S–11S

Li D, Yin X, Zmuda EJ, Wolford CC, Dong X, White MF, Hai T (2008) The repression of IRS2 gene by ATF3, a stress-inducible gene, contributes to pancreatic β-cell apoptosis. Diabetes 57:635–644

Ludvik B, Nolan JJ, Baloga J, Sacks D, Olefsky J (1995) Effect of obesity on insulin resistance in normal subjects and patients with NIDDM. Diabetes 44:1121–1125

Masiello P (2006) Animal models of type 2 diabetes with reduced pancreatic β-cell mass. Int J Biochem Cell Biol 38:873–893

Muoio DM, Newgard CB (2008) Mechanisms of disease: molecular and metabolic mechanisms of insulin resistance and β-cell failure in type 2 diabetes. Nat Rev Mol Cell Biol 9:193–205

Navarro-Tableros V, Fiordelisio T, Hernandez-Cruz A, Hiriart M (2007) Physiological development of insulin secretion, calcium channels and GLUT2 expression of pancreatic rat β-cells. Am J Physiol Endocrinol Metab 292:E1018–E1029

Ng SF, Lin RC, Laybutt DR, Barres R, Owens JA, Morris MJ (2010) Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. Nature 467:963–966
Park JH, Stoffers DA, Nicholls RD, Simmons RA (2008) Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. J Clin Invest 118:2316–2324
Pick A, Clark J, Kubstrup C, Levisetti M, Pugh W, Bonner-Weir S, Polonsky KS (1998) Role of apoptosis in failure of β-cell mass compensation for insulin resistance and β-cell defects in the male Zucker diabetic fatty rat. Diabetes 47:358–364
Poitout V, Robertson RP (2002) Minireview: secondary β-cell failure in type 2 diabetes - a convergence of glucotoxicity and lipotoxicity. Endocrinology 143:339–342
Poitout V, Robertson RP (2008) Glucolipotoxicity: fuel excess and β-cell dysfunction. Endocr Rev 29:351–366
Popkin BM (2001) Nutrition in transition: the changing global nutrition challenge. Asia Pac J Clin Nutr 10:S13–S18
Portha B, Chavey A, Movassat J (2011) Early-life origins of type 2 diabetes: fetal programming of the β-cell mass. Exp Diabetes Res 2011:105076
Prentice AM, Moore SE (2005) Early programming of adult diseases in resource poor countries. Arch Dis Child 90:429–432
Prentki M, Nolan CJ (2006) Islet β cell failure in type 2 diabetes. J Clin Invest 116:1802–1812
Prentki M, Joly E, El-Assaad W, Roduit R (2002) Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in β-cell adaptation and failure in the etiology of diabetes. Diabetes 51: S405–S413
Reimer MK, Ahren B (2002) Altered β-cell distribution of pdx-1 and GLUT-2 after a short-term challenge with a high-fat diet in C57BL/6 J mice. Diabetes 51:S138–S143
Reusens B, Theys N, Dumortier O, Goosse K, Remacle C (2011) Maternal malnutrition programs the endocrine pancreas in progeny. Am J Clin Nutr 94:1824S–1829S
Rhodes CJ (2005) Type 2 diabetes-a matter of β-cell life and death? Science 307:380–384
Ruderman N, Prentki M (2004) AMP kinase and malonyl-CoA: targets for therapy of the metabolic syndrome. Nat Rev Drug Discov 3:340–351
Samuel VT, Shulman GI (2012) Mechanisms for insulin resistance: common threads and missing links. Cell 148:852–871
Siebel AL, Mibus A, De Blasio MJ, Westcott KT, Morris MJ, Prior L, Owens JA, Wlodek ME (2008) Improved lactational nutrition and postnatal growth ameliorates impairment of glucose tolerance by uteroplacental insufficiency in male rat offspring. Endocrinology 149:3067–3076
Skelly RH, Bollheimer LC, Wicksteed BL, Corkey BE, Rhodes CJ (1998) A distinct difference in the metabolic stimulus–response coupling pathways for regulating proinsulin biosynthesis and insulin secretion that lies at the level of a requirement for fatty acyl moieties. Biochem J 331:553–561
Sone H, Kagawa Y (2005) Pancreatic β cell senescence contributes to the pathogenesis of type 2 diabetes in high-fat diet-induced diabetic mice. Diabetologia 48:58–67
Terauchi Y, Sakura H, Yasuda K, Iwamoto K, Takahashi N, Ito K, Kasai H, Suzuki H, Ueda O, Kamada N (1995) Pancreatic β-cell-specific targeted disruption of glucokinase gene. Diabetes mellitus due to defective insulin secretion to glucose. J Biol Chem 270:30253–30256
Terauchi Y, Takamoto I, Kubota N, Matsui J, Suzuki R, Komeda K, Hara A, Toyoda Y, Miwa I, Aizawa S, Tsutsumi S, Tsubamoto Y, Hashimoto S, Eto K, Nakamura A, Noda M, Tobe K, Aburatani H, Nagai R, Kadowaki T (2007) Glucokinase and IRS-2 are required for compensatory β cell hyperplasia in response to high-fat diet-induced insulin resistance. J Clin Invest 117:246–257
Utzschneider KM, Carr DB, Hull RL, Kodama K, Shofer JB, Retzlaff BM, Knopp RH, Kahn SE (2004) Impact of intra-abdominal fat and age on insulin sensitivity and β-cell function. Diabetes 53:2867–2872
Van Raalte DH, Diamant M (2011) Glucolipotoxicity and β cells in type 2 diabetes mellitus: target for durable therapy? Diabetes Res Clin Pract 93:S37–S46

Weyer C, Bogardus C, Mott DM, Pratley RE (1999) The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J Clin Invest 104:787–794

Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, White MF (1998) Disruption of IRS-2 causes type 2 diabetes in mice. Nature 391:900–904

Zambrano E, Bautista CJ, Deas M, Martinez-Samayoa PM, Gonzalez-Zamorano M, Ledesma H, Morales J, Larrea F, Nathanielsz PW (2006) A low maternal protein diet during pregnancy and lactation has sex- and window of exposure-specific effects on offspring growth and food intake, glucose metabolism and serum leptin in the rat. J Physiol 571:221–230

Exercise-Induced Pancreatic Islet Adaptations in Health and Disease

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Contents

Abstract

According, to the World Health Organization (WHO), overweight and obesity represent a rapidly growing threat to worldwide health. Currently, more than 1.4 billion adults are overweight. Although genetic factors account for some cases of obesity, it is evident that a drastic change in lifestyle is a main cause that accounts for the worldwide obesity and type 2 diabetes (T2D) prevalence. Physical exercise prevents or attenuates main obesity outcomes such as fat accumulation, insulin resistance, dyslipidemia, hypertension, and glucose intolerance. Considering the relevance of cells and the benefits of exercise to the onset of T2D,

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_32,

in the present chapter, we review several studies that have evaluated the effects of exercise training on β-cell function and survival in health, obesity, and diabetes. Although the literature still lacks conclusive data in this field, exercise training that enhances β-cell survival is a common outcome in all of the studies. Exercise training-induced alterations on β-cell functions are more controversial. Generally, the studies indicate that in healthy and obese insulin-resistant subjects, exercise decreases nutrient-induced insulin secretion (associated with a correspondent increase in insulin action); however, increased insulin secretion occurs in T2D.

Keywords
Pancreatic β cell • type 1 diabetes • obesity • type 2 diabetes

Introduction

Pancreatic β cells (β-cells) are central elements in the maintenance of glucose homeostasis. To execute this function, β-cells are equipped with specialized transport mechanisms and complex metabolic pathways that couple glucose metabolism with depolarization events, culminating in calcium influx and insulin secretion (Henquin 2009; Maechler and Wollheim 2001; Ashcroft and Rorsman 1989). Several conditions such as overnutrition, malnutrition, and inflammatory processes may alter insulin sensitivity and β-cell functions, leading to impaired glucose-stimulated insulin secretion (GSIS) (Porte 2001; Kahn 2003). In this sense, physical exercise prevents or attenuates the main obesity outcomes, such as fat accumulation in visceral adipose tissue, insulin resistance, dyslipidemia, hypertension, and glucose intolerance (Ruderman et al. 2013; Roberts et al. 2013; Chiasson and Rabasa-Lhoret 2004). Thus, exercise is an important tool to reduce the incidence of type 2 diabetes (T2D) and other metabolic diseases. However, studies investigating the direct effects of exercise on β-cell function and survival are scarce and still controversial. Considering the relevance of β-cells to T2D onset and the benefits from exercise in this matter, in the present chapter, we review several studies that have evaluated the effects of exercise training on β-cell function and survival. In the first section, we review the mechanisms of insulin secretion. We next review studies concerning the actions of exercise in healthy β-cells. Finally, we explore the impact of exercise on β-cell function in obesity and diabetes.

Blood Glucose Signal Transduction and Insulin Secretion Overview

Insulin is the most important hormone for glycemic control because it modulates glucose disposal from peripheral tissues, particularly skeletal muscle, adipose tissue, and liver (Thorens 2010, 2011; Khan et al. 2012). In this context, the tight control of insulin secretion by β-cells is critical to preserve glucose homeostasis.

An important aspect of β-cells function is its ability to adequately adjust insulin secretion in response to variations in plasma nutrients, especially glucose. Generally, the mechanisms by which nutrients acutely regulate insulin secretion from β-cells depend on metabolic pathways and membrane depolarization (Henquin 2009; Ravier et al. 2010; Seino 2012).

Several nutrients, primarily glucose, stimulate insulin secretion. When blood glucose reaches critical values, glucose transporters mediate rapid glucose diffusion, thereby allowing β-cells to establish direct proportionality between blood glucose levels and insulin secretion. Most glucose is metabolized by glycolysis and further aerobically oxidized inside the mitochondria (Malaisse et al. 2004; Fridlyand and Philipson 2010; Doliba et al. 2012); thus, although ATP is also generated by cytosolic reactions, it is mostly derived from mitochondrial glucose-derived pyruvate oxidation (Westerlund and Bergsten 2001). The Krebs cycle in β-cells is also specialized as a distribution system through which a substantial fraction of the carbon skeleton is exported in anaplerotic/cataplerotic reactions that can generate signals to amplify insulin secretion (Henquin 2009; Malaisse et al. 2004; Ortsater et al. 2002; Bertram et al. 2007; Prentki et al. 2013).

Higher glucose metabolism rates increase the ATP/ADP ratio, closing the β-cell ATP-dependent potassium channel (K_{ATP}), resulting in intracellular potassium accumulation and subsequent membrane cell depolarization in an oscillatory pattern. Thus, K_{ATP} channels are essential to transduce increased glucose metabolism into depolarization changes and membrane potential oscillations (Henquin 2009; McTaggart et al. 2010). Membrane depolarization causes voltage-dependent calcium channel opening and calcium influx. The rise in intracellular calcium is the triggering signal for the exocytosis of insulin-containing granules (Seino 2012). Alone, this triggering signal is ineffective, but amplifying its signal via glucose metabolism improves its efficacy. The amplifying pathway glucose influences insulin secretion independent of the K_{ATP} channels (Henquin 2009; Ravier et al. 2010).

Finally, the secretory response to glucose is also modulated by a number of other agents including hormones that are released by the digestive tract such as glucagon-like, peptide 1 (GLP1), and autonomic nervous system (ANS)-derived neurotransmitters (Gilon and Henquin 2001; Rajan et al. 2012). The sympathetic and parasympathetic branches of the autonomic nervous system are involved in blood glucose homeostasis in response to changes in energy demands. Generally, sympathetic neuron-released norepinephrine inhibits insulin secretion, whereas parasympathetic neuron-released acetylcholine potentiates β-cell insulin secretion (Gilon and Henquin 2001). In addition, incretins such as GLP1 enhance β-cell secretory ability and proliferation, thus improving glycemic control (Drucker 2013).

Effects of Exercise in Lean Healthy Rodent Pancreatic Islets

Insulin secretion and action is essential for glucose homeostasis, and defects in either process are involved in several metabolic disorders such as obesity, metabolic syndrome, and diabetes (Porte 2001; Thorens 2011; Khan et al. 2012).

Thus, preserving β-cell mass and function is fundamental to avoid these pathologies. Regular physical activity is widely recognized to improve muscle and liver insulin sensitivity as well as overall glycemic control (Chiasson and Rabasa-Lhoret 2004), features that indirectly help preserve β-cells. However, studies assessing the direct effects of exercise training on healthy β-cell function and survival are scarce and still controversial.

In the 1980s, studies investigating the effect of exercise training on insulin secretion were published. Healthy endurance-trained humans reportedly displayed lower plasma insulin responses to identical glucose stimuli than their untrained counterparts (King et al. 1987; Wirth et al. 1981). Since then, some studies have been conducted using primarily rodent models to provide evidence of pancreatic islet adaptations from a mechanistic point of view in exercise-trained animals.

In this sense, endurance exercise reportedly increased β-cell mass and survival in healthy islets (Choi et al. 2006; Park et al. 2008). Pathophysiological conditions such as obesity, pregnancy, and glucocorticoid-induced insulin resistance increase β-cell mass mainly by hypertrophy (Rafacho et al. 2011; Jacovetti et al. 2012; Ribeiro et al. 2012). Conversely, exercise training reportedly increased β-cells mass by hyperplasia (Choi et al. 2006), most likely by increasing transcription factor PDX-1 expression (Choi et al. 2006; Park et al. 2008). Exercise training was reportedly associated with IRS-2, mTOR, and ERK 1/2 pathway activation in lean trained rat islets (Choi et al. 2006; Park et al. 2008; Calegari et al. 2012). Trained rats also display lower rates of apoptosis, which is another important finding associated with β-cell survival (Choi et al. 2006; Park et al. 2008). In agreement, proapoptotic markers such as cleaved caspase-3 and Bax were decreased, whereas the antiapoptotic marker Bcl-2 was enhanced in healthy trained rat islets.

This effect could also be attributed to improvements in islet redox balance, which demonstrated lower reactive species production and increased catalase content (Calegari et al. 2012). Furthermore, it was proposed that the reportedly increased expression of pyruvate-citrate cycle enzymes in trained rats would improve intracellular redox balance and favor islet survival by producing NADPH at higher rates, which is an important substrate for intracellular redox cycle enzymes such as glutathione reductase and NADPH oxidase (Zoppi et al. 2011).

Thus, a combination of increased proliferation pathways and decrease of apoptosis could improve or make "stronger" β cells in healthy exercised subjects, most likely by increasing the pancreatic β-cells' capacity to bear glucolipotoxicity at any time during life span. However, several other metabolic and signaling pathways that are involved in proliferation and β-cell survival as well as how the gene expression of these proteins is controlled need to be better understood.

Islet function in healthy subjects received more attention. However, the results are still controversial. One of these conflicting issues refers to the total islet insulin content, which was reportedly increased (Calegari et al. 2012; Oliveira et al. 2010) or unaltered (Tsuchiya et al. 2013) in trained rats. In addition, data concerning GSIS alterations in exercise-trained subjects remains a matter of debate. Whereas some studies have reported decreased GSIS, others have demonstrated no effect or increased GSIS in trained rats.

The reduction of GSIS in exercise-trained rats was first attributed to decreased glucose uptake and metabolism in β-cells because of reduced GLUT2 content and lower glucokinase activity (Koranyi et al. 1991; Ueda et al. 2003). However, functional data did not support this hypothesis since no differences in glucose uptake and oxidation between islets from trained and sedentary rats were observed (Oliveira et al. 2010). In addition, other studies reported increased AMPK activation and higher UCP2 expression in islets from trained rats. The increased mitochondrial uncoupling associated with reduced ATP synthesis was hypothesized to be a possible mechanism that could explain the exercise-induced GSIS reduction (Calegari et al. 2011).

The mechanisms by which exercise training would increase GSIS are even less understood. Some studies reported increased static and perfused insulin secretion in exercised rat islets (Oliveira et al. 2010; Tsuchiya et al. 2013; Fluckey et al. 1995). Among the studies demonstrating increased GSIS, few conducted a molecular analysis and reported that the expression of several proteins involved in insulin exocytosis such as the K_{ATP} channel KIR 6.2 subunit, the voltage-sensitive calcium channel Ca_v 2.1 subunit, SNAP25, VAMP2, and syntaxin 1 was not altered (Tsuchiya et al. 2013). It was concluded that exercise might signal membrane depolarization to more downstream proteins, which remain to be identified. Higher levels of anaplerotic enzymes were also positively related with the control of GSIS, and increased pyruvate carboxylase and glutamate dehydrogenase levels were reported after exercise training. However, the same study demonstrated lower insulin secretion in the trained group (Zoppi et al. 2011).

Current knowledge regarding this issue indicates that chronic exercise exerts direct effects upon pancreatic islet function and survival of lean, healthy subjects, apparently improving their capacity to handle stressful conditions such as glucolipotoxicity and inflammatory responses. In addition, the present data indicate a paradox between the molecular response to exercise and β-cell function, which needs to be investigated.

Exercise and β-Cell Function in Obese and Diabetic Humans

Although genetic factors account for some cases of obesity, it is evident that a drastic change in lifestyle is a main cause for the prevalence of worldwide obesity and T2D. Reduced physical activity and abundant energy intake are the two most common factors leading to uncontrolled body weight gain (Maarbjerg et al. 2011). T2D is the most common form of diabetes and is characterized by the progressive loss of peripheral insulin sensitivity in target tissues such as muscle, liver, and adipose tissue culminating with altered glucose homeostasis. During T2D progression, peripheral insulin resistance is compensated by increased β-cell capacity to secrete insulin. However, fasting hyperglycemia occurs when β-cells can no longer sustain high insulin demands (Kahn 2003; Chiasson and Rabasa-Lhoret 2004; Fridlyand and Philipson 2010; Meier and Bonadonna 2013).

It is clear that exercise improves glucose homeostasis by enhancing glucose uptake in obese and diabetic rodents and humans (Maarbjerg et al. 2011;

Kahn et al. 1990; Holloszy 2011; Boule et al. 2001). Regular exercise also creates an anti-inflammatory environment that favors β-cell survival (Nielsen and Pedersen 2007; Brandt and Pedersen 2010). Taken together, regular exercise offers protection for β-cells via insulin sensitization and reduced glucolipotoxicity, inflammation, and oxidative stress. Various alterations in response to exercise have been reported in obese and diabetic patients and in rodent pancreatic islets (Dela et al. 2004; Schneider et al. 1984; Pold et al. 2005; Sennott et al. 2008; Wagener et al. 2012; Leite Nde et al. 2013).

Concerning obese and diabetic humans, the current data suggest that diabetes prevention by exercise training occurs because of a primary effect on peripheral tissue insulin sensitivity rather than on insulin secretion (Chiasson and Rabasa-Lhoret 2004). However, the divergent exercise-elicited responses on β-cell function may also be dependent on the diabetes state and β-cell conditions (Dela et al. 2004; Larson-Meyer et al. 2006; Krotkiewski et al. 1985; Burns et al. 2007). For instance, the effects of 6 weeks of training thrice weekly on glycemic control in 20 sedentary T2D patients and 11 control subjects who had been matched by previous physical activity were analyzed. Oral and intravenous glucose tolerance tests were performed 72 h after the last exercise session demonstrated only minimal improvement (Schneider et al. 1984). Conversely, physical training (1 h/day, 7 days/week, for 6 weeks at 50–60 % maximum oxygen uptake) improved blood glucose control, glucose tolerance, and insulin secretion and action in five T2D patients (Trovati et al. 1984).

The effect of training on insulin secretion in T2D patients has been investigated by measuring plasma insulin concentrations during a glucose tolerance test. Most of these studies found an effect of training on insulin or C-peptide secretion in response to oral or intravenous glucose tolerance tests (Schneider et al. 1984). In contrast, others have reported improved insulin secretion in T2D subjects after exercise training (Krotkiewski et al. 1985; Reitman et al. 1984). Taken together, it may be expected that the effect of physical training on β-cell functions varies greatly among T2D patients.

A possible cause of variations in the response of insulin secretion to exercise training may depend on the capacity of insulin secretion. This issue was addressed, providing evidence that the effects of exercise training on the response of β-cells to secretagogues in T2D patients depend on the previous β-cell secretory capacity. Patients were distributed into groups with either moderate or low β-cell secretory capacity. Training was performed at home in a cycle ergometer (five sessions/week for 12 weeks). The results demonstrated that exercise training enhances β-cell function in subjects displaying moderate insulin secretory capacity. In contrast, in T2D patients with low insulin secretory capacity, exercise training did not modify β-cell function (Dela et al. 2004).

Young T2D obese subjects were submitted to an exercise program (1 h/day, four times/week for 12 weeks) and did not demonstrate any outcome compared with control subjects who were matched for age and obesity levels after aerobic exercise intervention (Burns et al. 2007). In a randomized study to test the effects of changing lifestyle on glucose metabolism (insulin sensitivity, β-cell function, and glucose tolerance), Japanese Americans with impaired glucose tolerance were fed

an isocaloric diet containing low saturated fat and submitted to a 24-month stretching exercise program (1 h/three times a week) or endurance training (1 h of walking or jogging on a treadmill three times a week) at approximately 70 % of the individual's heart rate reserve. This study demonstrated that the above lifestyle modifications (reduced energy intake and endurance exercise) resulted in significant weight loss, reduced visceral and subcutaneous fat depots, and increased insulin sensitivity. However, endurance exercise program did not improve β-cell functions as evaluated by the disposition index, which was calculated by the insulin sensitivity index x acute insulin response to glucose (Carr et al. 2005).

The impact of exercise on β-cell functions was also investigated, taking obesity and aging into account. An obese older group took part in weekly behavioral therapy meetings and was submitted to a supervised exercise training program (90 min sessions 3 days/week). The exercise program focused on improving flexibility, endurance, strength, and balance. β-Cell functions were estimated by measuring plasma glucose and C-peptide concentrations during an oral glucose tolerance test (OGTT). Glucose and insulin area under curve (AUC) decreased significantly after the treatment (diet plus exercise). Although insulin secretion did not change significantly, the plasma insulin clearance rate was increased. A higher insulin sensitivity index was also reported. However, both static and dynamic glucose stimulation-induced β-cell insulin secretion indices did not change. Therefore, this study demonstrated that weight loss therapy improves β-cell function in obese older adults without altering the absolute rate of insulin secretion. As suggested by the authors, although the mechanism responsible for the observed improvement is not clear, it might involve metabolic processes that reduce β-cell glucolipotoxicity (Villareal et al. 2008).

The association of diet and exercise may also be critical in determining whether glucose tolerance and β-cell functions will improve. For example, short-term studies that achieved weight loss by caloric restriction or bariatric surgery demonstrated improved β-cell function, whereas exercise training without weight loss did not (Kahn et al. 1990; Guldstrand et al. 2003; Utzschneider et al. 2004). Healthy overweight males (25–50 years) and females (25–45 years) were recruited for the Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy (CALERIE) trial and were randomly assigned into groups for evaluating the effects of caloric restriction and exercise together or separately. The caloric restriction plus exercise group participants increased their energy expenditure by 12.5 % above resting by undergoing structured exercise (i.e., walking, running, or stationary cycling) 5 days per week. After the 6-month intervention, there was a significant improvement in the insulin sensitivity index. Similarly, the acute insulin response to glucose was significantly decreased from baseline in each of the groups. These authors demonstrated that calorie restriction by diet alone or in conjunction with exercise similarly improved insulin sensitivity and reduced β-cell sensitivity to glucose in overweight, glucose-tolerant subjects (Larson-Meyer et al. 2006).

Another central aspect involving exercise training and β-cell function in obese subjects is the exercise profile. The Studies of a Targeted Risk Reduction Intervention through Defined Exercise (STRRIDE) study was a large, randomized, controlled

clinical trial that investigated the effects of different amounts and intensities of exercise training on numerous cardiometabolic risk factors. The subjects were aged 40–65 years, sedentary, overweight or mildly obese (BMI 25–35 kg/m^2), and moderately dyslipidemic. All of the subjects were randomly assigned to one of three training groups or to a sedentary control group. The exercise groups were (1) high amount/vigorous intensity, (2) low amount/vigorous intensity, and (3) low amount/moderate intensity. In middle-aged, overweight/obese, and moderately dyslipidemic individuals, 8 months of a moderate-intensity exercise program (40–55 % VO2 peak; 1,220 kcal/week) reportedly improved β-cell function three times more than vigorous-intensity exercise (65–80 % VO2 peak; 1,230–2,020 kcal/week). It was concluded that moderate-intensity exercise improves β-cells' function to a better extent than vigorous-intensity exercise (Slentz et al. 2011).

Taken together, these data indicate that in obese and/or T2D patients, exercise training may enhance β-cell function. However, the exercise-induced benefits on β-cells depend on the exercise intensity and the remaining β-cell secretory capacity.

Type 1 diabetes mellitus (T1D) is also a chronically progressive disease, which, in contrast to T2D, is triggered by autoimmune and inflammatory processes that are directed specifically to β-cells. These events cause a loss of β-cell mass and function (Krause Mda and De Bittencourt 2008). Several studies have demonstrated that increased physical activity reduces insulin needs, thus allowing for better glycemic control in T1D subjects (Salem et al. 2010; Chimen et al. 2012). Similarly, an improvement in insulin secretion could be an important mechanism by which exercise could directly affect blood glucose regulation in these patients. However, specific studies investigating the relationship between exercise and β-cell function in T1D subjects are scarce. To date, there is some evidence demonstrating that physical exercise exerts a protective role against the autoimmune process that is directed to β-cells, promoting anti-inflammatory cytokines upregulation (Krause Mda and De Bittencourt 2008). However, further studies are necessary to clarify whether the anti-inflammatory actions of exercise training on β-cells could increase their functions and postpone T1D onset.

Exercise and β-Cell Function in Obese and Diabetic Rodents

Considering the difficulties in obtaining human samples, rodent models of obesity, insulin resistance, and diabetes are important tools to evaluate the impact of exercise on functional, morphological, and molecular β-cell alterations. Here, we review studies that have reported the effects of exercise on pancreatic islets using several obesity and/or diabetes rat and mouse models.

Streptozotocin-Induced Diabetic Rats and Exercise

In the 1980s and 1990s, several studies were conducted to evaluate the relationship between exercise, insulin sensitivity, and insulin secretion in streptozotocin

(STZ)-induced diabetic rats. In healthy rats, exercise training induced a sharp decrease in the basal insulin levels without any significant changes in the glucose levels, whereas the basal glucose level was higher in STZ group, compared to healthy group, with a significant decrease after exercise in the diabetic rats, whereas the basal insulin values were similar in healthy and diabetic rats after training. The improvement in the diabetic trained rat glucose tolerance was further confirmed by the significant increase in the glucose disappearance rate constant (Tancrede et al. 1982). The effect of physical training and detraining on diabetic rat glucose homeostasis was also investigated. Intravenous glucose tolerance tests that were performed 64 h (trained rats) or 12 days (detrained rats) after the exercise training program demonstrated that basal glucose levels were significantly lower in the trained but not after the detraining period. Similar differences in the plasma glucose levels were observed after glucose loading, though the glucose disappearance rate constant was not significantly improved by training. The basal insulin levels were significantly higher in trained than in sedentary diabetic rats, but this alteration disappeared in detrained rats. It was suggested that the training-induced improvement in diabetic rat glucose homeostasis was a transient phenomenon, which is associated with increased circulating insulin levels (Rousseau-Migneron et al. 1988).

However, when diabetic rats started a heavy running (1 h/day, 5 days/week at a speed of 18 m/min, for 12 weeks) exercise program 1 week after STZ treatment, insulin, glucagon, somatostatin, and pancreatic islet cell polypeptide labeling was not altered by exercise in either the diabetic or the control healthy rats (Howarth et al. 2009). In contrast, diabetic Wistar rats that were submitted to swimming training prior to STZ administration displayed significantly decreased markers of oxidative stress such as malonaldehyde and nitric oxide and increased antioxidant enzyme activity. Exercise training also moderately increased insulin antigen positivity in β-cells. This effect was more evident in diabetic rats that were submitted to moderate-intensity exercise training (Coskun et al. 2004).

Another study investigated the effects of exercise training on islet morphology, density, size, cell composition, and insulin secretion and content 3 days after the second STZ injection. Compared with the sedentary diabetic group, the exercised diabetic mice displayed significantly lower glucose levels during the first 2 weeks of exercise. However, the difference was not statistically significant at later time points. Cellular atrophy and extensive vacuoles were present in 80 % of the islets from sedentary and exercised diabetic mice. Diabetes negatively affected the islet number, and exercise did not block this outcome. In contrast, exercise increased the insulin content by more than threefold. Interestingly, in the exercised group, insulin-labeling intensity was increased. Under low glucose conditions (3 mM), GSIS in the exercised diabetic group was significantly higher than that in the sedentary diabetic group. However, there was no significant difference between the exercised and sedentary diabetic groups under high glucose conditions (16 mM). Morphological analysis of islets from trained and sedentary diabetic groups did not reveal any differences. Voluntary exercise did not improve the proportion of insulin-producing β cells in the islets of diabetic animals; however, it improved the insulin content in isolated islets (Huang et al. 2011).

Taken together, exercise may have a protective effect if initiated prior to the onset of the disease; however, exercise introduced after β-cell failure was ineffective.

Pancreatectomized Diabetic Rats and Exercise

Several studies evaluating the effects of exercise on β-cell functions were performed using a diabetes model induced by partial pancreatectomy. Pancreatectomized rats display T2D characteristics associated with insulin deficiency and insulin resistance. Using this diabetic model, Farrell and colleagues investigated the effects of exercise training on GSIS during hyperglycemic clamps. During the hyperglycemic clamps, exercise training improved GSIS in mildly and moderately pancreatectomized diabetic rats but did not alter insulin secretion in more severely diabetic rats (Farrell et al. 1991).

Male Sprague-Dawley rats that had 90 % of their pancreas removed were submitted to exercise (20 m/min for 30 min treadmill run four times a week), which was associated or not with daily oral high- or low-dose dexamethasone administration for 8 weeks. Hyperglycemic clamps were performed, and pancreatic islets were isolated to determine the β-cell function and morphology. β-Cell function and mass were increased in both dexamethasone-treated and exercise-trained pancreatectomized and sham rats. Exercise restored β-cell function not only by reducing insulin resistance but also by increasing the β-cell number. Exercise also induced higher IRS2 expression in islets, leading to an enhanced insulin/IGF-I signaling cascade, which possibly improved β-cell function and mass expansion (Choi et al. 2006).

Another study investigated a mechanism to promote insulinotropic actions by supplying exendin-4 and/or exercise training in 90 % pancreatectomized rats that were fed a 40 % fat diet. Exercised groups ran on an uphill treadmill with a 15° slope at 20 m/min for 30 min, 5 days a week, for 8 weeks. Long-term exendin-4 administration and/or training increased the first phase of insulin secretion, whereas the second phase of insulin secretion was not altered in pancreatectomized diabetic rats. The expression of glucokinase in islets and the percentage of β-cell area, which is related to the pancreas area, were significantly increased in both the exendin-4 and exercise groups. Exendin-4 and exercise decreased the individual cell size compared with the controls. Both treatments stimulated β-cell proliferation, which was associated with reduced apoptosis. Exendin-4 treatment and exercise also improved β-cell function and mass through a common pathway involving IRS2-PI3K-Akt activation, resulting in higher PDX1 expression. Finally, long-term exendin-4 treatment and exercise training also improved GSIS and β-cell survival via a cAMP-dependent pathway (Park et al. 2008).

Similar studies were performed with pancreatectomized rats that received a high-fat diet (HFD) and were submitted to exercise. Exercised and sedentary HFD-fed rats ran uphill (treadmill at 20 m/min for 30 min, 5 days/week). Exercise reportedly improved the first phase of insulin secretion at stimulatory glucose concentrations, but it did not alter second phase of insulin secretion. Diabetic trained rats suppressed basal insulin secretion at 5 mM and the second phase of

insulin secretion response at 19.4 mM glucose in pancreatectomized rats that were fed HFD. Exercise training also increased total pancreatic insulin content and the percentage of β-cell area in both diets. It was also reported IRS2-PI3K-AKT pathway activation and PDX1 expression. Finally, this study demonstrated higher glucokinase and GLUT2 expression in the diabetic group submitted to exercise training (Park et al. 2007).

Zucker (fa/fa) Rats and Exercise

Zucker diabetic fatty (ZDF) rats are a genetic model of insulin resistance that display β-cell failure and obesity-related T2D (Tokuyama et al. 1995; Chentouf et al. 2011). This model is excellent for evaluating the benefits of exercise training in obesity-related β-cell dysfunction. In a study investigating the effects of exercise or AICAR (0.5 mg/kg) administration subcutaneously on β-cells, AICAR administration and exercise training reportedly increased peripheral insulin action. However, β-cell mass in the exercised rats was higher than in the AICAR-treated rats (Pold et al. 2005). Six-week-old HFD-fed Zucker rats were exercised daily by swimming for 4 weeks; β-cell function was investigated by measuring GSIS, glucose phosphorylation, and free fatty acid oxidation in cultured islets. Neither exercise nor HFD alone affected β-cell function; however, exercise plus HFD reduced glucokinase activity and increased the islet cell response to the inhibitory action of mannoheptulose (Kibenge and Chan 2002).

Oligonucleotide microarray gene chip technology was employed to investigate the effects of running exercise (20 m/min, 1 h/day, 6 days a week for 5 weeks) in ZDF diabetic male rats. As expected, reduced glycemia, plasma free fatty acid, insulin, and glucagon levels were reported. However, exercise training did not markedly influence pancreatic islet gene expression (Colombo et al. 2005). A swimming exercise program (once a day for 1 h, 5 days/week for 6 weeks) also helped maintain euglycemia, which attenuated the loss of β-cell function, as judged by the increased proliferation rates and mass and reduced protein ubiquitin pathway activity in β-cells (Kiraly et al. 2007; Kiraly et al. 2008).

Volitional wheel running also contributed to pancreatic β-cell protection in this model (Shima et al. 1997). Voluntary running in the ZDF rats increased the plasma insulin response compared with ZDF sedentary rats and improved glucose tolerance. Improved pancreatic islet β-cell functions were also observed. Partial but significant insulin store preservation was registered in ZDF exercised rats. Islets from physically active ZDF rats demonstrated enhanced glucose- and fatty acid-potentiated insulin secretion. Although voluntary exercise did not reverse hyperphagia and obesity, it prevented hyperglycemia in ZDF rats. Pancreatic islet hypertrophy with increased non-endocrine cells, fibrosis, and reduced insulin immunostaining were also frequently observed in ZDF obese rat pancreas, but they were reversed in islets from ZDF exercised rats. However, exercise did not restore fatty acid oxidation and lipid metabolism in islets from ZDF rats (Delghingaro-Augusto et al. 2012).

MSG-Obesity Model and Exercise

Several observations led to the identification of the hypothalamic arcuate nucleus (ARC) as a major integrative site for energy homeostasis inputs (Luquet et al. 2005; Vianna and Coppari 2011). High glutamate monosodium (MSG) doses administered during the neonatal phase induce ARC neuronal damage in rodents (Olney 1969; Dawson et al. 1997). After these neuronal lesions, an ensemble of neuronal and hormonal abnormalities occurs, which cause the development of obesity. MSG-fed obese animals demonstrate hyperinsulinemia, glucose intolerance, insulin resistance, dyslipidemia, and cardiovascular alterations (Balbo et al. 2007; Grassiolli et al. 2006; Macho et al. 2000). Thus, this hypothalamic obesity model reproduces all of the characteristics present in obese humans with metabolic syndrome. Considering that pancreatic islets from MSG-obese rodents display altered GSIS (Grassiolli et al. 2006; Balbo et al. 2002), MSG-obese rodents have also been used to investigate the effects of exercise on β-cell functions. Thus, MSG-obese male rats were submitted to swimming training (1 h/day, 5 days/week with a 5 % body weight overload for 10 weeks). Pancreatic islets from MSG rats displayed higher insulin secretion in response to low (2.8 mM) and moderate (8.3 mM) glucose concentrations compared with their controls. Exercise training counteracted the hypersecretion that was observed in MSG rats without disrupting glycemic control (de Souza et al. 2003).

Similar results were obtained in another study with MSG-obese mice that were also submitted to swimming training. MSG-obese mice swam over a period of 8 weeks for 15 min a day, 3 days a week bearing a load corresponding to 2.5 % of their body weight attached to the tail. Similar to what was observed for low and moderate GSIS concentrations, in the presence of high glucose concentrations (16.7 mM), islets from exercised MSG-obese mice secreted 1.83 times more insulin than islets from sedentary mice, and exercise training reverted this outcome (Andreazzi et al. 2009). In addition to MSG rats, exercise training reportedly also had effects in MSG-treated Swiss mice. Pancreatic islets from MSG-obese mice demonstrated impaired insulin signaling, which was restored by exercise (Miranda et al. 2013).

MSG rats that were submitted to a swimming training program (1 h/day, three times/week for 10 weeks) demonstrated reduced GSIS and islet hypertrophy that was associated with GLUT2 expression and mitochondrial complex III function reestablishment (Leite Nde et al. 2013). Using an identical swim training protocol, the exercise-induced reduction in GSIS was also reportedly associated with the insulinotropic actions of GLP1 in islets from MSG-obese rats (Svidnicki et al. 2013).

Other Obesity Models and Exercise

Although leptin-deficient *ob*/*ob* mice are frequently used to study obesity and diabetes, few studies investigated the effects of exercise on β-cell function in these genetic obese models. Dubuc et al. (1984) analyzed the levels of several hormones as well as the body composition of C57BL/6 J *ob*/*ob* mice following

25 days of limited caloric intake, voluntary exercise, or combined treatment. When diet was combined with exercise, fasting glycemia and glucagonemia were reduced to values that were similar to lean mice, but the plasma insulin and corticosterone levels remained elevated.

Another study submitted *ob*/*ob* obese mice to forced treadmill exercise training, and despite a similar degree of chronic exercise, reported in the previous study, any response, after 12 weeks of exercise training, in mitochondrial biogenesis or insulin sensitivity indicators in the *ob*/*ob* mice was reported (Li et al. 2011).

The available data indicate that leptin-deficient mice are resistant to exercise training benefits. However, few studies have evaluated the direct effects of exercise on insulin secretion in *ob*/*ob* mouse islets. Because leptin is an important regulator of insulin secretion, further studies are necessary to clarify the impact of exercise in pancreatic islets from this obese model.

Concluding Remarks

Despite controversies, the data from the literature strongly suggest that exercise training has beneficial effects on pancreatic islets. Most studies reported that exercise training enhanced β-cell proliferation and reduced apoptosis in healthy, obese or diabetic subjects leading to increased β-cell mass. Conversely, the effect of exercise on β-cell function depends on its secretory capacity. In healthy and obese insulin-resistant subjects, exercise decreases nutrient-responsive insulin secretion (associated with a correspondent increased insulin action), whereas β-cell function increases in T2D subjects. However, further studies are needed to better understand the mechanisms by which exercise training signals the islet β-cells to cope with the necessary adaptations.

References

Andreazzi AE, Scomparin DX, Mesquita FP, Balbo SL, Gravena C, De Oliveira JC, Rinaldi W, Garcia RM, Grassiolli S, Mathias PC (2009) Swimming exercise at weaning improves glycemic control and inhibits the onset of monosodium L-glutamate-obesity in mice. Eur J Endocrinol 201:351–359

Ashcroft FM, Rorsman P (1989) Electrophysiology of the pancreatic β-cell. Prog Biophys Mol Biol 54:87–143

Balbo SL, Bonfleur ML, Carneiro EM, Amaral ME, Filiputti E, Mathias PC (2002) Parasympathetic activity changes insulin response to glucose and neurotransmitters. Diabetes Metab 28:3S13–3S17 (discussion 13S108-112)

Balbo SL, Grassiolli S, Ribeiro RA, Bonfleur ML, Gravena C, Brito MN, Andreazzi AE, Mathias PC, Torrezan R (2007) Fat storage is partially dependent on vagal activity and insulin secretion of hypothalamic obese rat. Endocrine 31:142–148

Bertram R, Satin LS, Pedersen MG, Luciani DS, Sherman A (2007) Interaction of glycolysis and mitochondrial respiration in metabolic oscillations of pancreatic islets. Biophys J 92:1544–1555

Boule NG, Haddad E, Kenny GP, Wells GA, Sigal RJ (2001) Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. J Am Med Assoc 286:1218–1227

Brandt C, Pedersen BK (2010) The role of exercise-induced myokines in muscle homeostasis and the defense against chronic diseases. J Biomed Biotechnol 2010:520258

Burns N, Finucane FM, Hatunic M, Gilman M, Murphy M, Gasparro D, Mari A, Gastaldelli A, Nolan JJ (2007) Early-onset type 2 diabetes in obese white subjects is characterised by a marked defect in β cell insulin secretion, severe insulin resistance and a lack of response to aerobic exercise training. Diabetologia 50:1500–1508

Calegari VC, Zoppi CC, Rezende LF, Silveira LR, Carneiro EM, Boschero AC (2011) Endurance training activates AMP-activated protein kinase, increases expression of uncoupling protein 2 and reduces insulin secretion from rat pancreatic islets. J Endocrinol 208:257–264

Calegari VC, Abrantes JL, Silveira LR, Paula FM, Costa JM Jr, Rafacho A, Velloso LA, Carneiro EM, Bosqueiro JR, Boschero AC, Zoppi CC (2012) Endurance training stimulates growth and survival pathways and the redox balance in rat pancreatic islets. J Appl Physiol 112:711–718

Carr DB, Utzschneider KM, Boyko EJ, Asberry PJ, Hull RL, Kodama K, Callahan HS, Matthys CC, Leonetti DL, Schwartz RS, Kahn SE, Fujimoto WY (2005) A reduced-fat diet and aerobic exercise in Japanese Americans with impaired glucose tolerance decreases intra-abdominal fat and improves insulin sensitivity but not β-cell function. Diabetes 54:340–347

Chentouf M, Dubois G, Jahannaut C, Castex F, Lajoix AD, Gross R, Peraldi-Roux S (2011) Excessive food intake, obesity and inflammation process in Zucker fa/fa rat pancreatic islets. PLoS One 6:e22954

Chiasson JL, Rabasa-Lhoret R (2004) Prevention of type 2 diabetes: insulin resistance and β-cell function. Diabetes 53(Suppl 3):S34–S38

Chimen M, Kennedy A, Nirantharakumar K, Pang TT, Andrews R, Narendran P (2012) What are the health benefits of physical activity in type 1 diabetes mellitus? A literature review. Diabetologia 55:542–551

Choi SB, Jang JS, Hong SM, Jun DW, Park S (2006) Exercise and dexamethasone oppositely modulate β-cell function and survival via independent pathways in 90 % pancreatectomized rats. J Endocrinol 190:471–482

Colombo M, Gregersen S, Kruhoeffer M, Agger A, Xiao J, Jeppesen PB, Orntoft T, Ploug T, Galbo H, Hermansen K (2005) Prevention of hyperglycemia in Zucker diabetic fatty rats by exercise training: effects on gene expression in insulin-sensitive tissues determined by high-density oligonucleotide microarray analysis. Metab Clin Exp 54:1571–1581

Coskun O, Ocakci A, Bayraktaroglu T, Kanter M (2004) Exercise training prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat pancreas. Tohoku J Exp Med 203:145–154

Dawson R, Pelleymounter MA, Millard WJ, Liu S, Eppler B (1997) Attenuation of leptin-mediated effects by monosodium glutamate-induced arcuate nucleus damage. Am J Physiol 273:E202–E206

de Souza CT, Nunes WM, Gobatto CA, de Mello MA (2003) Insulin secretion in monosodium glutamate (MSG) obese rats submitted to aerobic exercise training. Physiol Chem Phys Med NMR 35:43–53

Dela F, von Linstow ME, Mikines KJ, Galbo H (2004) Physical training may enhance β-cell function in type 2 diabetes. Am J Physiol Endocrinol Metab 287:E1024–E1031

Delghingaro-Augusto V, Decary S, Peyot ML, Latour MG, Lamontagne J, Paradis-Isler N, Lacharite-Lemieux M, Akakpo H, Birot O, Nolan CJ, Prentki M, Bergeron R (2012) Voluntary running exercise prevents β-cell failure in susceptible islets of the Zucker diabetic fatty rat. Am J Physiol Endocrinol Metab 302:E254–E264

Doliba NM, Qin W, Najafi H, Liu C, Buettger CW, Sotiris J, Collins HW, Li C, Stanley CA, Wilson DF, Grimsby J, Sarabu R, Naji A, Matschinsky FM (2012) Glucokinase activation repairs defective bioenergetics of islets of Langerhans isolated from type 2 diabetics. Am J Physiol Endocrinol Metab 302:E87–E102

Drucker DJ (2013) Incretin action in the pancreas: potential promise, possible perils, and pathological pitfalls. Diabetes 62(10):3316–3323

Dubuc PU, Cahn PJ, Willis P (1984) The effects of exercise and food restriction on obesity and diabetes in young *ob*/*ob* mice. Int J Obes 8:271–278

Farrell PA, Caston AL, Rodd D (1991) Changes in insulin response to glucose after exercise training in partially pancreatectomized rats. J Appl Physiol 70:1563–1568

Fluckey JD, Kraemer WJ, Farrell PA (1995) Pancreatic-islet insulin-secretion is increased after resistance exercise in rats. J Appl Physiol 79:1100–1105

Fridlyand LE, Philipson LH (2010) Glucose sensing in the pancreatic β cell: a computational systems analysis. Theor Biol Med Model 7:15

Gilon P, Henquin JC (2001) Mechanisms and physiological significance of the cholinergic control of pancreatic β-cell function. Endocr Rev 22:565–604

Grassiolli S, Bonfleur ML, Scomparin DX, de Freitas Mathias PC (2006) Pancreatic islets from hypothalamic obese rats maintain K_{ATP} channel-dependent but not -independent pathways on glucose-induced insulin release process. Endocrine 30:191–196

Guldstrand M, Ahren B, Adamson U (2003) Improved β-cell function after standardized weight reduction in severely obese subjects. Am J Physiol Endocrinol Metab 284:E557–E565

Henquin JC (2009) Regulation of insulin secretion: a matter of phase control and amplitude modulation. Diabetologia 52:739–751

Holloszy JO (2011) Regulation of mitochondrial biogenesis and GLUT4 expression by exercise. Compr Physiol 1:921–940

Howarth FC, Marzouqi FM, Al Saeedi AM, Hameed RS, Adeghate E (2009) The effect of a heavy exercise program on the distribution of pancreatic hormones in the streptozotocin-induced diabetic rat. J Pancreas 10:485–491

Huang HH, Farmer K, Windscheffel J, Yost K, Power M, Wright DE, Stehno-Bittel L (2011) Exercise increases insulin content and basal secretion in pancreatic islets in type 1 diabetic mice. Exp Diabetes Res 2011:481427

Jacovetti C, Abderrahmani A, Parnaud G, Jonas JC, Peyot ML, Cornu M, Laybutt R, Meugnier E, Rome S, Thorens B, Prentki M, Bosco D, Regazzi R (2012) MicroRNAs contribute to compensatory β cell expansion during pregnancy and obesity. J Clin Invest 122:3541–3551

Kahn SE (2003) The relative contributions of insulin resistance and β-cell dysfunction to the pathophysiology of type 2 diabetes. Diabetologia 46:3–19

Kahn SE, Larson VG, Beard JC, Cain KC, Fellingham GW, Schwartz RS, Veith RC, Stratton JR, Cerqueira MD, Abrass IB (1990) Effect of exercise on insulin action, glucose tolerance, and insulin secretion in aging. Am J Physiol 258:E937–E943

Khan A, Raza S, Khan Y, Aksoy T, Khan M, Weinberger Y, Goldman J (2012) Current updates in the medical management of obesity. Recent Patents Endocr Metab Immune Drug Discov 6:117–128

Kibenge MT, Chan CB (2002) The effects of high-fat diet on exercise-induced changes in metabolic parameters in Zucker fa/fa rats. Metab Clin Exp 51:708–715

King DS, Dalsky GP, Staten MA, Clutter WE, Van Houten DR, Holloszy JO (1987) Insulin action and secretion in endurance-trained and untrained humans. J Appl Physiol 63:2247–2252

Kiraly MA, Bates HE, Yue JT, Goche-Montes D, Fediuc S, Park E, Matthews SG, Vranic M, Riddell MC (2007) Attenuation of type 2 diabetes mellitus in the male Zucker diabetic fatty rat: the effects of stress and non-volitional exercise. Metab Clin Exp 56:732–744

Kiraly MA, Bates HE, Kaniuk NA, Yue JT, Brumell JH, Matthews SG, Riddell MC, Vranic M (2008) Swim training prevents hyperglycemia in ZDF rats: mechanisms involved in the partial maintenance of β-cell function. Am J Physiol Endocrinol Metab 294:E271–E283

Koranyi LI, Bourey RE, Slentz CA, Holloszy JO, Permutt MA (1991) Coordinate reduction of rat pancreatic islet glucokinase and proinsulin mRNA by exercise training. Diabetes 40:401–404

Krause Mda S, De Bittencourt PI Jr (2008) Type 1 diabetes: can exercise impair the autoimmune event? The L-arginine/glutamine coupling hypothesis. Cell Biochem Funct 26:406–433

Krotkiewski M, Lonnroth P, Mandroukas K, Wroblewski Z, Rebuffescrive M, Holm G, Smith U, Bjorntorp P (1985) The effects of physical-training on insulin-secretion and effectiveness and on glucose-metabolism in obesity and type-2 (non-insulin-dependent) diabetes-mellitus. Diabetologia 28:881–890

Larson-Meyer DE, Heilbronn LK, Redman LM, Newcomer BR, Frisard MI, Anton S, Smith SR, Alfonso A, Ravussin E (2006) Effect of calorie restriction with or without exercise on insulin sensitivity, β-cell function, fat cell size, and ectopic lipid in overweight subjects. Diabetes Care 29:1337–1344

Leite Nde C, Ferreira TR, Rickli S, Borck PC, Mathias PC, Emilio HR, Grassiolli S (2013) Glycolytic and mitochondrial metabolism in pancreatic islets from MSG-treated obese rats subjected to swimming training. Cell Physiol Biochem: Int J Exp Cell Physiol Biochem Pharmacol 31:242–256

Li L, Pan R, Li R, Niemann B, Aurich AC, Chen Y, Rohrbach S (2011) Mitochondrial biogenesis and peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) deacetylation by physical activity: intact adipocytokine signaling is required. Diabetes 60:157–167

Luquet S, Perez FA, Hnasko TS, Palmiter RD (2005) NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. Science 310:683–685

Maarbjerg SJ, Sylow L, Richter EA (2011) Current understanding of increased insulin sensitivity after exercise – emerging candidates. Acta Physiol (Oxf) 202:323–335

Macho L, Fickova M, Jezova ZS (2000) Late effects of postnatal administration of monosodium glutamate on insulin action in adult rats. Physiol Res/Acad Sci Bohemoslovaca 49(Suppl 1): S79–S85

Maechler P, Wollheim CB (2001) Mitochondrial function in normal and diabetic β-cells. Nature 414:807–812

Malaisse WJ, Zhang Y, Jijakli H, Courtois P, Sener A (2004) Enzyme-to-enzyme channelling in the early steps of glycolysis in rat pancreatic islets. Int J Biochem Cell Biol 36:1510–1520

McTaggart JS, Clark RH, Ashcroft FM (2010) The role of the K_{ATP} channel in glucose homeostasis in health and disease: more than meets the islet. J Physiol 588:3201–3209

Meier JJ, Bonadonna RC (2013) Role of reduced β-cell mass versus impaired β-cell function in the pathogenesis of type 2 diabetes. Diabetes Care 36(Suppl 2):S113–S119

Miranda RA, Branco RC, Gravena C, Barella LF, da Silva Franco CC, Andreazzi AE, de Oliveira JC, Picinato MC, de Freitas Mathias PC (2013) Swim training of monosodium L-glutamate-obese mice improves the impaired insulin receptor tyrosine phosphorylation in pancreatic islets. Endocrine 43:571–578

Nielsen AR, Pedersen BK (2007) The biological roles of exercise-induced cytokines: IL-6, IL-8, and IL-15. Appl Physiol Nutr Meta 32:833–839

Oliveira CA, Paiva MF, Mota CA, Ribeiro C, Leme JA, Luciano E, Mello MA (2010) Exercise at anaerobic threshold intensity and insulin secretion by isolated pancreatic islets of rats. Islets 2:240–246

Olney JW (1969) Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science 164:719–721

Ortsater H, Liss P, Akerman KE, Bergsten P (2002) Contribution of glycolytic and mitochondrial pathways in glucose-induced changes in islet respiration and insulin secretion. Pflug Arch Eur J Physiol 444:506–512

Park SM, Hong SM, Lee JE, Sung SR (2007) Exercise improves glucose homeostasis that has been impaired by a high-fat diet by potentiating pancreatic β-cell function and mass through IRS2 in diabetic rats. J Appl Physiol 103:1764–1771

Park S, Hong SM, Sung SR (2008) Exendin-4 and exercise promotes β-cell function and mass through IRS2 induction in islets of diabetic rats. Life Sci 82:503–511

Pold R, Jensen LS, Jessen N, Buhl ES, Schmitz O, Flyvbjerg A, Fujii N, Goodyear LJ, Gotfredsen CF, Brand CL, Lund S (2005) Long-term AICAR administration and exercise prevents diabetes in ZDF rats. Diabetes 54:928–934

Porte D Jr (2001) Clinical importance of insulin secretion and its interaction with insulin resistance in the treatment of type 2 diabetes mellitus and its complications. Diabetes Metab Res Rev 17:181–188

Prentki M, Matschinsky FM, Madiraju SR (2013) Metabolic signaling in fuel-induced insulin secretion. Cell Metab 18:162–185

Rafacho A, Abrantes JL, Ribeiro DL, Paula FM, Pinto ME, Boschero AC, Bosqueiro JR (2011) Morphofunctional alterations in endocrine pancreas of short- and long-term dexamethasone-treated rats. Horm Metab Res 43:275–281

Rajan S, Torres J, Thompson MS, Philipson LH (2012) SUMO downregulates GLP-1-stimulated cAMP generation and insulin secretion. Am J Physiol Endocrinol Metab 302:E714–E723

Ravier MA, Cheng-Xue R, Palmer AE, Henquin JC, Gilon P (2010) Subplasmalemmal Ca^{2+} measurements in mouse pancreatic β cells support the existence of an amplifying effect of glucose on insulin secretion. Diabetologia 53:1947–1957

Reitman JS, Vasquez B, Klimes I, Nagulesparan M (1984) Improvement of glucose-homeostasis after exercise training in non-insulin-dependent diabetes. Diabetes Care 7:434–441

Ribeiro RA, Santos-Silva JC, Vettorazzi JF, Cotrim BB, Mobiolli DD, Boschero AC, Carneiro EM (2012) Taurine supplementation prevents morpho-physiological alterations in high-fat diet mice pancreatic β-cells. Amino Acids 43:1791–1801

Roberts CK, Hevener AL, Barnard RJ (2013) Metabolic syndrome and insulin resistance: underlying causes and modification by exercise training. Compr Physiol 3:1–58

Rousseau-Migneron S, Turcotte L, Tancrede G, Nadeau A (1988) Transient increase in basal insulin levels in severely diabetic rats submitted to physical training. Diabetes Res 9:97–100

Ruderman NB, Carling D, Prentki M, Cacicedo JM (2013) AMPK, insulin resistance, and the metabolic syndrome. J Clin Invest 123:2764–2772

Salem MA, Aboelasrar MA, Elbarbary NS, Elhilaly RA, Refaat YM (2010) Is exercise a therapeutic tool for improvement of cardiovascular risk factors in adolescents with type 1 diabetes mellitus? A randomised controlled trial. Diabetol Metab Syndr 2:47

Schneider SH, Amorosa LF, Khachadurian AK, Ruderman NB (1984) Studies on the mechanism of improved glucose control during regular exercise in type 2 (non-insulin-dependent) diabetes. Diabetologia 26:355–360

Seino S (2012) Cell signalling in insulin secretion: the molecular targets of ATP, cAMP and sulfonylurea. Diabetologia 55:2096–2108

Sennott J, Morrissey J, Standley PR, Broderick TL (2008) Treadmill exercise training fails to reverse defects in glucose, insulin and muscle GLUT4 content in the db/db mouse model of diabetes. Pathophysiol 15:173–179

Shima K, Zhu M, Noma Y, Mizuno A, Murakami T, Sano T, Kuwajima M (1997) Exercise training in Otsuka Long-Evans Tokushima Fatty rat, a model of spontaneous non-insulin-dependent diabetes mellitus: effects on the β-cell mass, insulin content and fibrosis in the pancreas. Diabetes Res Clin Pract 35:11–19

Slentz CA, Bateman LA, Willis LH, Shields AT, Tanner CJ, Piner LW, Hawk VH, Muehlbauer MJ, Samsa GP, Nelson RC, Huffman KM, Bales CW, Houmard JA, Kraus WE (2011) Effects of aerobic vs. resistance training on visceral and liver fat stores, liver enzymes, and insulin resistance by HOMA in overweight adults from STRRIDE AT/RT. Am J Physiol Endocrinol Metab 301:E1033–E1039

Svidnicki PV, de Carvalho LN, Venturelli AC, Camargo RL, Vicari MR, de Almeida MC, Artoni RF, Nogaroto V, Grassiolli S (2013) Swim training restores glucagon-like peptide-1 insulinotropic action in pancreatic islets from monosodium glutamate-obese rats. Acta Physiol (Oxf) 209:34–44

Tancrede G, Rousseau-Migneron S, Nadeau A (1982) Beneficial effects of physical training in rats with a mild streptozotocin-induced diabetes mellitus. Diabetes 31:406–409

Thorens B (2010) Central control of glucose homeostasis: the brain-endocrine pancreas axis. Diabetes Metab 36(Suppl 3):S45–S49

Thorens B (2011) Of fat, β cells, and diabetes. Cell Metab 14:439–440

Tokuyama Y, Sturis J, DePaoli AM, Takeda J, Stoffel M, Tang J, Sun X, Polonsky KS, Bell GI (1995) Evolution of β-cell dysfunction in the male Zucker diabetic fatty rat. Diabetes 44:1447–1457

Trovati M, Carta Q, Cavalot F, Vitali S, Banaudi C, Lucchina RG, Fiocchi F, Emanuelli G, Lenti G (1984) Influence of physical-training on blood-glucose control, glucose-tolerance, insulin-secretion, and insulin action in non-insulin-dependent diabetic-patients. Diabetes Care 7:416–420

Tsuchiya M, Manabe Y, Yamada K, Furuichi Y, Hosaka M, Fujii NL (2013) Chronic exercise enhances insulin secretion ability of pancreatic islets without change in insulin content in non-diabetic rats. Biochem Biophys Res Commun 430:676–682

Ueda H, Urano Y, Sakurai T, Kizaki T, Hitomi Y, Ohno H, Izawa T (2003) Enhanced expression of neuronal nitric oxide synthase in islets of exercise-trained rats. Biochem Biophys Res Commun 312:794–800

Utzschneider KM, Carr DB, Barsness SM, Kahn SE, Schwartz RS (2004) Diet-induced weight loss is associated with an improvement in β-cell function in older men. J Clin Endocrinol Metab 89:2704–2710

Vianna CR, Coppari R (2011) A treasure trove of hypothalamic neurocircuitries governing body weight homeostasis. Endocrinology 152:11–18

Villareal DT, Banks MR, Patterson BW, Polonsky KS, Klein S (2008) Weight loss therapy improves pancreatic endocrine function in obese older adults. Obesity (Silver Spring) 16:1349–1354

Wagener A, Schmitt AO, Brockmann GA (2012) Early and late onset of voluntary exercise have differential effects on the metabolic syndrome in an obese mouse model. Exp Clin Endocr Diab 120:591–597

Westerlund J, Bergsten P (2001) Glucose metabolism and pulsatile insulin release from isolated islets. Diabetes 50:1785–1790

Wirth A, Diehm C, Mayer H, Morl H, Vogel I, Bjorntorp P, Schlierf G (1981) Plasma C-peptide and insulin in trained and untrained subjects. J Appl Physiol 50:71–77

Zoppi CC, Calegari VC, Silveira LR, Carneiro EM, Boschero AC (2011) Exercise training enhances rat pancreatic islets anaplerotic enzymes content despite reduced insulin secretion. Eur J Appl Physiol 111:2369–2374

Molecular Basis of cAMP Signaling in Pancreatic β Cells

20

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_25,

Abstract

Recent advances in conditional gene targeting and cyclic nucleotide research further our understanding of how the incretin hormone GLP-1 exerts a therapeutically important action to restore pancreatic insulin secretion in patients with type 2 diabetes mellitus (T2DM). These studies demonstrate that the pancreatic β-cell GLP-1 receptor has the capacity to signal through two distinct branches of the adenosine 3′,5′-cyclic monophosphate (cAMP) signal transduction network; one branch activates protein kinase A (PKA), and the second engages a cAMP-regulated guanine nucleotide exchange factor designated as Epac2. Under normal dietary conditions, specific activation of the cAMP-PKA branch in mice dramatically augments glucose-stimulated insulin secretion (GSIS). However, under conditions of diet-induced insulin resistance, cAMP-Epac2 signaling in the control of GSIS becomes prominent. This chapter provides an update on GLP-1 receptor signaling in the islets of Langerhans, with special emphasis on key molecular events that confer "plasticity" in the β-cell cAMP signal transduction network. The reader is reminded that an excellent review of β-cell cAMP signaling can also be found in the prior first edition of this book.

Keywords

Cyclic AMP • Protein kinase A • Epac2 • GLP-1 • Diabetes

Introduction

The cytosolic second messenger cAMP is a key activating signaling molecule supporting insulin exocytosis from pancreatic β-cells located in the islets of Langerhans (Holz 2004a; Leech et al. 2010a; Tengholm 2012). cAMP exerts its insulin secretagogue actions by binding to and activating either protein kinase A (PKA, a serine/threonine protein kinase) or Epac2 (a guanine nucleotide exchange factor which in turn activates Rap1 GTPase). cAMP modulates insulin exocytosis so that it potentiates glucose-stimulated insulin secretion (GSIS) from the β-cells (Holz and Habener 1992). As illustrated in Figs. 1 and 2, the downstream targets of PKA, Epac2, and glucose that are relevant to insulin secretion include proteins that control β-cell membrane excitability (ATP-sensitive K^+ channels, K_{ATP}), Ca^{2+} influx (voltage-dependent Ca^{2+} channels, VDCCs; nonselective cation channels, NSCCs), intracellular Ca^{2+} mobilization (IP_3 receptors, IP_3R; ryanodine receptors, RYR), as well as secretory granule and SNARE complex-associated proteins that promote Ca^{2+}-dependent exocytosis of insulin (syntaxin, SNAP-25, VAMP2, RIM2, Piccolo, Munc13-1) (Seino and Shibasaki 2005; Holz et al. 2006; Kwan and Gaisano 2007; Seino et al. 2009; Vikman et al. 2009; Leech et al. 2011; Song et al. 2011, 2013; Hussain et al. 2012; Kasai et al. 2012).

cAMP biosynthesis in β-cells is catalyzed by transmembrane adenylyl cyclases (TMACs) that use ATP as a substrate in order to generate cAMP, and it is

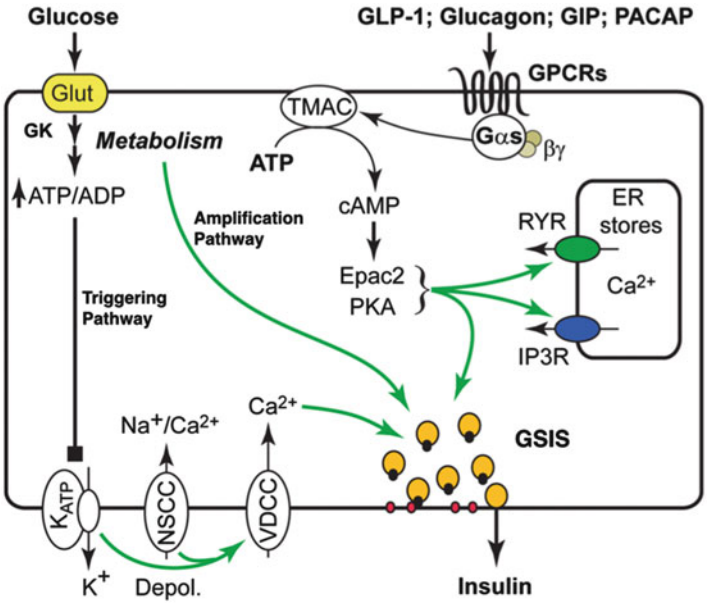

Fig. 1 β-cell GPCR activation by GLP-1, glucagon, GIP, or PACAP results in cAMP production catalyzed by TMACs. cAMP activates Epac2 and PKA in order to potentiate glucose-stimulated insulin secretion (*GSIS*). Glucose sensing by the β-cell requires glucose uptake mediated by glucose transporters (*Glut*), whereas cytosolic glucokinase (*GK*) acts as the rate-limiting enzyme for oxidative glucose metabolism. A triggering pathway for GSIS involves K_{ATP} channel closure, membrane depolarization (*Depol.*), and Ca^{2+} influx that occurs in response to the increase of cytosolic ATP/ADP concentration ratio that glucose metabolism produces. Ca^{2+} triggers exocytosis of insulin, and this action of Ca^{2+} is enhanced by PKA. Activation of Epac2 facilitates glucose-dependent closure of K_{ATP} channels, thereby sensitizing β-cells to the stimulatory effect of glucose. Thus, GLP-1 is a β-cell glucose sensitizer. Note that the Ca^{2+} signal important to exocytosis is generated by Ca^{2+} entry through VDCCs or by the mobilization of Ca^{2+} from intracellular Ca^{2+} stores. Intracellular Ca^{2+} release channels (*IP_3R*, *RYR*) located on the ER are targets of PKA and Epac2, thereby allowing cAMP to facilitate glucose-dependent release of Ca^{2+} from the ER. Nonselective Ca^{2+} channels (*NSCC*) activated in response to ER Ca^{2+} mobilization generate a depolarizing inward Na^+/Ca^{2+} current in order to increase β-cell excitability. Resultant action potential generation leads to additional Ca^{2+} influx and insulin exocytosis. These established mechanisms of "triggered" insulin secretion are reinforced by a K_{ATP} channel-independent amplification pathway. Although less well understood, it couples glucose metabolism to the recruitment of secretory granules to the plasma membrane where they undergo exocytosis in response to Ca^{2+}

terminated by cyclic nucleotide phosphodiesterases (PDEs) that hydrolyze cAMP to 5′-AMP (Furman et al. 2010). Since cAMP-elevating agents have little or no insulin secretagogue action in the absence of glucose, and since insulin secretion can be stimulated by glucose in the absence of cAMP-elevating agents, it is generally accepted that the primary stimulus for insulin secretion is glucose, whereas cAMP acts to potentiate GSIS from β-cells (Henquin 2000).

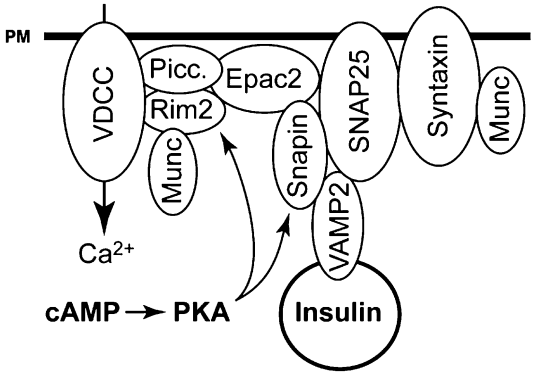

Fig. 2 SNARE complex and secretory granule-associated proteins interact in order to mediate the action of cAMP to potentiate GSIS. These proteins include syntaxin and SNAP-25 located on the plasma membrane (*PM*) and VAMP2 located on the secretory granules. High concentrations of glucose promote the interaction of SNAP-25 with VAMP2, and this interaction is enhanced under conditions in which PKA and Epac2 are activated. Snapin is a substrate for PKA, and its phosphorylation on Ser-50 facilitates its interactions with SNAP-25 and Epac2. Direct binding of cAMP to Epac2 promotes its interaction with SNAP-25. When both PKA and Epac2 are activated, SNARE complex assembly is enhanced so that insulin exocytosis may occur in response to depolarization-induced entry of Ca^{2+} through VDCCs. Rim2 (Rab3-interacting molecule2), Piccolo (a Ca^{2+} sensor), and Munc (a PKA substrate) are Epac2-interacting proteins that also participate in the cAMP-dependent control of insulin secretion

Pharmacological agents that increase levels of β-cell cAMP in order to potentiate GSIS include stimulators of TMAC activity (forskolin, cholera toxin, pertussis toxin) or inhibitors of PDE activity (IBMX) (Holz et al. 2000; Pyne and Furman 2003). The incretin hormone glucagon-like peptide-1 (GLP-1) acting at the β-cell GLP-1 receptor (GLP-1R) stimulates TMACs in order to potentiate GSIS (Thorens 1992; Mojsov et al. 1987; Orskov et al. 1988; Gromada et al. 1998b; Holz 2004b), whereas neurotransmitters such as galanin and norepinephrine inhibit TMACs to inhibit insulin secretion (Sharp 1996; Straub and Sharp 2012). The hormone leptin acting via the β-cell Ob-Rb receptor stimulates PDE isoform 3B (PDE3B) in order to inhibit insulin secretion (Zhao et al. 1998; Emilsson et al. 1997; Kieffer et al. 1997; Kulkarni et al. 1997), and a targeted knockout (KO) of Ob-Rb in β-cells of mice leads to a marked enhancement of GSIS (Morioka et al. 2012).

Class II GTP-binding protein-coupled receptors (GPCRs) expressed on β-cells are coupled to cAMP production (Winzell and Ahrén 2007; Ahrén 2009; Couvineau and Laburthe 2012), and they bind GLP-1, glucagon, glucose-dependent insulinotropic peptide (GIP), and pituitary adenylyl cyclase-activating polypeptide (PACAP). The Gila monster lizard *Heloderma* is the source of GLP-1R agonist exendin-4, and its fragment exendin-(9–39) is a GLP-1R antagonist that inhibits cAMP production and insulin secretion (De Leon et al. 2008). Unexpectedly, Class II GPCRs are structurally related to CIRL (the Ca^{2+}-independent receptor for α-latrotoxin), whereas GLP-1 shares structural homology with α-latrotoxin, a venom derived from the black widow spider *Latrodectus*. These findings have

prompted efforts to develop chimeric peptides that are comprised of amino acid sequences found in both GLP-1 and α-latrotoxin. For example, human islet insulin secretion is stimulated by one such peptide designated as black widow GLP-1 (Holz and Habener 1998).

GPR119 is a Class I GPCR that mediates stimulatory effects of 2-oleoyl glycerol, lysophosphatidylcholine, and fatty acid amides (e.g., oleoylethanolamide; OEA) on cAMP production and β-cell insulin secretion (Soga et al. 2005; Overton et al. 2008; Chu et al. 2007; Hansen et al. 2011). Synthetic small molecules that activate GPR119 (e.g., AR231453) are orally administrable and are currently under investigation for use in the treatment of T2DM (Jones et al. 2009; Shah and Kowalski 2010; Hansen et al. 2012). The potential usefulness of GPR119 agonists for this purpose is emphasized by the fact that they also stimulate intestinal GLP-1 release (Chu et al. 2008; Lan et al. 2009, 2012; Hansen et al. 2011).

Drug discovery efforts have yielded β-cell cAMP-elevating GLP-1R agonists such as exenatide and liraglutide that mimic the action of GLP-1 to lower levels of blood glucose in patients with T2DM (Gutniak et al. 1992; Nathan et al. 1992; Drucker and Nauck 2006; Lovshin and Drucker 2009). In contrast to endogenous GLP-1, these GLP-1R agonists are resistant to degradation by dipeptidyl peptidase-IV (DPP-IV) (Kieffer et al. 1995). Thus, they have an extended duration of action when administered by subcutaneous injection.

Orally administrable DPP-IV inhibitors such as sitagliptin and vildagliptin also exert insulin secretagogue and blood glucose-lowering actions in patients with T2DM (Drucker and Nauck 2006; Karagiannis et al. 2012). These agents delay metabolic degradation of GLP-1, thereby enabling endogenously secreted GLP-1 to more effectively raise levels of cAMP in β-cells (Dalle et al. 2013). Current drug discovery efforts seek to broaden the base of GLP-1R-targeted therapeutics by developing an orally administrable form of GLP-1 that is a conjugate of vitamin B_{12} (Clardy-James et al. 2013). B_{12}-GLP-1 exploits the vitamin B_{12} uptake system that utilizes intrinsic factor (IF) in order to achieve intestinal absorption of the conjugate.

Non-peptide orally administrable GLP-1R agonists may also broaden the incretin-based therapeutic armamentarium. This approach might yield GLP-1R agonists that allosterically activate the receptor by binding to sites on the receptor that are not identical to sites that bind GLP-1 (Koole et al. 2010). Further, such small molecules may be designed to activate the receptor in a manner that "biases" its signal transduction properties. Thus, GLP-1R agonists might be tailored to selectively activate either the cAMP signaling mechanism or growth factor signaling mechanisms important to β-cell function (Koole et al. 2010).

The safety profile of GLP-1R agonists and DPP-IV inhibitors is recently questioned in reports that link their use in humans to an increased incidence of pancreatitis, exocrine pancreas dysplasia, and islet α-cell hyperplasia (Butler et al. 2013; Singh et al. 2013). These findings need to be substantiated, but it is of interest that prior in vivo studies of rodents or in vitro studies of human islets demonstrate that GLP-1R agonists enhance β-cell neogenesis, proliferation, and survival (Xu et al. 1999; Tourrel et al. 2001; Li et al. 2003; Farilla et al. 2003).

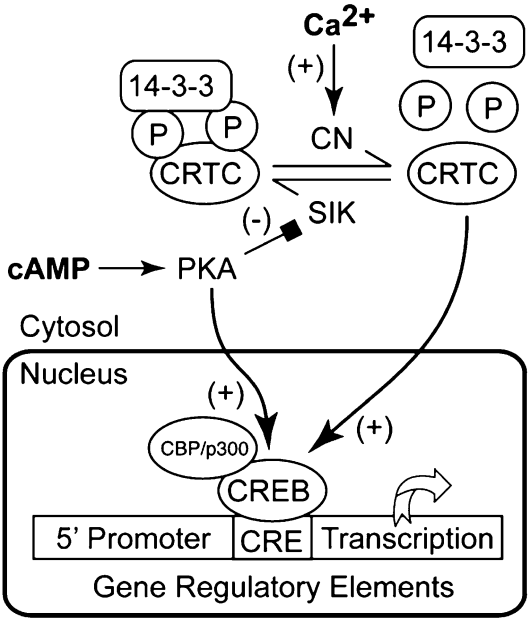

Fig. 3 cAMP-stimulated gene expression in β-cells results from PKA holoenzyme activation with consequent translocation of PKA catalytic subunits to the nucleus where PKA phosphorylates CREB on Ser-133. Histone acetyltransferases p300 and CBP are transcriptional co-activators that enhance binding of Ser-133-CREB to cAMP response elements (*CRE*) located in 5′ gene promoter sequences. Ser-133-CREB binding to CREs is also enhanced by cAMP-regulated transcriptional co-activators (*CRTC*). At low glucose and in the absence of cAMP-elevating agents, CRTC is phosphorylated by salt-inducible kinase (*SIK*) to promote its association with 14-3-3 proteins, thereby sequestering CRTC in the cytoplasm. High glucose stimulates an increase of $[Ca^{2+}]_i$ that activates the phosphatase calcineurin (*CN*) in order to dephosphorylate CRTC, whereas cAMP-elevating agents act via PKA to inhibit SIK activity in order to slow phosphorylation of CRTC. The net effect is that dephosphorylated CRTC dissociates from 14-3-3 proteins so that it may translocate to the nucleus, bind Ser-133-CREB, and co-activate transcription. Note that CRTC is a cAMP and Ca^{2+} coincidence detector important to β-cell gene expression

In rodents, GLP-1R agonists produce an increase of β-cell mass, but it is not clear if such a potentially beneficial effect occurs in patients with T2DM (Friedrichsen et al. 2006; Song et al. 2008; Lavine and Attie 2010; Tschen et al. 2011).

When considering how GLP-1R agonists act as β-cell trophic factors, there is evidence for an insulinotropic action at the transcriptional level that is either PKA dependent (Drucker et al. 1987) or PKA independent (Skoglund et al. 2000; Chepurny et al. 2002). The PKA-dependent action of GLP-1R agonists is mediated by cAMP response elements (CREs) located in the human insulin gene (Hay et al. 2005). As illustrated in Fig. 3, CREs bind the cAMP response element-binding protein (CREB), a basic region leucine zipper transcription factor (bZIP) that is regulated by PKA and co-activators p300 and CRTC in β-cells (Altarejos and Montminy 2011; Dalle et al. 2011b). These CREs also bind bZIPs that mediate

PKA-independent actions of GLP-1R agonists, and in this regard the insulinotropic action of GLP-1 is sensitive to Ro 318220, a serine/threonine protein kinase inhibitor that inhibits MAPK-activated kinases (RSKs) and mitogen/stress-activated kinases (MSK) that serve as CREB kinases (Chepurny et al. 2002). Transcriptional activation of insulin gene expression by GLP-1 is also accompanied by GLP-1-stimulated translational biosynthesis of proinsulin (Fehmann and Habener 1992).

PKA-mediated induction of insulin receptor substrate-2 (IRS-2) expression promotes β-cell growth in response to GLP-1 (Jhala et al. 2003; Park et al. 2006), and studies of β-cell lines or neonatal β-cells indicate that PKA also mediates transcriptional induction of cyclin D1 by GLP-1 in order to stimulate proliferation (Kim et al. 2006; Friedrichsen et al. 2006). Furthermore, a proliferative action of GLP-1 results from PKA-mediated phosphorylation of β-catenin, thereby indicating that the β-cell cAMP-PKA signaling branch exhibits signal transduction cross talk with a noncanonical Wnt signaling pathway that uses the transcription factor TCF7L2 to control gene expression (Liu and Habener 2008). PKA also mediates the action of GLP-1 to promote nuclear localization of transcription factor PDX-1, thereby enhancing the differentiated state of β-cells (Wang et al. 2001). SAD-A kinase is reported to be under the control of PKA in order for cAMP to stimulate insulin secretion (Nie et al. 2013).

A surprising finding is that a truncated GLP-1 designated as GLP-1(28–36) amide stimulates cAMP production in β-cells, thereby activating the β-catenin/TCF7L2 signaling pathway (Shao et al. 2013). Furthermore, GLP-1(28–36)amide protects against β-cell glucotoxicity by improving mitochondrial function (Liu et al. 2012). GLP-1(28–36)amide is a cell-penetrating peptide that does not exert its effects by binding to the GLP-1R, but instead acts intracellularly. Thus, it is not clear how GLP-1(28–36) amide stimulates cAMP production.

Since there is evidence that the β-cell GLP-1R signals through cAMP sensor Epac2, the possibility exists that this cAMP-regulated guanine nucleotide exchange factor participates not only in the control of insulin secretion but also β-cell growth. However, recent studies demonstrate that β-cell mass is preserved in mice with a whole-body knockout (KO) of Epac2 gene expression (Song et al. 2013). Still, additional findings demonstrate a role for Epac2 in the protection of β-cells from cytotoxicity induced by reactive oxygen species (ROS) (Mukai et al. 2011). Redox control in β-cells is under the control of thioredoxin (TxN), and TxNIPs are thioredoxin-interacting proteins that downregulate the ROS buffering capacity of thioredoxin. Thus, it is significant that GLP-1 acts via Epac2 to suppress TxNIP expression in β-cells (Shao et al. 2010).

cAMP-independent actions of GLP-1 exist, and they are also of significance when considering how GLP-1 maintains β-cells in a healthy state (Holz and Chepurny 2005). Such actions include the ability of GLP-1R agonists to counteract endoplasmic reticulum stress (Yusta et al. 2006) and to signal via the GLP-1R through β-arrestin (Sonoda et al. 2008; Dalle et al. 2011a) and epidermal growth factor (EGF) receptor transactivation (Buteau et al. 2003) in order to downregulate the activities of proapoptotic protein Bad (Quoyer et al. 2010), the SirT1

deacetylase (Bastien-Dionne et al. 2011), and transcription factor FoxO1 (Buteau et al. 2006). GLP-1 also upregulates the activities of c-Src kinase (Talbot et al. 2012), phosphatidylinositol 3-kinase (PI-3-kinase) (Buteau et al. 1999), protein kinase B (PKB) (Wang et al. 2004), protein kinase c-ζ (PKCζ) (Buteau et al. 2001), and extracellular signal-regulated protein kinases (ERK1/2) (Arnette et al. 2003). As alluded to above, it may be possible to develop allosteric GLP-1R agonists with biased signaling properties that preferentially activate these various signaling pathways.

In Vivo Actions Of cAMP-Elevating Agents In Humans

GLP-1 and GIP are released from enteroendocrine L-cells and K-cells, respectively (Kieffer and Habener 1999; Baggio and Drucker 2007; Holst 2007; McIntosh et al. 2010). These cells are located in the intestinal wall where they act as nutrient sensors such that nutrient ingestion stimulates the release of GLP-1 and GIP into the systemic circulation. During the postprandial increase of blood glucose concentration, released GLP-1 and GIP potentiate GSIS from β-cells. Thus, GLP-1 and GIP mediate the "incretin effect" whereby gut-derived signals synergize with intestinally absorbed glucose to potentiate insulin secretion (Creutzfeldt 2005). In patients with T2DM that undergo Roux-en-Y gastric bypass surgery (RYGB), an improvement of β-cell function and glucose tolerance is observed, and these beneficial effects are related to an exaggerated release of GLP-1 from L-cells (Jorgensen et al. 2013).

It is especially interesting that T2DM can be treated with GLP-1R agonists, whereas GIP receptor agonists are ineffective (Nauck et al. 1993). Why this is the case is not clear, but it is possible that in T2DM, β-cell GIP receptor expression is reduced (Lynn et al. 2001). Alternatively, the action of GIP at the β-cell may require a cofactor that is absent in T2DM. For example, xenin-25, a peptide co-secreted with GIP from K-cells, activates local enteric nervous system reflexes that enhance β-cell GIP sensitivity in healthy individuals but not in patients with T2DM (Wice et al. 2010, 2012). Dysfunctional xenin-25 action could therefore explain why GIP is not an insulin secretagogue in T2DM.

GLP-1 receptors are expressed not only on β-cells but also on vagal sensory nerve endings that innervate the intestinal wall where L-cells are located (Ahrén 2000). Thus, locally secreted GLP-1 may act via vagal-vagal reflex pathways in which afferent sensory neuron activity is transmitted to the central nervous system, with consequent efferent activity transmitted to islets by the parasympathetic autonomic nervous system (Burcelin 2010; Hayes 2012). Parasympathetic ganglia neurons release the neurotransmitter PACAP in order to stimulate cAMP production in β-cells (Ahrén 2008), so it is possible that intestinally released GLP-1 acts indirectly via neuronally released PACAP to stimulate insulin secretion. Since GLP-1 has a short half-life in the systemic circulation (<5 min in humans), and since it is secreted in close proximity to vagal sensory nerve endings located in the wall of the intestine, a circumstance may exist in which the indirect action of GLP-1 mediated by the GLP-1R on the vagus nerve overshadows the direct action of

circulating GLP-1 at the GLP-1R on β-cells. However, a different situation exists when considering the actions of DPP-IV-resistant GLP-1R agonists since these peptides have an extended duration of action in the circulation (>30 min). Studies of mice that express the GLP-1R only in the pancreas demonstrate that a direct action of GLP-1R agonists at the β-cell GLP-1R is sufficient to potentiate GSIS and to improve glucoregulation in the absence of vagal neuron stimulation (Lamont et al. 2012). Thus, it seems likely that the β-cell GLP-1R mediates a direct insulin secretagogue action of DPP-IV-resistant GLP-1R agonists in humans.

In Vitro Evidence That Glucose Metabolism Stimulates cAMP Production

Surprisingly, cAMP production is stimulated by β-cell glucose metabolism (Landa et al. 2005; Dyachok et al. 2006, 2008; Kim et al. 2008a; Idevall-Hagren et al. 2010; Tian et al. 2011), and in the 1970s it was proposed that cAMP mediates the action of glucose to stimulate insulin secretion (Charles et al. 1975). Such an effect of glucose might be a consequence of its ability to stimulate Ca^{2+} influx and to raise levels of cytosolic Ca^{2+}, thereby stimulating TMACs that are under the control of Ca^{2+}/calmodulin (Ca^{2+}/CaM) (Delmiere et al. 2003; Roger et al. 2011). Alternatively, glucose metabolism might be coupled to HCO_3^- production that activates a soluble adenylyl cyclase (sAC) in β-cells (Ramos et al. 2008; Zippen et al. 2013).

Glucose metabolism provides ATP for TMAC-catalyzed cAMP production in β-cells. Levels of ATP at low concentrations of glucose are limiting for cAMP production such that an elevation of glucose concentration leads to increased ATP availability (Takahashi et al. 1999; Kasai et al. 2002). cAMP activates PKA, and PKA-mediated phosphorylation facilitates Ca^{2+}-dependent exocytosis of insulin (Thams et al. 2005; Hatakeyama et al. 2006, 2007). As illustrated in Fig. 4, cAMP generated by glucose metabolism also activates Epac2 (Idevall-Hagren et al. 2013), but it is uncertain if glucose and GLP-1 activate identical pools of PKA and Epac2. Finally, WFS1, an endoplasmic reticulum protein, supports glucose-stimulated TMAC activity in an as-yet-to-be determined manner (Fonseca et al. 2012).

Mathematical models predict how cytosolic levels of cAMP and Ca^{2+} oscillate under conditions in which β-cells are exposed to glucose and GLP-1 (Fridlyand et al. 2007; Ni et al. 2011; Takeda et al. 2011). In the absence of GLP-1, glucose metabolism has a modest stimulatory effect on cAMP production due to the fact that it provides substrate ATP, while also providing a cytosolic Ca^{2+} signal that stimulates Ca^{2+}/CaM-regulated TMACs. Simultaneously, Ca^{2+}-regulated PDEs are activated in order to lower levels of cAMP. Under these conditions, oscillations of cAMP and Ca^{2+} occur, and these oscillations are anti-phasic such that high levels of cAMP coincide with low levels of Ca^{2+} (Landa et al. 2005). An important prediction of these mathematical models is that exposure of β-cells to GLP-1 in the presence of glucose results in a reversal of the oscillatory activity such that high levels of cAMP coincide with high levels of Ca^{2+}. This reversal is explained by the fact that TMAC activity is also stimulated by Gs proteins linked to GLP-1

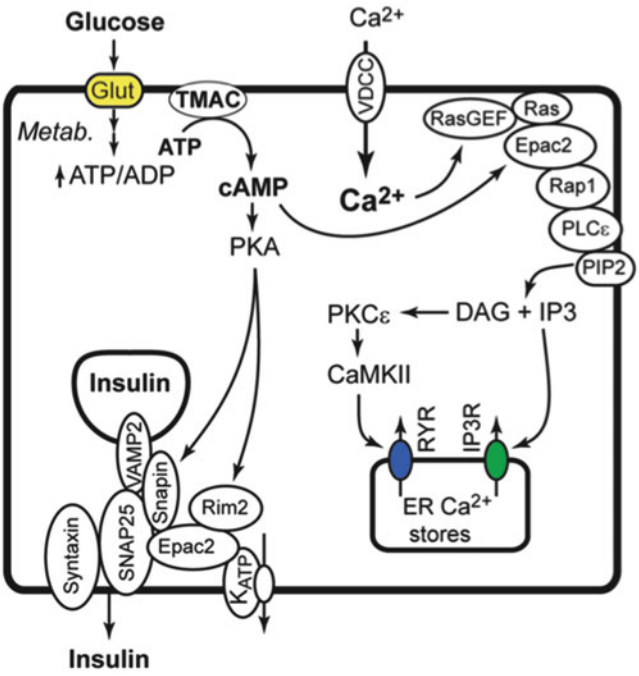

Fig. 4 Glucose metabolism provides ATP for TMAC-catalyzed cAMP production, PKA activation, and Epac2A activation. Glucose metabolism also stimulates an increase of $[Ca^{2+}]_i$ that activates guanine nucleotide exchange factors for Ras GTPase (*Ras-GEF*). Activated Ras-GTP binds to the Ras-association domain of Epac2 and recruits it to the plasma membrane where it activates Rap1 GTPase. Activated Rap1-GTP then binds the Rap-association domain of PLCϵ in order to stimulate its intrinsic catalytic activity, thereby initiating PIP_2 hydrolysis with consequent production of DAG and IP_3. DAG activates protein kinase C-ε (*PKCε*) in order to activate CaM-KII which then phosphorylates and activates RYR located on the ER. Simultaneously, IP_3 activates IP_3R on the ER, and Ca^{2+} released from the ER acts to promote additional Ca^{2+}-induced Ca^{2+} release from the ER. Ca^{2+} released in this manner acts as a direct trigger for insulin secretion under conditions in which PKA activity sensitizes the release mechanism to Ca^{2+}

receptors. Thus, TMACs act as molecular coincidence detectors for Ca^{2+}/CaM and Gs in order to generate synchronous inphase oscillations of cAMP and Ca^{2+} that are of importance to insulin secretion from β-cells (Holz et al. 2008b).

Insulin Exocytosis Is Stimulated Directly By cAMP: The Role Of PKA

An established literature documents the role of cAMP as a stimulator of insulin secretion, as measured in studies of isolated islets (Prentki and Matschinski 1987; Howell et al. 1994), or in live-cell imaging and patch clamp-based assays of exocytosis

occurring in single β-cells (Seino et al. 2009; Kasai et al. 2010; Dolenšek et al. 2011). The action of cAMP occurs at "late" or "distal" steps of β-cell stimulus-secretion coupling in which cAMP has a direct action to enhance secretory granule exocytosis (Ämmälä et al. 1993; Gillis and Misler 1993; Barnett et al. 1994). As illustrated in Figs. 1, 2, and 4, this action of cAMP is both PKA dependent and PKA independent (Renstrom et al. 1997), and evidence exists that the SNARE complex-associated protein snapin mediates the PKA-dependent component (Song et al. 2011), whereas Epac2 mediates the PKA-independent component (Ozaki et al. 2000; Eliasson et al. 2003). It is presently unclear whether compartmentalized cAMP signaling results in a situation in which certain Class II GPCRs preferentially couple to either the PKA-dependent or PKA-independent branches of this cAMP signaling network.

PKA-mediated phosphorylation has diverse stimulatory effects on insulin exocytosis. In one model illustrated in Fig. 5, ATP-dependent "priming" of secretory granules located within a readily releasable pool (RRP) renders them competent to undergo exocytosis. PKA then acts at a postpriming step to enhance their Ca^{2+}-dependent fusion with the plasma membrane (Takahashi et al. 1999). Although the identity of the postpriming substrate protein phosphorylated by PKA remains to be determined, this PKA activity is stimulated by glucose metabolism and is permissive for exocytosis (Hatakeyama et al. 2006, 2007). In fact, the ability of selective Epac activator 8-pCPT-2′-*O*-Me-cAMP-AM to potentiate GSIS from human islets requires concomitant permissive PKA activity (Chepurny et al. 2010).

PKA activity also renders secretory granules within the RRP more sensitive to the stimulatory action of Ca^{2+} so that they have an increased probability to undergo exocytosis in response to Ca^{2+} (Skelin and Rupnik 2011). This action of PKA is complemented by its ability to recruit a reserve pool of secretory granules from the cytoplasm to the plasma membrane so that the RRP may be refilled under conditions of sustained exocytosis (Renstrom et al. 1997). Simultaneously, PKA activity increases the number of highly Ca^{2+}-sensitive secretory granules, some of which are located outside of the RRP (Wan et al. 2004; Yang and Gillis 2004).

Another model seeks to explain how GLP-1 potentiates GSIS in a Ca^{2+}-dependent manner (Kang et al. 2003; Holz 2004b). In the absence of GLP-1, glucose metabolism stimulates the exocytosis of secretory granules located within "active zones" where microdomains of high cytosolic [Ca^{2+}] form at VDCCs. When β-cells are exposed to GLP-1, PKA activity sensitizes secretory granules to the action of Ca^{2+}, thereby ensuring that exocytosis will also occur at regions of the plasma membrane located outside of active zones. This Ca^{2+} sensitization allows a new larger source of granules to undergo exocytosis. For example, PKA activity enables additional secretory granules to undergo exocytosis in response to Ca^{2+} mobilized via a mechanism of Ca^{2+}-induced Ca^{2+} release (CICR) (Holz et al. 1999; Kang and Holz 2003).

Conceivably, all of the above-summarized processes act in concert to enable GLP-1 to potentiate GSIS. However, much of what we know concerning PKA signaling in the β-cell is based on studies using cAMP analogs in order to selectively activate PKA. New studies reveal the dangers of such an approach since Epac2 can be activated by 6-Bn-cAMP-AM, an *N*6-Benzyladenine-substituted

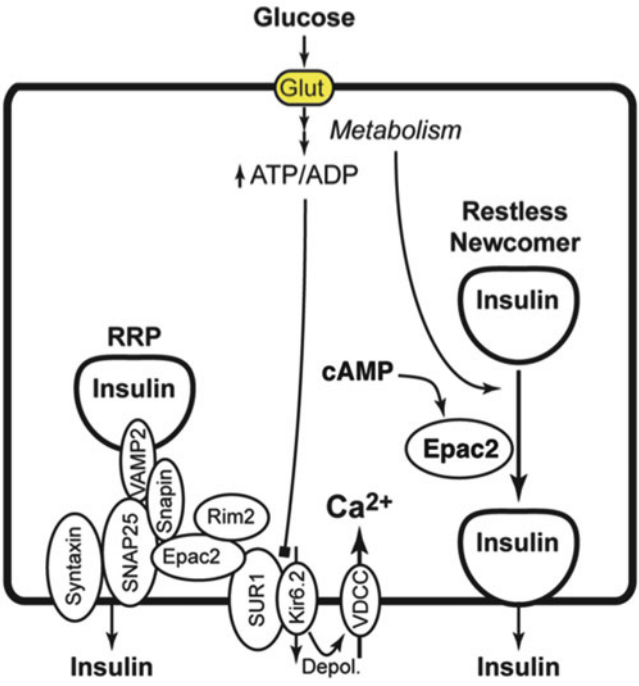

Fig. 5 Electrophysiological studies of β-cells define a readily releasable pool (*RRP*) of secretory granules that are prepositioned at the plasma membrane in order to undergo exocytosis in response to Ca^{2+} (*left side* of illustration). In marked contrast, imaging studies using total internal reflection microscopy (*TIRF*) of secretory granule trafficking indicate that secretory granules located in the cytoplasm move to the plasma membrane and immediately undergo exocytosis in response to Ca^{2+}. These secretory granules are designated as "restless newcomers," and they do not require propositioning at the plasma membrane (*right side* of illustration). This mechanism of restless newcomer exocytosis is dually regulated by glucose metabolism and cAMP, and it plays a prominent role in first phase GSIS. Studies with Epac2 KO mice demonstrate that expression of Epac2 is necessary in order for cAMP to potentiate first phase restless newcomer exocytosis. Glucose metabolism may not only provide a Ca^{2+} signal for exocytosis, but it may also induce remodeling of a cortical actin barrier so that secretory granules within the cytoplasm may transit to the plasma membrane

cAMP analog that was considered to be PKA selective (G. G. Holz, unpublished studies). As summarized below, studies using a molecular approach involving gene targeting provide new evidence for a role of PKA in the control of GSIS.

Insulin Exocytosis Is Stimulated Directly By cAMP: The Role Of Epac2

Epac2 participates in the direct control of insulin exocytosis by cAMP, and this action of Epac2 may also mediate the action of GLP-1 to potentiate GSIS (Kashima et al. 2001). Live-cell imaging studies of single β-cells provide key insights into

how these effects are achieved. By imaging the movement of β-cell secretory granules in response to glucose, it is possible to demonstrate that secretory granules fuse with the plasma membrane quickly (first phase) or with a delay (second phase). Under these conditions, cAMP potentiates first phase exocytosis in an Epac2-mediated manner (Shibasaki et al. 2007). This action of Epac2 correlates with its binding to SNARE protein SNAP-25 (Vikman et al. 2009) and SNARE complex-associated proteins Rim2 and Piccolo (Ozaki et al. 2000; Fujimoto et al. 2002; Shibasaki et al. 2004). It also correlates with Epac2-mediated phosphorylation of a microtubule-associated protein (syntabulin) that influences secretory granule trafficking (Ying et al. 2012). However, Epac2 is primarily an activator of Rap1 GTPase, so it is not yet clear how these signaling events lead to a potentiation of first phase exocytosis.

Epac2 also mediates cAMP-dependent acidification of β-cell secretory granules, thereby rendering them competent to undergo fast exocytosis in response to Ca^{2+} influx through VDCCs (Eliasson et al. 2003). This action of Epac2 is specific for an immediately releasable pool (IRP) of secretory granules that undergo exocytosis during first phase GSIS. Mechanistically, the activation of Epac2 promotes granule acidification by establishing a Cl^- concentration gradient that enables entry of protons across the secretory granule membrane. Surprisingly, a KO of the SUR1 subunit of K_{ATP} channels disrupts this action of Epac2. Since SUR1 is present in the secretory granule membrane where Cl^- channels are present (Geng et al. 2003), it could be that Epac2 and SUR1 mediate an action of cAMP to control secretory granule Cl^- channel function.

There is also evidence for cAMP-dependent stimulation of Cl^- channel activity in the plasma membrane of β-cells (Kinard and Satin 1995). Opening of these Cl^- channels generates β-cell depolarization due to the fact that the reversal potential for the corresponding Cl^- current is −34 mV (Kinard and Satin 1995). This Cl^- current is activated not only by cAMP but also by the sulfonylurea glyburide. Thus, it could be that Cl^- channels present in the β-cell plasma membrane, as well as in the secretory granule membrane, are under the control of SUR1 serving in its role as an Epac2-interacting protein (Shibasaki et al. 2004). Still, it remains to be determined if and how the guanine nucleotide exchange factor activity of Epac2 leads to Rap1-dependent opening of Cl^- channels.

When considering how Rap1 might mediate a direct action of Epac2 to control exocytosis, it is significant that there is expression of a Rap1-regulated phospholipase C-ε (PLCε) in mouse β-cells (Dzhura et al. 2010). PLCϵ contains a Rap1-association domain, thereby allowing cAMP to act via Epac2 and Rap1 to stimulate its catalytic activity. PLCϵ catalyzes hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), and it links cAMP signaling to diacylglycerol (DAG) production and PKC activation (Smrcka et al. 2012). DAG formed in the plasma membrane may then bind SNARE complex-associated proteins such as Munc13-1 in order to facilitate exocytosis (Betz et al. 1998). Simultaneously, activated PKC recruited to the plasma membrane may phosphorylate SNAP-25 in order to facilitate exocytosis (Yang et al. 2007). In this regard, it is noteworthy that sulfonylureas directly stimulate β-cell exocytosis in a PKC-dependent manner (Eliasson et al. 1996).

Since sulfonylureas are reported to directly activate Epac2 (Zhang et al. 2009), an unexpected situation may exist in which Epac2, Rap1, and PLCϵ mediate a direct stimulatory action of sulfonylureas at β-cell secretory granules.

Contrasting Roles Of PKA And Epac2 In The Control Of GSIS

Glucose metabolism in β-cells is coupled to K_{ATP} channel closure, with resultant depolarization-induced entry of Ca^{2+} through VDCCs (Henquin 2000). In healthy β-cells this Ca^{2+} entry produces an increase of $[Ca^{2+}]_i$ that stimulates insulin exocytosis, and one study using genetically engineered mice in which there is a KO of PKA regulatory subunit 1a (PKArs1a) demonstrates that enhanced PKA activity potentiates GSIS (Song et al. 2011).

Epac2 also plays a role in the control of insulin secretion. New findings demonstrate that in healthy mice fed with a normal diet, a whole-body KO of Epac2 does not disrupt GSIS, but it does impair the action of GLP-1R agonist exendin-4 to potentiate first phase GSIS, both in vitro and in vivo (Song et al. 2013). Remarkably, a different situation exists when mice are fed with a high-fat diet that induces insulin resistance. In these unhealthy mice, Epac2 ablation disrupts insulin secretion in response to glucose alone (Song et al. 2013). Thus, a role for Epac2 in the control of GSIS is measurable in a rodent model of obesity-related T2DM.

In human T2DM an unhealthy situation also exists in which there is reduced coupling of β-cell glucose metabolism to K_{ATP} channel closure so that glucose fails to fully generate the Ca^{2+} signal that triggers insulin exocytosis (Doliba et al. 2012a). This pathology might be explained by aberrant glucose sensing by β-cell glucokinase (Doliba et al. 2012b), or by defects of mitochondrial metabolism (Wiederkehr and Wollheim 2008; Mulder and Ling 2009; Patti and Corvera 2010). The pathology might also be explained by a reduced capacity of K_{ATP} channels to close in response to the increase of cytosolic ATP/ADP concentration ratio that glucose metabolism produces. With these points in mind, we propose that Epac2 activation corrects for metabolic defects in T2DM, thereby restoring the Ca^{2+} signal that triggers insulin exocytosis. Thus, we predict that in T2DM, Epac2 participates in the restoration of GSIS by GLP-1R agonists.

Restoration Of GSIS In T2DM: A Role For Ca^{2+} Influx

A loss of first phase GSIS is one of the earliest indicators of β-cell dysfunction in a prediabetic patient (Brunzell et al. 1976). However, first phase GSIS is quickly restored during intravenous infusion of GLP-1 receptor agonist exenatide to these patients (Fehse et al. 2005). Such observations indicate that β-cells of early-stage T2DM patients have sufficient quantities of insulin available for exocytosis, yet first phase GSIS is somehow disturbed. Importantly, the secretory defect occurring in β-cells of T2DM patients might not be generalized since adequate quantities of

insulin are secreted in response to sulfonylureas (Seino et al. 2011). Sulfonylureas inhibit β-cell K_{ATP} channels to produce Ca^{2+} influx, so it might be that the fundamental mechanisms of Ca^{2+}-dependent exocytosis are not disrupted in β-cells of T2DM patients. These observations lead us to hypothesize that in T2DM, the coupling of β-cell glucose metabolism to K_{ATP} channel closure is reduced so that glucose fails to generate the necessary Ca^{2+} signal that initiates insulin secretion. When GLP-1R agonists are administered, the coupling of glucose metabolism to K_{ATP} channel closure is restored so that Ca^{2+}-dependent exocytosis of insulin may occur.

Restoration of K_{ATP} channel closure by GLP-1 is measurable under experimental conditions in which β-cells are initially exposed to a glucose-free solution that depletes intracellular ATP (Holz et al. 1993). Under these conditions, transient reintroduction of glucose weakly inhibits K_{ATP} channel activity, and this action of glucose is greatly potentiated by GLP-1, thereby generating "bursts" of action potentials (Holz et al. 1993). Such a restorative action of GLP-1 might reflect its ability to stimulate β-cell glucose metabolism. Alternatively, it might reflect an ability of GLP-1 to alter the adenine nucleotide sensitivity of K_{ATP} channels so that these channels will close more efficiently in response to an increase of cytosolic ATP/ADP concentration ratio that glucose metabolism produces (Tarasov et al. 2013).

Studies of mice lacking SUR1 and Kir6.2 subunits of K_{ATP} channels provide evidence for a K_{ATP} channel-dependent action of GLP-1 to stimulate insulin secretion. In these SUR1 and Kir6.2 KO mice, GLP-1 potentiation of GSIS is absent (Nakazaki et al. 2002; Shiota et al. 2002) or reduced (Miki et al. 2005). Furthermore, in mice harboring a tyrosine-to-stop codon (Y12STOP) mutation in the gene coding for Kir6.2, K_{ATP} channel expression and GLP-1-stimulated insulin secretion are absent (Hugill et al. 2010). Important findings are also provided by a study of patients with neonatal diabetes mellitus (NDM) owing to gain-of-function mutations (C435R; R1380) in the gene coding for SUR1 (Bourron et al. 2012). These mutations lead to overactive K_{ATP} channels and a consequent reduction of GSIS. Remarkably, the administration of a GLP-1R agonist restores insulin secretion in these patients.

PKA and Epac2 mediate the action of GLP-1 to close K_{ATP} channels such that PKA reduces the stimulatory action of Mg-ADP at SUR1 (Light et al. 2002), whereas Epac2 enhances the inhibitory action of ATP at Kir6.2 (Kang et al. 2008). The net effect is that GLP-1 produces a left shift in the dose-response relationship describing how an increase of cytosolic ATP/ADP concentration ratio (x-axis) inhibits K_{ATP} channel activity (y-axis). This mechanism of K_{ATP} channel modulation underlies the ability of GLP-1 to act as a β-cell glucose sensitizer so that it can facilitate glucose metabolism-dependent depolarization of β-cells (Holz et al. 1993). Numerous studies of human, rat, and mouse β-cells demonstrate that the glucose-dependent depolarizing action of GLP-1 in β-cells is reproduced by cAMP-elevating agents such as forskolin, IBMX, and glucagon, or by membrane-permeant cAMP analogs (Henquin and Meissner 1983; Henquin et al. 1983; Henquin and Meissner 1984a, b; Eddlestone et al. 1985; Barnett et al. 1994; He et al. 1998; Gromada et al. 1998a; Fernandez and Valdeolmillos 1999;

Suga et al. 2000; Ding et al. 2001; McQuaid et al. 2006; Kang et al. 2006, 2008; Chepurny et al. 2010; Leech et al. 2010b, 2011).

Under conditions of K_{ATP} channel closure in which β-cell depolarization initiates bursts of action potentials, there also exists an effect of GLP-1 to inhibit the delayed rectifier voltage-dependent K^+ current (MacDonald et al. 2003). This action of GLP-1 in β-cells is mediated by the PKA signaling pathway in conjunction with epidermal growth factor transactivation signaling that stimulates PI-3K and PKCζ activities (MacDonald et al. 2003). By inhibiting the voltage-dependent K^+ current (K_v), GLP-1 prolongs the action potential duration, thereby enhancing Ca^{2+} influx through VDCCs (Yada et al. 1993).

Nonselective cation channels (NSCCs) activated by GLP-1 in β-cells provide a depolarizing inward Na^+ current that is also important to action potential generation (Holz et al. 1995; Leech and Habener 1997). These channels are dually stimulated by cAMP and Ca^{2+}, and they appear to be a subtype of Ca^{2+} -activated NSCC, although their molecular identities remain to be ascertained. New data indicate that β-cell NSCCs are activated by Epac2 (Yoshida et al. 2012; Jarrard et al. 2013). Therefore, Epac2 might mediate a stimulatory action of GLP-1 at these channels in order to promote Ca^{2+} influx.

GLP-1 might also promote Ca^{2+} influx by upregulating β-cell glucose metabolism that closes K_{ATP} channels. For example, GLP-1 is reported to signal through cAMP and Epac2 to increase β-cell glucokinase (GK) activity (Ding et al. 2011; Park et al. 2012). Since GK activity constitutes the rate-limiting step in β-cell glucose sensing, any Epac2-mediated action of GLP-1 at GK is expected to be of major physiological significance. Potentially just as important is one report that GLP-1 stimulates mitochondrial ATP production in a β-cell line (MIN6 cells) (Tsuboi et al. 2003). However, studies using human and rodent islets dispute all of these findings (Peyot et al. 2009; Doliba et al. 2012a; Song et al. 2013), leaving it unclear whether GLP-1 does in fact stimulate β-cell glucose metabolism.

Restoration Of GSIS In T2DM: A Role For Ca^{2+} Mobilization

GLP-1 and various cAMP-elevating agents such as forskolin and PACAP mobilize an intracellular source of Ca^{2+} in β-cells (Leech et al. 2011). Thus, a Ca^{2+} mobilizing action of GLP-1 is expected to become important under conditions of T2DM in which the ability of glucose metabolism to stimulate β-cell Ca^{2+} influx is impaired. Furthermore, since β-cell mitochondrial ATP production is stimulated by Ca^{2+} released from endoplasmic reticulum (ER) Ca^{2+} stores (Tsuboi et al. 2003), the ER Ca^{2+} mobilizing action of GLP-1 might lead to a restoration of ATP production in β-cells of patients with T2DM. Therefore, it is of interest to summarize what is known concerning how GLP-1 acts via cAMP, PKA, and Epac2 to mobilize Ca^{2+} in β-cells.

As illustrated in Fig. 1, the Ca^{2+} mobilizing action of GLP-1 is explained by PKA-mediated phosphorylation of IP_3 receptor (IP_3R) and ryanodine receptor (RYR) intracellular Ca^{2+} release channels located on the ER (Holz et al. 1999;

Dyachok and Gylfe 2004; Islam et al. 1998). When considering the IP_3R, the second messenger IP_3 acts as a co-agonist with Ca^{2+} to gate the opening of IP_3R, and this process is facilitated by GLP-1 in a PKA-dependent manner. Similarly, GLP-1 sensitizes RYR to the stimulatory action of Ca^{2+} in order to facilitate Ca^{2+}-induced Ca^{2+} release (CICR) from the ER. When β-cells are exposed only to glucose, resultant Ca^{2+} influx has a limited ability to promote Ca^{2+} release from the ER. However, ER Ca^{2+} release is more efficiently triggered under conditions in which β-cells are simultaneously exposed to glucose and GLP-1. These findings lead us to propose that in T2DM, there is weak Ca^{2+} influx initiated by unhealthy β-cell glucose sensing and that GLP-1 compensates for this defect by facilitating ER Ca^{2+} release, thereby restoring a cytosolic Ca^{2+} signal important to GSIS.

As illustrated in Fig. 4, an Epac2-mediated action of GLP-1 complements these PKA-dependent mechanisms of Ca^{2+} mobilization (Kang et al. 2001, 2003, 2005). It mobilizes Ca^{2+} from the ER of β-cells, and it results from Epac2-dependent activation of a Rap1-regulated PLCϵ (Dzhura et al. 2010). Thus, PLCϵ links GLP-1R-stimulated cAMP production to PIP_2 hydrolysis with resultant IP_3 production, IP_3R activation, and ER Ca^{2+} mobilization. Simultaneously, DAG production and PKC activation initiate a signaling cascade that culminates with Ca^{2+}/calmodulin-dependent protein kinase-II (CaM-KII)-catalyzed phosphorylation of RYR in order to facilitate CICR (Dzhura et al. 2010). Remarkably, this Epac2-mediated action of GLP-1 to control RYR is similar to that which is described for ventricular cardiomyocytes in which RYR is under the control of β_1-adrenergic receptors (Oestreich et al. 2007, 2009). Just as intriguing, GLP-1 acts via Epac2 and PIP_2 hydrolysis in order to stimulate atrial natriuretic peptide (ANP) release from atrial cardiomyocytes (Kim et al. 2013). Thus, it appears that an evolutionarily conserved cAMP signaling "module" comprised of Epac2, Rap1, and PLCϵ controls CICR in β-cells and cardiomyocytes, while also promoting Ca^{2+}-dependent exocytosis of secretory granules in β-cells that contain insulin and in cardiomyocytes that contain ANP.

Less well understood is the action of GLP-1 to stimulate cyclic ADP-ribose (cADP-R) and nicotinic acid adenine dinucleotide phosphate (NAADP) production in order to mobilize Ca^{2+} from the ER, endosomes, and lysosomes of β-cells (Kim et al. 2008b). Evidently, cADP-R promotes RYR-mediated CICR, whereas NAADP acts directly at 2-pore Ca^{2+} release channels (TPCs). The NAADP receptor antagonist Ned-19 reduces GSIS from mouse islets, thereby demonstrating a clear functional link between intracellular Ca^{2+} mobilization and insulin exocytosis (Naylor et al. 2009).

When considering how cAMP-dependent intracellular Ca^{2+} mobilization influences insulin secretion, there is reason to believe that Ca^{2+} released in this manner promotes the activation of NSCCs located in the plasma membrane. Since NSCCs generate a depolarizing inward Na^+ current, their activation increases β-cell excitability in order to generate bursts of action potentials, especially under conditions of high membrane resistance in which K_{ATP} channels are closed (Cha et al. 2011). The ensuing increase of $[Ca^{2+}]_i$ is then reversed by cAMP-stimulated reuptake of Ca^{2+} into the ER (Yaekura and Yada 1998). Although ER Ca^{2+} depletion that

accompanies ER Ca^{2+} release is expected to activate store-operated Ca^{2+} channels in the plasma membrane, the existence of a cAMP-regulated store-operated Ca^{2+} current (SOC) in β-cells is questioned since cAMP fails to promote association of ER Ca^{2+} sensor Stim1 with the pore-forming subunit Orai1 of store-operated Ca^{2+} channels located in the plasma membrane (Tian et al. 2012).

As illustrated in Fig. 4, remarkable findings exist concerning PLCϵ KO mice. First, the Ca^{2+} mobilizing action of selective Epac activator 8-pCPT-2′-*O*-Me-cAMP-AM is nearly abolished in PLCϵ KO mice (Dzhura et al. 2010). Second, islets of PLCϵ KO mice are smaller in diameter and contain less insulin than control wild-type (WT) mice (Dzhura et al. 2011), a finding that is consistent with the established role of PLCϵ in growth control processes in other cell types (Smrcka et al. 2012). Especially interesting are findings that 8-pCPT-2′-*O*-Me-cAMP-AM stimulates β-cell PIP_2 hydrolysis (Leech et al. 2010b; Kumar et al. 2012) but that it has a reduced capacity to potentiate GSIS from islets of PLCϵ KO mice (Dzhura et al. 2011). Since the Ca^{2+} mobilizing action of 8-pCPT-2′-*O*-Me-cAMP-AM in WT mouse β-cells is disrupted by a Rap-GAP that inactivates Rap1 (Dzhura et al. 2010), it is clear that PLCϵ is a downstream target of Epac2 and Rap1 for cAMP-dependent control of insulin secretion (Shibasaki et al. 2007; Kelly et al. 2008).

In Vivo Studies Of The cAMP: PKA Signaling Branch In β-Cells

Defined genetic mouse models allow detailed in vivo analyses of the GLP-1 signaling pathways in β-cells. Specifically, the cAMP-PKA and cAMP-EPAC2A signaling branches within β-cells can be individually investigated using these models. Here, we adopt a standard classification scheme for naming the multiple isoforms of PKA regulatory subunits (Taylor et al. 2008), and we also adopt terminology in which Epac2A (i.e., full-length Epac2) is the predominant isoform of Epac2 expressed in β-cells (Niimura et al. 2009).

The PKA holoenzyme consists of the catalytic subunit (PKAcs) bound to four different regulatory subunits (PKARs 1a, 1b, 2a, and 2b). Among these, PKARs1a (prkar1a) is highly expressed in pancreatic islets (Petyuk et al. 2008). To investigate the cAMP-PKA signaling branch in β-cells, it is possible to use a mouse model specifically lacking pancreatic prkar1a (Δprkar1a) (Song et al. 2011) by interbreeding PDX1-CRE deleter mice (Gu et al. 2002) with prkar1a floxed mice (Kirschner et al. 2005). As expected, Δprkar1a islets do not contain prkar1a, whereas PKAcs activity is increased, as reflected by increased phosphorylation of PKAcs target CREB (Song et al. 2011). Thus, Δprkar1a mice exhibit PKA activity that is constitutively elevated in their islets. The islet and β-cell mass of Δprkar1a and control wild-type (WT) littermates are similar, indicating that constitutively increased islet PKA activity does not increase β-cell proliferation in vivo (Song et al. 2011). Furthermore, the proliferation marker Ki67 is also similar in Δprkar1a and WT littermates. Based on these observations, it appears that increased β-cell proliferation is not achieved after selective activation of the

cAMP-PKA signaling branch. Such findings are remarkable in view of the fact that β-cell proliferation is stimulated in WT mice during pharmacologic activation of the GLP-1R with exendin-4 (Song et al. 2008).

When examined at baseline fasting conditions, Δprkar1a mice do not show any abnormalities in glucose homeostasis (Song et al. 2011). Baseline glucose and insulin levels are similar to those in control littermates. However, Δprkar1a mice exhibit augmented insulin secretion, as measured in an intraperitoneal glucose tolerance test (ipGTT). GSIS is prompt and serum insulin concentrations after acute administration of glucose are eight- to tenfold higher than in littermate controls. These findings obtained with Δprkar1a mice are similar to findings obtained using mice that are engineered to allow inducible expression of a constitutively active PKAcs transgene specifically in β-cells (Kaihara et al. 2013). In these studies of transgenic mice, PKAcs activity can be induced in adult mice, which are then evaluated in an ipGTT at different glucose doses. As is the case for Δprkar1a mice, these transgenic mice with constitutively increased PKAcs activity show augmented GSIS at every glucose dose administered (Kaihara et al. 2013).

Collectively, these observations obtained with two mouse models show that specific upregulation of cAMP-PKA signaling – as found during pharmacologic GLP-1R stimulation – (a) retains β-cell glucose responsiveness and (b) allows insulin secretion to be shut off at glucose levels below physiologic fasting glycemia, and (c) at glucose levels above physiologic fasting levels, insulin secretion is dramatically augmented. Given that a whole-body KO of Epac2 does not disrupt GSIS in healthy mice (Song et al. 2013; see below), these findings suggest that in healthy β-cells, the cAMP-PKA signaling branch can in fact mediate the potentiation of endogenous incretin action. However, one caveat to this interpretation is that mouse models of constitutive PKA activity do not necessarily recapitulate compartmentalized cAMP signaling that is expected to occur in β-cells after pharmacologic GLP-1R agonist stimulation (Holz et al. 2008b). Furthermore, since these engineered mice have chronically elevated PKA activity, enhanced GSIS could reflect alterations of β-cell gene expression that are secondary to CREB activation (Dalle et al. 2011b).

It remains unclear how the cAMP-PKA signaling branch modulates glucose-stimulated Ca^{2+} handling under conditions of constitutive PKA activity. Based on findings obtained in single cell assays of β-cell depolarization, PKA should sensitize β-cells to the stimulatory action of glucose (Holz et al. 1993). Thus, the consequences of increased PKA activity need to be studied over a full range of glucose concentrations. With this limitation in mind, healthy β-cells with inducible and cell-specific transgenic PKAcs overexpression do not show any appreciable change in Ca^{2+} dynamics in response to a high (i.e., saturating) concentration of glucose (Kaihara et al. 2013). In contrast, islets from Δprkar1a mice show increased Ca^{2+} dynamics after glucose stimulation (Song et al. 2013). These divergent findings obtained using different mouse models may be explained by different experimental approaches such as nonidentical means of activating PKA, different glucose concentrations tested, and differences in the outcomes of single β-cell vs. whole islet measurements.

An important aspect of compartmentalized cAMP signaling in the β-cell is that PKA-anchoring proteins (A kinase-anchoring proteins; AKAPs) bind PKA regulatory subunits in order to control and define the subcellular location of PKAcs function/activity (Welch et al. 2010). It may be concluded that subcellular anchoring of PKA is required in order for GLP-1 to stimulate insulin secretion (Lester et al. 1997; Fraser et al. 1998). Thus, pharmacologic disruption of PKA anchoring impairs cAMP-dependent potentiation of GSIS (Lester et al. 1997). Still, it should be noted that AKAPs also anchor Epac proteins within defined subcellular compartments (Hong et al. 2008; Nijholt et al. 2008). Furthermore, AKAPs can anchor protein phosphatase 2B (PP2B), PKC, and PDEs (Scott and Santana 2010). It may be concluded that the potential exists for highly coordinate β-cell cAMP signaling involving PKA, Epac2, PP2B, PKC, and PDEs.

Global disruption of AKAP150 gene expression in mice impairs the ability of these mice to respond to a glucose challenge with insulin secretion while also inhibiting the action of cAMP to potentiate GSIS (Hinke et al. 2012). Furthermore, the lack of AKAP150 impairs the functionality of L-type Ca^{2+} channels and Ca^{2+} handling in the β-cell (Hinke et al. 2012). Surprisingly, in the absence of AKAP150 there is increased insulin sensitivity, thereby improving glucose tolerance in these AKAP150 KO mice (Hinke et al. 2012). Equally surprising are findings obtained using AKAP150 knock-in mice that harbor mutations in binding motifs of AKAP150 that normally permit it to interact with PKA regulatory subunits or PP2B. These studies demonstrate that a disruption of the PP2B-binding site, but not the PKA-binding site, replicates the metabolic phenotype of the whole-body AKAP150 KO (Hinke et al. 2012). This finding confirms the importance of AKAP150 in β-cell function, albeit surprisingly pointing toward a central role for the anchoring of PP2B by AKAP150. In this regard, it is noteworthy that GSIS is accompanied by PP2B-catalyzed dephosphorylation of kinesin heavy chain (Donelan et al. 2002), a component of the microtubule-associated motor protein kinesin that plays a role in the transport of secretory granules to the plasma membrane.

MyRIP (myosin and rab-interacting protein) is an AKAP that anchors PKA to the exocyst complex, an assembly of proteins that mediates secretory granule trafficking and targeting to the plasma membrane. In rat INS-1 insulin-secreting cells, an siRNA-mediated knockdown of MyRIP disrupts exocytosis in response to glucose and forskolin, and in these cells, MyRIP interacts with the Sec6 and Sec8 components of the exocyst complex (Goehring et al. 2007). Studies further indicate a reciprocal interplay between cAMP-PKA and MyRIP. While MyRIP anchors PKA to the exocyst complex, MyRIP is also phosphorylated in response to cAMP-PKA. Phospho-MyRIP in turn associates with Myosin Va (MyoVa), a motor protein involved in the transport of secretory granules (Brozzi et al. 2012). Phosphorylation of MyRIP also leads to increased phosphorylation of the MyoVa docking-receptor Rph-3A. Collectively, these data indicate that when cAMP levels are elevated, MyRIP forms a functional protein complex with MyoVa on secretory granules in order to promote secretory granule transport. Furthermore, MyRIP facilitates PKA-mediated phosphorylation of secretory granule-associated proteins to enhance exocytosis (Brozzi et al. 2012).

The SNARE complex-associated protein snapin is an established target of PKA in neurons (Chheda et al. 2001), and snapin is expressed at high levels in β-cells, where PKA activation induces its phosphorylation at serine residue 50 (Song et al. 2011). As illustrated in Fig. 2, snapin phosphorylation facilitates interactions between the vesicle-associated SNARE protein (v-SNARE; VAMP2) and the target cell surface-associated protein (t-SNARE; SNAP-25) (Chheda et al. 2001; Song et al. 2011). Interestingly, SNAP-25 also interacts with Epac2A in β-cells (Vikman et al. 2009; Song et al. 2011). These findings suggest a scenario in which multiple cAMP-dependent signaling pathways converge to assemble a complex in which each participating protein concentrates its functional role at the site of imminent exocytosis.

Another interesting aspect of snapin biology is that when mice are rendered glucose intolerant after receiving a lipid-enriched (high-fat) diet (60 % calories from saturated fats), snapin is hyperglycosylated with *N*-acetyl-glucosamine at amino acid residue serine 50 (O-GlcNac). Activation of GLP-1R signaling reverses snapin-*O*-GlcNacylation at serine 50 and favors S50 phosphorylation, thereby enabling snapin to associate with SNAP-25 and Epac2A (Song et al. 2011). This finding provides a unifying molecular mechanism for β-cell dysfunction, which occurs at the level of exocytosis and is rapidly and effectively reversed by pharmacologic GLP-1R agonists.

In Vivo Studies Of The cAMP: Epac2A Signaling Branch In β-Cells

The discovery of cAMP-regulated guanine nucleotide exchange factors designated as cAMP-GEF-I and cAMP-GEF-II (now known as Epac1 and Epac2) by two independent groups (de Rooij et al. 1998; Kawasaki et al. 1998) provides an explanation for PKA-independent control of insulin secretion by cAMP (Renstrom et al. 1997; Kashima et al. 2001; Nakazaki et al. 2002; Eliasson et al. 2003; Hashiguchi et al. 2006; Kwan et al. 2007; Kelley et al. 2009; Vikman et al. 2009; Chepurny et al. 2010; Idevall-Hagren et al. 2010; Dzhura et al. 2011). Consistent with the expression of Epac2 in β-cells (Leech et al. 2000; Ozaki et al. 2000), there exist PKA-independent stimulatory actions of cAMP to raise levels of Ca^{2+} in β-cells (Bode et al. 1999; Kang et al. 2001). As illustrated in Fig. 3, the PKA-independent action of cAMP to potentiate GSIS is mediated by Epac2 and its partner Rap1.

Epac2A, the full-length form of Epac2, is the predominant isoform of Epac expressed in β-cells (Niimura et al. 2009). An assessment of the role of Epac2A in the control of GSIS can be achieved using recently developed tools including membrane-permeable Epac-selective cAMP analogs (ESCAS) that activate Epac proteins but not PKA when used at low concentrations (Vliem et al. 2008; Chepurny et al. 2009), specific small molecular Epac2 inhibitors (Tsalkova et al. 2012; Chen et al. 2013), and whole-body Epac2A KO mice (Shibasaki et al. 2007; Dzhura et al. 2010), double Epac1 and Epac2 KO mice (Yang et al. 2012), as well as floxed Epac2A mice for the cell type-specific KO of Epac1 or Epac2 (Pereira et al. 2013). Thus, there exist new strategies with which to

assess the importance of Epac2 to the control of GSIS. Initial findings indicate that β-cell mass is preserved in whole-body Epac2 KO mice, whereas a defect of glucoregulation is measurable when these mice are fed with a high-fat diet (Song et al. 2013).

The generation of a mouse model with a whole-body KO of Epac2A gene expression greatly advances our understanding of how Epac2A influences insulin secretion (Song et al. 2013). β-cells and islets from Epac2A KO mice exhibit smaller elevations of cytosolic Ca^{2+} concentration in response to GLP-1R agonist exendin-4 (Dzhura et al. 2010; Song et al. 2013), and this impairment correlates with a reduced potentiation of first phase GSIS by exendin-4 in vitro (Song et al. 2013). Furthermore, in vivo assays demonstrate a reduced insulin secretagogue action of exendin-4, as measured in Epac2A KO mice following intraperitoneal administration of both glucose and exendin-4 (Song et al. 2013). Thus, Epac2A mediates, at least in part, GLP-1R agonist action to potentiate GSIS.

Epac2A interacts with secretory granule and SNARE complex-associated proteins that are important to insulin secretion (Seino et al. 2009). Since these interactions are absent in Epac2A KO mice, the reduced insulin secretagogue action of exendin-4 in Epac2A KO mice may be explained, at least in part, by the failure of cAMP to directly stimulate secretory granule exocytosis in β-cells. As illustrated in Fig. 5, imaging studies with β-cells of Epac2 KO mice indicate that Epac2 mediates cAMP-dependent potentiation of a novel mechanism of exocytosis in which secretory granules located in the cytoplasm transit to the plasma membrane where they undergo immediate release, a process of Ca^{2+}-dependent exocytosis designated as "restless newcomer" exocytosis (Shibasaki et al. 2007). Although the molecular basis for restless newcomer exocytosis is not known, it could be that this mechanism of exocytosis requires direct interactions of Epac2 with secretory granule or SNARE complex-associated proteins. Thus, the reduced capacity of exendin-4 to potentiate first phase GSIS in islets of Epac2 KO mice may be explained not only by defective Ca^{2+} handling in the β-cell but also by the failure of cAMP to directly promote restless newcomer exocytosis.

The understanding of Epac2A function in β-cells broadened dramatically when the Seino laboratory identified Epac2A as a direct cellular target of the sulfonylurea class of blood glucose-lowering agents (Zhang et al. 2009). While Epac2A KO mice exhibit normal oral and intraperitoneal glucose tolerance at baseline, they remarkably exhibit a reduced response to sulfonylureas (Zhang et al. 2009). Thus, the sulfonylureas are proposed to function by two distinct mechanisms: (a) they bind to SUR1 in order to close β-cell K_{ATP} channels and to promote Ca^{2+}-dependent insulin secretion independently of glucose metabolism, and (b) they bind to Epac2A in order to directly potentiate GSIS.

As illustrated in Fig. 4, under conditions in which β-cells are exposed to a low concentration of glucose, sulfonylureas directly inhibit K_{ATP} channels in order to generate a Ca^{2+} signal that stimulates exocytosis of secretory granules prepositioned at the plasma membrane where they are "docked" and "primed." When β-cells are exposed to a stimulatory concentration of glucose, it could be that sulfonylureas also act via Epac2A to enhance glucose-dependent restless newcomer exocytosis. These

considerations are of therapeutic significance in view of the finding that gliclazide is unique among sulfonylureas in that it does not activate Epac2A, but binds only to SUR1 to close K_{ATP} channels (Zhang et al. 2009). What remains to be determined is exactly how sulfonylureas activate Epac2A. Since Epac2-dependent Rap1 activation by sulfonylureas is not measurable in a solution assay using recombinant Epac2A and Rap1 (Tsalkova et al. 2011; Rehmann 2012), it could be that sulfonylureas act indirectly to activate Epac2 in living cells, as might be expected since high concentrations of sulfonylureas elevate levels of cAMP by inhibiting PDEs in islets (Goldfine et al. 1971). However, PDE inhibition may not be a factor since imaging studies of living cells indicate that sulfonylureas activate Epac2A but not Epac1 (Herbst et al. 2011).

Studies with Epac2A KO mice clarify distinctions between the functional roles of Epac2A and PKA in β-cells (Song et al. 2013). Epac2A KO mice, as compared to WT littermates, exhibit impaired adaptation of insulin secretion in response to insulin resistance induced by a short-term (1 month) high-fat content diet (60 % calories from saturated fats). In addition, when the cAMP-PKA branch is disinhibited in Δprkar1a mice, the additional absence of Epac2A blunts the augmented GSIS, which is seen in Δprkar1a.

Epac2A KO mice fed with a normal diet show reduced responsiveness to GLP-1R activation by exendin-4 in an in vivo assay of insulin secretion, and this is also the case for in vitro studies examining insulin secretion from isolated islets of Epac2A KO mice (Song et al. 2013). Remarkably, Epac2A KO mice also show reduced potentiation of GSIS in response to pharmacologic activation of GPR40, a GPCR for long-chain fatty acids (Song et al. 2013). This finding is unexpected because GPR40 activation potentiates GSIS in a cAMP-independent manner, one involving PLCβ, PIP_2 hydrolysis, and Ca^{2+} mobilization (Mancini and Poitout 2013). Clearly, a better understanding of GPR40 is warranted in view of the fact that GPR40 agonists are in early phases of clinical use for the treatment of T2DM (Mancini and Poitout 2013).

In summary, Epac2A appears to be a key molecule that is required for the β-cell to respond functionally to increased insulin resistance (as found after high-fat diet) as well as to a multitude of β-cell-targeted secretagogues (GLP-1R agonists, sulfonylureas, GPR40 activators). Thus, Epac2A selective activators may constitute a new class of blood glucose-lowering agents for pharmacological intervention in the treatment of T2DM. Future studies will also be required to examine the role of Epac2A in the pathogenesis of β-cell dysfunction in T2DM and/or the metabolic syndrome. Future studies using mouse models with cell- and tissue-specific Epac2A ablation will also be necessary to discriminate any metabolic effects of Epac2A in non-β-cells.

Conclusion

Plasticity in the β-cell cAMP signaling network is increasingly viewed as an adaptive response to metabolic demands imposed by changes in nutritional status, or in response to pathophysiological processes such as insulin resistance and

glucolipotoxicity (Hinke et al. 2004). The short-term outcomes of altered cAMP signaling include a restoration of Ca^{2+} handling and secretory granule exocytosis in experimental models of T2DM. These changes induced by cAMP occur within minutes, and they result from PKA-mediated phosphorylation of snapin accompanied by Epac2-mediated activation of Rap1 and PLCϵ. Less well understood are long-term changes of β-cell function in response to cAMP. These changes can occur on a time scale of hours, days, weeks, or months and are explained by changes in gene expression for key transcription factors, enzymes of glucose metabolism, and mediators of insulin exocytosis. Whereas PKA predominates as a stimulus for insulin secretion in healthy β-cells, a role for Epac2 in the control of GSIS is revealed under conditions of a high-fat diet (HFD).

The HFD mouse model of T2DM is characterized by compensatory islet hyperplasia with increased β-cell mass and intact β-cell glucose sensitivity, but exaggerated insulin secretion that counters peripheral insulin resistance (Winzell and Ahrén 2004). With continued administration of the HFD, there is reduced β-cell mass, diminished β-cell glucose sensitivity, and a loss of GSIS. GLP-1R agonists correct for these defects, either by preserving β-cell mass or by restoring β-cell glucose sensitivity. Compensatory processes induced by the HFD lead to a situation in which Epac2 becomes of critical importance to GSIS, even in the absence of administered GLP-1R agonists (Song et al. 2013). Why this is the case is not clear, but it might indicate that under conditions of the HFD, glucose metabolism is coupled to cAMP production and Epac2 activation in order to stimulate insulin secretion.

Equally intriguing is the finding that GLP-1R expression is upregulated in islets of mice fed with the HFD (Ahlkvist et al. 2013) and that Epac2 expression is stimulated after treatment of T2DM donor human islets with GLP-1R agonist exendin-4 (Lupi et al. 2008). Since insulin resistance is characteristic of both T2DM and the HFD mouse model, it is possible that trophic factors such as betatrophin released from the liver circulate in response to diminished insulin action (Yi et al. 2013) in order to control the expression and/or function of the GLP-1R and Epac2 in β-cells. Alternatively, the HFD might induce epigenetic control of cAMP signaling, as recently demonstrated for Epac2 (Lee et al. 2012).

Based on studies of rodents, it is possible that in human T2DM, there is an uncoupling of β-cell glucose metabolism to cAMP production (Abdel-Halim et al. 1996; Dachicourt et al. 1996). However, in the Goto-Kakazaki (GK) rat model of T2DM, a secretory defect exists in which GSIS is downregulated despite the fact that glucose-dependent cAMP production is elevated (Dolz et al. 2011). Treatment of GK islets with GLP-1 produces an exaggerated stimulation of cAMP production, thereby restoring GSIS (Dolz et al. 2011). Since Epac2 is less sensitive to cAMP in comparison with PKA (Holz et al. 2008a), it could be that Epac2 is recruited by GLP-1 into the β-cell cAMP signaling network in order to achieve this restoration of GSIS. Importantly, such an Epac2-mediated action of GLP-1 would be conditional on basal PKA activity that supports exocytosis (Chepurny et al. 2010). Thus, a new paradigm may be evident in which β-cell stimulus-secretion coupling under the control of glucose and cAMP exhibits plasticity such

that the relative importance of PKA and Epac2 to GSIS is determined by nutritional and metabolic status. The challenge now is to relate these findings concerning mice or rats to our understanding of human T2DM, while also seeking to identify new strategies with which to manipulate the β-cell cAMP signaling network.

Acknowledgments This work was supported by American Diabetes Association Basic Science Awards to GGH (7-12-BS-077) and CAL (1-12-BS-109). National Institutes of Health funding was provided to GGH (DK069575) and MAH (DK090245, DK090816, DK084949, DK079637). GGH and OGC acknowledge the support of SUNY Upstate Medical University.

Cross-References

▶ ATP-Sensitive Potassium Channels in Health and Disease
▶ Calcium Signaling in the Islets
▶ Electrical, Calcium, and Metabolic Oscillations in Pancreatic Islets
▶ Electrophysiology of Islet Cells
▶ Exocytosis in Islet β-Cells

References

Abdel-Halim SM, Guenifi A, Khan A, Larsson O, Berggren PO, Ostenson CG, Efendić S (1996) Impaired coupling of glucose signal to the exocytotic machinery in diabetic GK rats: a defect ameliorated by cAMP. Diabetes 45:934–940

Ahlkvist L, Brown K, Ahrén B (2013) Upregulated insulin secretion in insulin-resistant mice: evidence of increased islet GLP-1 receptor levels and GPR119-activated GLP-1 secretion. Endocr Connect 2:69–78

Ahrén B (2000) Autonomic regulation of islet hormone secretion – implications for health and disease. Diabetologia 43:393–410

Ahrén B (2008) Role of pituitary adenylate cyclase-activating polypeptide in the pancreatic endocrine system. Ann N Y Acad Sci 1144:28–35

Ahrén B (2009) Islet G protein-coupled receptors as potential targets for treatment of type 2 diabetes. Nat Rev Drug Discov 8:369–385

Altarejos JY, Montminy M (2011) CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. Nat Rev Mol Cell Biol 12:141–151

Ämmälä C, Ashcroft FM, Rorsman P (1993) Calcium-independent potentiation of insulin release by cyclic AMP in single β-cells. Nature 363:356–358

Arnette D, Gibson TB, Lawrence MC, January B, Khoo S, McGlynn K, Vanderbilt CA, Cobb MH (2003) Regulation of ERK1 and ERK2 by glucose and peptide hormones in pancreatic β cells. J Biol Chem 278:32517–32525

Baggio LL, Drucker DJ (2007) Biology of incretins: GLP-1 and GIP. Gastroenterology 132:2131–2157

Barnett DW, Pressel DM, Chern HT, Scharp DW, Misler S (1994) cAMP-enhancing agents "permit" stimulus-secretion coupling in canine pancreatic islet β-cells. J Membr Biol 138:113–120

Bastien-Dionne PO, Valenti L, Kon N, Gu W, Buteau J (2011) Glucagon-like peptide-1 inhibits the sirtuin deacetylase SirT1 to stimulate pancreatic β-cell mass expansion. Diabetes 60:3217–3222

Betz A, Ashery U, Rickmann M, Augustin I, Neher E, Südhof TC, Retting J, Brose N (1998) Munc13-1 is a presynaptic phorbol ester receptor that enhances neurotransmitter release. Neuron 21:123–136

Bode HP, Moormann B, Dabew R, Göke B (1999) Glucagon-like peptide-1 elevates cytosolic calcium in pancreatic β-cells independently of protein kinase A. Endocrinology 140:3919–3927

Bourron O, Chebbi F, Halbron M, Saint-Martin C, Bellanné-Chantelot C, Abed A, Charbit B, Magnan C, Lacorte JM, Hartemann A (2012) Incretin effect of glucagon-like peptide-1 receptor agonist is preserved in presence of ABCC8/SUR1 mutation in β-cell. Diabetes Care 35:e76

Brozzi F, Lajus S, Diraison F, Rajatileka S, Hayward K, Regazzi R, Molnár E, Váradi A (2012) MyRIP interaction with MyoVa on secretory granules is controlled by the cAMP-PKA pathway. Mol Biol Cell 23:4444–4455

Brunzell JD, Robertson RP, Lerner RL, Hazzard WR, Ensinck JW, Biermal FL, Porte D Jr (1976) Relationships between fasting plasma glucose levels and insulin secretion during intravenous glucose tolerance tests. J Clin Endocrinol Metab 42:222–229

Burcelin R (2010) The gut-brain axis: a major glucoregulatory player. Diabetes Metab 36(Suppl 3):S54–S58

Buteau J, Roduit R, Susini S, Prentki M (1999) Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in β (INS-1)-cells. Diabetologia 42:856–864

Buteau J, Foisy S, Rhodes CJ, Carpenter L, Biden TJ, Prentki M (2001) Protein kinase Cζ activation mediates glucagon-like peptide-1-induced pancreatic β-cell proliferation. Diabetes 50:2237–2243

Buteau J, Foisy S, Joly E, Prentki M (2003) Glucagon-like peptide-1 induces pancreatic β-cell proliferation via transactivation of the epidermal growth factor receptor. Diabetes 52:124–132

Buteau J, Spatz ML, Accili D (2006) Transcription factor FoxO1 mediates glucagon-like peptide-1 effects on pancreatic β-cell mass. Diabetes 55:1190–1196

Butler AE, Campbell-Thompson M, Gurlo T, Dawson DW, Atkinson M, Butler PC (2013) Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. Diabetes 62:2595–2604

Cha CY, Powell T, Noma A (2011) Analyzing electrical activities of pancreatic β cells using mathematical models. Prog Biophys Mol Biol 107:265–273

Charles MA, Lawecki J, Pictet R, Grodsky GM (1975) Insulin secretion. Interrelationships of glucose, cyclic adenosine 3′:5′-monophosphate, and calcium. J Biol Chem 250:6134–6140

Chen H, Tsalkova T, Chepurny OG, Mei FC, Holz GG, Cheng X, Zhou J (2013) Identification and characterization of small molecules as potent and specific EPAC2 antagonists. J Med Chem 56:952–962

Chepurny OG, Hussain MA, Holz GG (2002) Exendin-4 as a stimulator of rat insulin I gene promoter activity via bZIP/CRE interactions sensitive to serine/threonine protein kinase inhibitor Ro 31–8220. Endocrinology 143:2303–2313

Chepurny OG, Leech CA, Kelley GG, Dzhura I, Dzhura E, Li X, Rindler MJ, Schwede F, Genieser HG, Holz GG (2009) Enhanced Rap1 activation and insulin secretagogue properties of an acetoxymethyl ester of an Epac-selective cyclic AMP analog in rat INS-1 cells: studies with 8-pCPT-2′–O-Me-cAMP-AM. J Biol Chem 284:10728–10736

Chepurny OG, Kelley GG, Dzhura I, Leech CA, Roe MW, Dzhura E, Li X, Schwede F, Genieser HG, Holz GG (2010) PKA-dependent potentiation of glucose-stimulated insulin secretion by Epac activator 8-pCPT-2′–O-Me-cAMP-AM in human islets of Langerhans. Am J Physiol Endocrinol Metab 298:E622–E633

Chheda MG, Ashery U, Thakur P, Rettig J, Sheng ZH (2001) Phosphorylation of snapin by PKA modulates its interaction with the SNARE complex. Nat Cell Biol 3:331–338

Chu ZL, Jones RM, He H, Carroll C, Gutierrez V, Lucman A, Moloney M, Gao H, Mondala H, Bagnol D, Unett D, Liang Y, Demarest K, Semple G, Behan DP, Leonard J (2007) A role for β-cell-expressed G protein-coupled receptor 119 in glycemic control by enhancing glucose-dependent insulin release. Endocrinology 148:2601–2609

Chu ZL, Carroll C, Alfonso J, Gutierrez V, He H, Lucman A, Pedraza M, Mondala H, Gao H, Bagnol D, Chen R, Jones RM, Behan DP, Leonard J (2008) A role for intestinal endocrine cell-expressed G protein-coupled receptor 119 in glycemic control by enhancing glucagon-like peptide-1 and glucose-dependent insulinotropic peptide release. Endocrinology 149:2038–2047

Clardy-James S, Chepurny OG, Leech CA, Holz GG, Doyle RP (2013) Synthesis, characterization and pharmacodynamics of vitamin-B_{12}-conjugated glucagon-like peptide-1. ChemMedChem 8:582–586

Couvineau A, Laburthe M (2012) The family B1 GPCR: structural aspects and interaction with accessory proteins. Curr Drug Targets 13:103–115

Creutzfeldt W (2005) The [pre]-history of the incretin concept. Regul Pept 128:87–91

Dachicourt N, Serradas P, Giroix MH, Gangnerau MN, Portha B (1996) Decreased glucose-induced cAMP and insulin release in islets of diabetic rats: reversal by IBMX, glucagon, GIP. Am J Physiol 271:E725–E732

Dalle S, Ravier MA, Bertrand G (2011a) Emerging roles of β-arrestin-1 in the control of the pancreatic β-cell function and mass: new therapeutic strategies and consequences for drug screening. Cell Signal 23:522–528

Dalle S, Quoyer J, Varin E, Costes S (2011b) Roles and regulation of the transcription factor CREB in pancreatic β-cells. Curr Mol Pharmacol 4:187–195

Dalle S, Burcelin R, Gourdy P (2013) Specific actions of GLP-1 receptor agonists and DPP4 inhibitors for the treatment of pancreatic β-cell impairments in type 2 diabetes. Cell Signal 25:570–579

De Leon DD, Li C, Delson MI, Matschinsky FM, Stanley CA, Stoffers DA (2008) Exendin-(9–39) corrects fasting hypoglycemia in SUR-$1^{-/-}$ mice by lowering cAMP in pancreatic β-cells and inhibiting insulin secretion. J Biol Chem 283:25786–25793

de Rooij J, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer A, Bos JL (1998) Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. Nature 396:474–477

Delmeire D, Flamez D, Hinke SA, Cali JJ, Pipeleers D, Schuit F (2003) Type VIII adenylyl cyclase in rat β cells: coincidence signal detector/generator for glucose and GLP-1. Diabetologia 46:1383–1393

Ding WG, Kitasato H, Matsuura H (2001) Involvement of calmodulin in glucagon-like peptide 1 (7–36)amide-induced inhibition of the ATP-sensitive K^+ channel in mouse pancreatic β-cells. Exp Physiol 86:331–339

Ding SY, Nkobena A, Kraft CA, Markwardt ML, Rizzo MA (2011) Glucagon-like peptide-1 stimulates post-translational activation of glucokinase in pancreatic β cells. J Biol Chem 286:16768–16774

Dolenšek J, Skelin M, Rupnik MS (2011) Calcium dependencies of regulated exocytosis in different endocrine cells. Physiol Res 60(Suppl 1):S29–S38

Doliba NM, QinW NH, Liu C, Buettger CW, Sotiris J, Collins HW, Li C, Stanley CA, Wilson DF, Grimsby J, Sarabu R, Naji A, Matschinsky FM (2012a) Glucokinase activation repairs defective bioenergetics of islets of Langerhans isolated from type 2 diabetics. Am J Physiol Endocrinol Metab 302:E87–E102

Doliba NM, Fenner D, Zelent B, Bass J, Sarabu R, Matschinsky FM (2012b) Repair of diverse diabetic defects of β-cells in man and mouse by pharmacological glucokinase activation. Diabetes Obes Metab 14(Suppl 3):109–119

Dolz M, Movassat J, Balbé D, Le Stunff H, Giroix MH, Fradet M, Kergoat M, Portha B (2011) cAMP-secretion coupling is impaired in diabetic GK/Par rat β-cells: a defect counteracted by GLP-1. Am J Physiol Endocrinol Metab 301:E797–E806

Donelan MJ, Morfini G, Julyan R, Sommers S, Hays L, Kajo H, Braud I, Easom RA, Molkentin JD, Brady ST, Rhodes CJ (2002) Ca^{2+}-dependent dephosphorylation of kinesin heavy chain on β-granules in pancreatic β-cells. Implications for regulated β-granule transport and insulin exocytosis. J Biol Chem 277:24232–24242

Drucker DJ, Nauck MA (2006) The incretin system: glucagon-like peptide-1 agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet 368:1696–1705

Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF (1987) Glucagon-like peptide-1 stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. Proc Natl Acad Sci USA 84:3434–3438

Dyachock O, Gylfe E (2004) Ca^{2+}-Induced Ca^{2+} release via inositol 1,4,5-trisphosphate receptors is amplified by protein kinase A and triggers exocytosis in pancreatic β-cells. J Biol Chem 279:45455–45461

Dyachock O, Isakov Y, Sågetorp J, Tengholm A (2006) Oscillations of cyclic AMP in hormone-stimulated insulin-secreting β-cells. Nature 439:349–352

Dyachock O, Idevall-Hagren O, Sågetorp J, Tian J, Wuttke A, Arrieumerlou C, Akusjärvi G, Gylfe E, Tengholm A (2008) Glucose-induced cyclic AMP oscillations regulate pulsatile insulin secretion. Cell Metab 8:26–37

Dzhura I, Chepurny OG, Kelley GG, Leech CA, Roe MW, Dzhura E, Afshri P, Malik S, Rindler MJ, Xu X, Lu Y, Smrcka AV, Holz GG (2010) Epac2-dependent mobilization of intracellular Ca^{2+} by glucagon-like peptide-1 receptor agonist exendin-4 is disrupted in β-cells of phospholipase Cϵ knockout mice. J Physiol 588:4871–4889

Dzhura I, Chepurny OG, Leech CA, Roe MW, Dzhura E, Xu X, Lu Y, Schwede F, Genieser HG, Smrcka AV, Holz GG (2011) Phospholipase Cϵ links Epac2 activation of the potentiation of glucose-stimulated insulin secretion from mouse islets of Langerhans. Islets 3:121–128

Eddlestone GT, Oldham SB, Lipson LG, Premdas FH, Beigelman PM (1985) Electrical activity, cAMP concentration, and insulin release in mouse islets of Langerhans. Am J Physiol 248: C145–C153

Eliasson L, Renström E, Ämmälä C, Berggren PO, Bertorello AM, Bokvist K, Chibalin A, Deeney JT, Flatt PR, Gäbel J, Gromada J, Larsson O, Lindström P, Rhodes CJ, Rorsman P (1996) PKC-dependent stimulation of exocytosis by sulfonylureas in pancreatic β cells. Science 271:813–815

Eliasson L, Ma S, Renström E, Barg S, Berggren PO, Galvanovskis J, Gromada J, Jing X, Lundquist I, Salehi A, Sewing S, Rorsman P (2003) SUR1 regulates PKA-independent cAMP-induced granule priming in mouse pancreatic β-cells. J Gen Physiol 121:181–197

Emilsson V, Liu YL, Cawthorne MA, Morton NM, Davenport M (1997) Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. Diabetes 46:313–316

Farilla L, Bulotta A, Hirshberg B, Li Calzi S, Khoury N, Noushmehr H, Bertolotto C, Di Mario U, Harlan DM, Perfetti R (2003) Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. Endocrinology 144:5149–5158

Fehmann HC, Habener JF (1992) Insulinotropic hormone glucagon-like peptide-1(7–37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma β TC-1 cells. Endocrinology 130:159–166

Fehse F, Trautmann M, Holst JJ, Halseth AE, Nanayakkara N, Nielsen LL, Fineman MS, Kim DD, Nauck MA (2005) Exenatide augments first- and second-phase insulin secretion in response to intravenous glucose in subjects with type 2 diabetes. J Clin Endocrinol Metabol 90:5991–5997

Fernandez J, Valdeolmillos M (1999) Glucose-dependent stimulatory effect of glucagon-like peptide 1(7–36) amide on the electrical activity of pancreatic β-cells recorded in vivo. Diabetes 48:754–757

Fonseca SG, Urano F, Weir GC, Gromada J, Burcin M (2012) Wolfram syndrome 1 and adenylyl cyclase 8 interact at the plasma membrane to regulate insulin production and secretion. Nat Cell Biol 14:1105–1112

Fraser IDC, Tavalin SJ, Lester LB, Langeberg LK, Westphal AM, Dean RA, Marrion NV, Scott JD (1998) A novel lipid-anchored A-kinase anchoring protein facilitates cAMP-responsive membrane events. EMBO J 17:2261–2272

Fridlyand LE, Harbeck MC, Roe MW, Philipson LH (2007) Regulation of cAMP dynamics by Ca^{2+} and G protein-coupled receptors in the pancreatic β-cell: a computational approach. Am J Physiol Cell Physiol 293:C1924–C1933

Friedrichsen BN, Neubauer N, Lee YC, Gram VK, Blume N, Petersen JS, Nielsen JH, Møldrap A (2006) Stimulation of pancreatic β-cell replication by incretin involves transcriptional induction of cyclin D1 via multiple signaling pathways. J Endocrinol 188:481–492

Fujimoto K, Shibasaki T, Yokoi N, Kashima Y, Matsumoto M, Sasaki T, Tajima N, Iwanaga T, Seino S (2002) Piccolo, a Ca^{2+} sensor in pancreatic β-cells. Involvement of cAMP-GEFII. Rim2.Piccolo complex in cAMP-dependent exocytosis. J Biol Chem 277:50497–50502

Furman B, Ong WK, Pyne NJ (2010) Cyclic AMP signaling in pancreatic islets. Adv Exp Med Biol 654:281–304

Geng X, Li L, Watkins S, Robbins PD, Drain P (2003) The insulin secretory granule is the major site of K_{ATP} channels of the endocrine pancreas. Diabetes 52:767–776

Gillis KD, Misler S (1993) Enhancers of cytosolic cAMP augment depolarization-induced exocytosis from pancreatic β-cells: evidence for effects distal to Ca^{2+} entry. Pflugers Arch 424:195–197

Goehring AS, Pedroja BS, Hinke SA, Langeberg LK, Scott JD (2007) MyRIP anchors protein kinase A to the exocyst complex. J Biol Chem 282:33155–33167

Goldfine ID, Perlman R, Roth J (1971) Inhibition of cyclic 3′,5′-AMP phosphodiesterases in islet cells and other tissues by tolbutamide. Nature 234:295–297

Gromada J, Bokvist K, Ding WG, Holst JJ, Nielsen JH, Rorsman P (1998a) Glucagon-like peptide 1(7–36) amide stimulates exocytosis in human pancreatic β-cells by both proximal and distal regulatory steps in stimulus-secretion coupling. Diabetes 47:57–65

Gromada J, Holst JJ, Rorsman P (1998b) Cellular regulation of islet hormone secretion by the incretin hormone glucagon-like peptide 1. Pflugers Arch 435:583–594

Gu G, Dubauskaite J, Melton DA (2002) Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. Development 129:2447–2457

Gutniak M, Ørskov C, Holst JJ, Ahrén B, Efendić S (1992) Antidiabetic effect of glucagon-like peptide-1 (7–36) amide in normal subjects and patients with diabetes mellitus. N Engl J Med 326:1316–1322

Hansen HS, Rosenkilde MM, Knop FK, Wellner N, Diep TA, Rehfeld JF, Andersen UB, Holst JJ, Hansen HS (2011) 2-Oleoyl glycerol is a GPR119 agonist and signals GLP-1 release in humans. J Clin Endocrinol Metab 96:E1409–E1417

Hansen HS, Rosenkilde MM, Holst JJ, Schwartz TW (2012) GPR119 as a fat sensor. Trends Pharmacol Sci 33:374–381

Hashiguchi H, Nakazaki M, Koriyama N, Fukudome M, Aso K, Tei C (2006) Cyclic AMP/cAMP-GEF pathway amplifies insulin exocytosis induced by Ca^{2+} and ATP in rat islet β-cells. Diabetes Metab Res Rev 22:64–71

Hatakeyama H, Kishimoto T, Nemoto T, Kasai H, Takahashi N (2006) Rapid glucose sensing by protein kinase A for insulin exocytosis in mouse pancreatic islets. J Physiol 570:271–282

Hatakeyama H, Takahashi N, Kishimoto T, Nemoto T, Kasai H (2007) Two cAMP-dependent pathways differentially regulate exocytosis of large dense-core and small vesicles in mouse β-cells. J Physiol 582:1087–1098

Hay CW, Sinclair EM, Bermano G, Durward E, Tadayyon M, Docherty K (2005) Glucagon-like peptide-1 stimulates human insulin promoter activity in part through cAMP-responsive elements that lie upstream and downstream of the transcription start site. J Endocrinol 186:353–365

Hayes MR (2012) Neuronal and intracellular signaling pathways mediating GLP-1 energy balance and glycemic effects. Physiol Behav 106:413–416

He LP, Mears D, Atwater I, Kitasato H (1998) Glucagon induces suppression of ATP-sensitive K^+ channel activity through a Ca^{2+}/calmodulin-dependent pathway in mouse pancreatic β-cells. J Membr Biol 166:237–244

Henquin JC (2000) Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 49:1751–1760

Henquin JC, Meissner HP (1983) Dibutyryl cyclic AMP triggers Ca^{2+} influx and Ca^{2+}-dependent electrical activity in pancreatic B cells. Biochem Biophys Res Commun 112:614–620

Henquin JC, Meissner HP (1984a) Effects of theophylline and dibutyryl cyclic adenosine monophosphate on the membrane potential of mouse pancreatic β-cells. J Physiol 351:595–612

Henquin JC, Meissner HP (1984b) The ionic, electrical, and secretory effects of endogenous cyclic adenosine monophosphate in mouse pancreatic B cells: studies with forskolin. Endocrinology 115:1125–1134

Henquin JC, Schmeer W, Meissner HP (1983) Forskolin, an activator of adenylate cyclases, increases Ca^{2+}-dependent electrical activity induced by glucose in mouse pancreatic B cells. Endocrinology 112:2218–2220

Herbst KJ, Coltharp C, Amzel LM, Zhang J (2011) Direct activation of Epac by sulfonylurea is isoform selective. Chem Biol 18:243–251

Hinke SA, Hellemans K, Schuit FC (2004) Plasticity of the β cell insulin secretory competence: preparing the pancreatic β cell for the next meal. J Physiol 558:369–380

Hinke SA, Navedo MF, Ulman A, Whiting JL, Nygren PJ, Tian G, Jimenez-Caliani AJ, Langeberg LK, Cirulli V, Tengholm A, Dell'Acqua ML, Santana LF, Scott JD (2012) Anchored phosphatases modulate glucose homeostasis. EMBO J 31:3991–4004

Holst JJ (2007) The physiology of glucagon-like peptide 1. Physiol Rev 87:1409–1439

Holz GG (2004a) Epac: a new cAMP-binding protein in support of glucagon-like peptide-1 receptor-mediated signal transduction in the pancreatic β-cell. Diabetes 53:5–13

Holz GG (2004b) New insights concerning the glucose-dependent insulin secretagogue action of glucagon-like peptide-1 in pancreatic β-cells. Horm Metab Res 36:787–794

Holz GG, Chepurny OG (2005) Diabetes outfoxed by GLP-1? Sci STKE 268:pe2

Holz GG, Habener JF (1992) Signal transduction crosstalk in the endocrine system: pancreatic β-cells and the glucose competence concept. Trends Biochem Sci 17:388–393

Holz GG, Habener JF (1998) Black widow spider α-latrotoxin: a presynaptic neurotoxin that shares structural homology with the glucagon-like peptide-1 family of insulin secretagogic hormones. Comp Biochem Physiol B Biochem Mol Biol 121:177–184

Holz GG 4th, Kuhtreiber WM, Habener JF (1993) Pancreatic β-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7–37). Nature 361:362–365

Holz GG, Leech CA, Habener JF (1995) Activation of a cAMP-regulated Ca^{2+}-signaling pathway in pancreatic β-cells by the insulinotropic hormone glucagon-like peptide-1. J Biol Chem 270:17749–17757

Holz GG, Leech CA, Heller RS, Castonguay M, Habener JF (1999) cAMP-dependent mobilization of intracellular Ca^{2+} stores by activation of ryanodine receptors in pancreatic β-cells. A Ca^{2+} signaling system stimulated by the insulinotropic hormone glucagon-like peptide-1-(7–37). J Biol Chem 274:14147–14156

Holz GG, Leech CA, Habener JF (2000) Insulinotropic toxins as molecular probes for analysis of glucagon-like peptide-1 receptor-mediated signal transduction in pancreatic β-cells. Biochimie 82:915–926

Holz GG, Kang G, Harbeck M, Roe MW, Chepurny OG (2006) Cell physiology of cAMP sensor Epac. J Physiol 577:5–15

Holz GG, Chepurny OG, Schwede F (2008a) Epac-selective cAMP analogs: new tools with which to evaluate the signal transduction properties of cAMP-regulated guanine nucleotide exchange factors. Cell Signal 20:10–20

Holz GG, Heart E, Leech CA (2008b) Synchronizing Ca^{2+} and cAMP oscillations in pancreatic β-cells: a role for glucose metabolism and GLP-1 receptors? Focus on "regulation of cAMP dynamics by Ca^{2+} and G protein-coupled receptors in the pancreatic β-cell: a computational approach". Am J Physiol Cell Physiol 294:C4–C6

Hong K, Lou L, Gupta S, Ribeiro-Neto F, Altschuler DL (2008) A novel Epac-Rap-PP2A signaling module controls cAMP-dependent Akt regulation. J Biol Chem 283:23129–23138

Howell SL, Jones PM, Persaud SJ (1994) Regulation of insulin secretion: the role of second messengers. Diabetologia 37(Suppl 2):S30–S35

Hugill A, Shimomura K, Ashcroft FM, Cox RD (2010) A mutation in KCNJ11 causing human hyperinsulinism (Y12X) results in a glucose-intolerant phenotype in the mouse. Diabetologia 53:2352–2356

Hussain MA, Stratakis C, Kirschner L (2012) Prkar1a in the regulation of the insulin secretion. Horm Metab Res 44:759–765

Idevall-Hagren O, Barg S, Gylfe E, Tengholm A (2010) cAMP mediators of pulsatile insulin secretion from glucose-stimulated single β-cells. J Biol Chem 285:23007–23018

Idevall-Hagren O, Jakobsson I, Xu Y, Tengholm A (2013) Spatial control of Epac2 activity by cAMP and Ca^{2+}-mediated activation of Ras in pancreatic β cells. Sci Signal 6:ra29

Islam MS, Leibiger I, Leibiger B, Rossi D, Sorrentino V, Ekström TJ, Westerblad H, Andrade FH, Berggren PO (1998) In situ activation of the type 2 ryanodine receptor in pancreatic β cells requires cAMP-dependent phosphorylation. Proc Natl Acad Sci USA 95:6145–6160

Jarrard RE, Wang Y, Salyer AE, Pratt EP, Soderling IM, Guerra ML, Lange AM, Broderick HJ, Hockerman GH (2013) Potentiation of sulfonylurea action by an EPAC-selective cAMP analog in INS-1 cells: comparison of tolbutamide and gliclazide and a potential role for EPAC activation of a 2-APB-sensitive Ca^{2+} influx. Mol Pharmacol 83:191–205

Jhala US, Canettieri G, Screaton RA, Kulkarni RN, Krajewski S, Reed J, Walker J, Lin X, White M, Montminy M (2003) cAMP promotes pancreatic β-cell survival via CREB-mediated induction of IRS2. Genes Dev 17:1575–1580

Jones RM, Leonard JN, Buzard DJ, Lehmann J (2009) GPR119 agonists for the treatment of type 2 diabetes. Expert Opin Ther Pat 19:1339–1359

Jørgensen NB, Dirksen C, Bojsen-Møller KN, Jacobsen SH, Worm D, Hansen DL, Kristiansen VB, Naver L, Madsbad S, Holst JJ (2013) The exaggerated glucagon-like peptide-1 response is important for the improved β-cell function and glucose tolerance after Roux-en-Y gastric bypass in patients with type 2 diabetes. Diabetes 62:3044–3052

Kaihara KA, Dickson LM, Jacobson DA, Tamarina N, Roe MW, Philipson LH, Wicksteed B (2013) β-cell-specific protein kinase A activation enhances the efficiency of glucose control by increasing acute-phase insulin secretion. Diabetes 62:1527–1536

Kang G, Holz GG (2003) Amplification of exocytosis by Ca^{2+}-induced Ca^{2+} release in INS-1 pancreatic β cells. J Physiol 546:175–189

Kang G, Chepurny OG, Holz GG (2001) cAMP-regulated guanine nucleotide exchange factor II (Epac2) mediates Ca^{2+}-induced Ca^{2+} release in INS-1 pancreatic β-cells. J Physiol 536:375–385

Kang G, Joseph JW, Chepurny OG, Monaco M, Wheeler MB, Bos JL, Schwede F, Genieser HG, Holz GG (2003) Epac-selective cAMP analog 8-pCPT-2′–O-Me-cAMP as a stimulus for Ca^{2+}-induced Ca^{2+} release and exocytosis in pancreatic β-cells. J Biol Chem 278:8279–8285

Kang G, Chepurny OG, Rindler MJ, Collis L, Chepurny Z, Li WH, Harbeck M, Roe MW, Holz GG (2005) A cAMP and Ca^{2+} coincidence detector in support of Ca^{2+}-induced Ca^{2+} release in mouse pancreatic β cells. J Physiol 566:173–188

Kang G, Chepurny OG, Malester B, Rindler MJ, Rehmann H, Bos JL, Schwede F, Coetzee WA, Holz GG (2006) cAMP sensor Epac as a determinant of ATP-sensitive potassium channel activity in human pancreatic β cells and rat INS-1 cells. J Physiol 573:595–609

Kang G, Leech CA, Chepurny OG, Coetzee WA, Holz GG (2008) Role of the cAMP sensor Epac as a determinant of K_{ATP} channel ATP sensitivity in human pancreatic β cells and rat INS-1 cells. J Physiol 586:1307–1319

Karagiannis T, Paschos P, Paletas K, Matthews DR, Tsapas A (2012) Dipeptidyl peptidase-4 inhibitors for treatment of type 2 diabetes mellitus in the clinical setting: systematic review and meta-analysis. BMJ 344:e1369

Kasai H, Suzuki T, Liu TT, Kishimoto T, Takahashi N (2002) Fast and cAMP-sensitive mode of Ca^{2+}-dependent exocytosis in pancreatic β-cells. Diabetes 51(Suppl 1):S19–S24

Kasai H, Hatakeyama H, Ohno M, Takahashi N (2010) Exocytosis in islet β-cells. Adv Exp Med Biol 654:305–338

Kasai H, Takahashi N, Tokumaru H (2012) Distinct initial SNARE configurations underlying the diversity of exocytosis. Physiol Rev 92:1915–1964

Kashima Y, Miki T, Shibasaki T, Ozaki N, Miyazaki M, Yano H, Seino S (2001) Critical role of cAMP-GEFII-Rim2 complex in incretin-potentiated insulin secretion. J Biol Chem 276:46046–46053

Kawasaki H, Springett GM, Mochizuki N, Toki S, Nakaya M, Matsuda M, Housman DE, Graybiel AM (1998) A family of cAMP-binding proteins that directly activate Rap1. Science 282:2275–2279

Kelley GG, Chepurny OG, Schwede F, Genieser HG, Leech CA, Roe MW, Li X, Dzhura I, Dzhura E, Afshari P, Holz GG (2009) Glucose-dependent potentiation of mouse islet insulin secretion by Epac activator 8-pCPT-2′–O-Me-cAMP-AM. Islets 1:260–265

Kelly P, Bailey CL, Fueger PT, Newgard CB, Casey PJ, Kimple ME (2008) Rap1 promotes multiple pancreatic islet cell functions and signals through mammalian target of rapamycin complex 1 to enhance proliferation. J Biol Chem 285(21):15777–15785

Kieffer TJ, Habener JF (1999) The glucagon-like peptides. Endocr Rev 20:876–913

Kieffer TJ, McIntosh CH, Pederson RA (1995) Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. Endocrinology 136:3585–3596

Kieffer TJ, Heller RS, Leech CA, Holz GG, Habener JF (1997) Leptin suppression of insulin secretion by activation of ATP-sensitive K^+ channels in pancreatic β-cells. Diabetes 46:1087–1093

Kim MJ, Kang JH, Park YG, Ryu GR, Ko SH, Jeong IK, Koh KH, RhieDJ YSH, Hahn SJ, Kim MS, Jo YH (2006) Exendin-4 induction of cyclin D1 expression in INS-1 β-cells: involvement of cAMP-responsive element. J Endocrinol 188:623–633

Kim JW, Roberts CD, Berg SA, Caicedo A, Roper SD, Chaudhari N (2008a) Imaging cyclic AMP changes in pancreatic islets of transgenic reporter mice. PLoS One 3:e2127

Kim BJ, Park KH, Yim CY, Takasawa S, Okamoto H, Im MJ, Kim UH (2008b) Generation of nicotinic acid adenine dinucleotide phosphate and cyclic ADP-ribose by glucagon-like peptide-1 evokes Ca^{2+} signal that is essential for insulin secretion in mouse pancreatic islets. Diabetes 57:868–878

Kim M, Platt MJ, Shibasaki T, Quaggin SE, Backx PH, Seino S, Simpson JA, Drucker DJ (2013) GLP-1 receptor activation and Epac2 link atrial natriuretic peptide secretion to control of blood pressure. Nat Med 19:567–575

Kinard TA, Satin LS (1995) An ATP-sensitive Cl^- channel current that is activated by cell swelling, cAMP, and glyburide in insulin-secreting cells. Diabetes 44:1461–1466

Kirschner LS, Kuzewitt DF, Matyakhina L, Towns WH 2nd, Carney JA, Westphal H, Stratakis CA (2005) A mouse model for the Carney complex tumor syndrome develops neoplasia in AMP-responsive tissues. Cancer Res 65:4506–4514

Koole C, Wootten D, Simms J, Valant C, Sridhar R, Woodman OL, Miller LJ, Summers RJ, Christopoulos A, Sexton PM (2010) Allosteric ligands of the glucagon-like peptide 1 receptor (GLP-1R) differentially modulate endogenous and exogenous peptide responses in a pathway-selective manner: implications for drug screening. Mol Pharmacol 78:456–465

Kulkarni RN, Wang ZL, Wang RM, Hurley JD, Smith DM, Ghatei MA, Withers DJ, Gardiner JV, Bailey CJ, Bloom SR (1997) Leptin rapidly suppresses insulin release from insulinoma cells, rat and human islets and, in vivo, in mice. J Clin Invest 100:2729–2736

Kumar DP, Rajagopal S, Mahavadi S, Mirshahi F, Grider JR, Murthy KS, Sanyal AJ (2012) Activation of transmembrane bile acid receptor TGR5 stimulates insulin secretion in pancreatic β-cells. Biochem Biophys Res Commun 427:600–6005

Kwan EP, Gaisano HY (2007) New insights into the molecular mechanisms of priming of insulin exocytosis. Diabetes Obes Metab Suppl 2:99–108

Kwan EP, Gao X, LeungYM GHY (2007) Activation of exchange protein directly activated by cyclic adenosine monophosphate and protein kinase A regulate common and distinct steps in promoting plasma membrane exocytic and granule-to-granule fusion in rat islet β cells. Pancreas 35:e45–e54

Lamont BJ, Li Y, Kwan E, Brown TJ, Gaisano H, Drucker DJ (2012) Pancreatic GLP-1 receptor activation is sufficient for incretin control of glucose metabolism in mice. J Clin Invest 122:388–402

Lan H, Vassileva G, Corona A, Liu L, Baker H, Golovko A, Abbondanzo SJ, Hu W, Yang S, Ning Y, Del Vecchio RA, Poulet F, Laverty M, Gustafson EL, Hedrick JA, Kowalski TJ (2009) GPR119 is required for physiological regulation glucagon-like peptide-1 secretion but not for metabolic homeostasis. J Endocrinol 201:219–230

Lan H, Lin HV, Wang CF, Wright MJ, Xu S, Kang L, Juhl K, Hedrick JA, Kowalski TJ (2012) Agonists at GPR119 mediate secretion of GLP-1 from mouse enteroendocrine cells through glucose-independent pathways. Br J Pharmacol 165:2799–2807

Landa LR Jr, Harbeck M, Kaihara K, Chepurny O, Kitiphongspattana K, Graf O, Nikolaev VO, Lohse MJ, Holz GG, Roe MW (2005) Interplay of Ca^{2+} and cAMP signaling in the insulin-secreting MIN6 β-cell line. J Biol Chem 280:31294–31302

Lavine JA, Attie AD (2010) Gastrointestinal hormones and the regulation of β-cell mass. Ann N Y Acad Sci 1212:41–58

Lee H, Jaffe AE, Feinberg JI, Tryggvadottir R, Brown S, Montano C, Aryee MJ, Irizarry RA, Herbstman J, Witter FR, Goldman LR, Feinberg AP, Fallin MD (2012) DNA methylation shows genome-wide association of NFIX, RAPGEF2 and MSRB3 with gestational age at birth. Int J Epidemiol 41:188–199

Leech CA, Habener JF (1997) Insulinotropic glucagon-like peptide-1-mediated activation of non-selective cation currents in insulinoma cells is mimicked by maitotoxin. J Biol Chem 272:17987–17993

Leech CA, Holz GG, Chepurny OG, Habener JF (2000) Expression of cAMP-regulated guanine nucleotide exchange factors in pancreatic β-cells. Biochem Biophys Res Commun 278:44–47

Leech CA, Chepurny OG, Holz GG (2010a) Epac2-dependent Rap1 activation and the control of islet insulin secretion by glucagon-like peptide-1. Vitam Horm 84:279–302

Leech CA, Dzhura I, Chepurny OG, Schwede F, Genieser HG, Holz GG (2010b) Facilitation of β-cell K_{ATP} channel sulfonylurea sensitivity by a cAMP analog selective for the cAMP-regulated guanine nucleotide exchange factor Epac. Islets 2:72–81

Leech CA, Dzhura I, Chepurny OG, Kang G, Schwede F, Genieser HG, Holz GG (2011) Molecular physiology of glucagon-like peptide-1 insulin secretagogue action in pancreatic β cells. Prog Biophys Mol Biol 107:236–247

Lester LB, Langeberg LK, Scott JD (1997) Anchoring of protein kinase A facilitates hormone-mediated insulin secretion. Proc Natl Acad Sci USA 94:14942–14947

Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ (2003) Glucagon-like peptide-1 receptor signaling modulates β cell apoptosis. J Biol Chem 278:471–478

Light PE, Manning Fox JE, Riedel MJ, Wheeler MB (2002) Glucagon-like peptide-1 inhibits pancreatic ATP-sensitive potassium channels via a protein kinase A- and ADP-dependent mechanism. Mol Endocrinol 16:2135–2144

Liu Z, Habener JF (2008) Glucagon-like peptide-1 activation of TCF7L2-dependent Wnt signaling enhances pancreatic β cell proliferation. J Biol Chem 283:8723–8735

Liu Z, Stanojevic V, Brindamour LJ, Habener JF (2012) GLP1-derived nonapeptide GLP1(28–36)amide protects pancreatic β-cells from glucolipotoxicity. J Endocrinol 213:143–154

Lovshin JA, Drucker DJ (2009) Incretin-based therapies for type 2 diabetes mellitus. Nat Rev Endocrinol 5:262–269

Lupi R, Mancarella R, Del Guerra S, Bugliani M, Del Prato S, Boggi U, Mosca F, Filipponi F, Marchetti P (2008) Effects of exendin-4 on islets from type 2 diabetes patients. Diabetes Obes Metab 10:515–519

Lynn FC, Pamir N, Ng EH, McIntosh CH, Kieffer TJ, Pederson RA (2001) Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. Diabetes 50:1004–1011

MacDonald PE, Wang X, Xia F, El-kholy W, Targonsky ED, Tsushima RG, Wheeler MB (2003) Antagonism of rat β-cell voltage-dependent K^+ currents by exendin 4 requires dual activation of the cAMP/protein kinase A and phosphatidylinositol 3-kinase signaling pathways. J Biol Chem 278:52446–52453

Mancini AD, Poitout V (2013) The fatty acid receptor FFA1/GPR40 a decade later: how much we know? Trends Endocrinol Metab 24:398–407

McIntosh CH, Widenmaier S, Kim SJ (2010) Pleiotropic actions of the incretin hormones. Vitam Horm 84:21–79

McQuaid TS, Saleh MC, Joseph JW, GyukhandanyanA M-FJE, MacLellan JD, Wheeler MB, Chan CB (2006) cAMP-mediated signaling normalizes glucose-stimulated insulin secretion in uncoupling protein-2 overexpressing β-cells. J Endocrinol 190:669–680

Miki T, Minami K, Shinozaki H, Matsumura K, Saraya A, Ikeda H, Yamada Y, Holst JJ, Seino S (2005) Distinct effects of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 on insulin secretion and gut motility. Diabetes 54:1056–1063

Mojsov S, Weir GC, Habener JF (1987) Insulinotropin: glucagon-like peptide I (7–37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. J Clin Invest 79:616–619

Morioka T, Dishinger JF, Reid KR, Liew CW, Zhang T, Inaba M, Kennedy RT, Kulkarni RN (2012) Enhanced GLP-1- and sulfonylurea-induced insulin secretion in islets lacking leptin signaling. Mol Endocrinol 26:967–976

Mudler H, Ling C (2009) Mitochondrial dysfunction in pancreatic β-cells in type 2 diabetes. Mol Cell Endocrinol 297:34–40

Mukai E, Fujimoto S, Sato H, Oneyama C, Kominato R, Sato Y, Sasaki M, Nishi Y, Okada M, Inagaki N (2011) Exendin-4 suppresses Src activation and reactive oxygen species production in diabetic Goko-Kakizaki rat islets in an Epac-dependent manner. Diabetes 60:218–226

Nakazaki M, Crane A, Hu M, Seghers V, Ullrich S, Aguilar-Bryan L, Bryan J (2002) cAMP-activated protein kinase-independent potentiation of insulin secretion by cAMP is impaired in SUR1 null islets. Diabetes 51:3440–3449

Nathan DM, Schreiber E, Fogel H, Mojsov S, Habener JF (1992) Insulinotropic action of glucagonlike peptide-I-(7–37) in diabetic and nondiabetic subjects. Diabetes Care 15:270–276

Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W (1993) Preserved incretin activity of glucagon-like peptide 1 [7–36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest 91:301–307

Naylor E, Arredouani A, Vasudevan SR, Lewis AM, Parkesh R, Mizote A, Rosen D, Thomas JM, Izumi M, Ganesan A, Galione A, Churchill GC (2009) Identification of a chemical probe for NAADP by virtual screening. Nat Chem Biol 5:220–226

Ni Q, Ganesan A, Aye-Han NN, Gao X, Allen MD, Levchenko A, Zhang J (2011) Signaling diversity of PKA achieved via a Ca^{2+}-cAMP-PKA oscillatory circuit. Nat Chem Biol 7:34–40

Nie J, Lilley BN, Pan YA, Faruque O, Liu X, Zhang W, Sanes JR, Han X, Shi Y (2013) SAD-A kinase enhances insulin secretion as a downstream target of GLP-1 signaling in pancreatic β-cells. Mol Cell Biol 13:2527–2534

Niimura M, MikiT ST, Fujimoto W, Iwanaga T, Seino S (2009) Critical role of the *N*-terminal cyclic AMP-binding domain of Epac2 in its subcellular localization and function. J Cell Physiol 219:652–658

Nijholt IM, Dolga AM, Ostroveanu A, Luiten PG, Schmidt M, Eisel UL (2008) Neuronal AKAP150 coordinates PKA and Epac-mediated PKB/Akt phosphorylation. Cell Signal 20:1715–1724

Oestreich EA, Wang H, Malik S, Kaproth-Joslin KA, Blaxall BC, Kelley GG, Dirksen RT, Smrcka AV (2007) Epac-mediated activation of phospholipase Cϵ plays a critical role in β-adrenergic receptor-dependent enhancement of Ca^{2+} mobilization in cardiac myocytes. J Biol Chem 282:5488–5495

Oestreich EA, Malik S, Goonasekera SA, Blaxall BC, Kelley GG, Dirksen RT, Smrcka AV (2009) Epac and phospholipase Cϵ regulate Ca^{2+} release in the heart by activation of protein kinase Cϵ and calcium-calmodulin kinase II. J Biol Chem 284:1514–1522

Ørskov C, Holst JJ, Nielsen OV (1988) Effect of truncated glucagon-like peptide-1 [proglucagon-(78–107) amide] on endocrine secretion from pig pancreas, antrum, and nonantral stomach. Endocrinology 123:2009–2013

Overton HA, Fyfe MC, Reynet C (2008) GPR119, a novel G protein-coupled receptor target for the treatment of type 2 diabetes and obesity. Br J Pharmacol 153(Suppl 1):S76–S81

Ozaki N, Shibasaki T, Kashima Y, Miki T, Takahashi K, Ueno H, Sunaga Y, Yano H, Matsuura Y, Iwanaga T, Takai Y, Seino S (2000) cAMP-GEFII is a direct target of cAMP in regulated exocytosis. Nat Cell Biol 2:805–811

Park S, Dong X, Fisher TL, Dunn S, Omer AK, Weir G, White MF (2006) Exendin-4 uses Irs2 signaling to mediate pancreatic β cell growth and function. J Biol Chem 281:1159–1168

Park JH, Kim SJ, Park SH, Son DG, Bae JH, Kim HK, Han J, Song DK (2012) Glucagon-like peptide-1 enhances glucokinase activity in pancreatic β-cells through the association of Epac2 with Rim2 and Rab3A. Endocrinology 153:574–582

Patti ME, Corvera S (2010) The role of mitochondria in the pathogenesis of type 2 diabetes. Endocr Rev 31:364–395

Pereira L, Cheng H, Lao DH, Na L, van Oort RJ, Brown JH, Wehrens XH, Chen J, Bers DM (2013) Epac2 mediates cardiac β1-adrenergic-dependent sarcoplasmic reticulum Ca^{2+} leak and arrhythmia. Circulation 127:913–922

Petyuk VA, Qian WJ, Hinault C, Gritsenko MA, Singhal M, Monroe ME, Camp DG 2nd, Kulkarni RN, Smith RD (2008) Characterization of the mouse pancreatic islet proteome and comparative analysis with other mouse tissues. J Proteome Res 7:3114–3126

Peyot ML, Gray JP, Lamontagne J, Smith PJ, Holz GG, Madiraju SR, Prentki M, Heart E (2009) Glucagon-like peptide-1 induced signaling and insulin secretion do not drive fuel and energy metabolism in primary rodent pancreatic β-cells. PLoS One 4:e6221

Prentki M, Matschinsky FM (1987) Ca^{2+}, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. Physiol Rev 67:1185–1248

Pyne NJ, Furman BL (2003) Cyclic nucleotide phosphodiesterases in pancreatic islets. Diabetologia 46:1179–1189

Quoyer J, Longuet C, Broca C, Linck N, Costes S, Varin E, Brockaert J, Bertrand G, Dalle S (2010) GLP-1 mediates antiapoptotic effect by phosphorylating Bad through a β arrestin 1-mediated ERK1/2 activation in pancreatic β-cells. J Biol Chem 285:1989–2002

Ramos LS, Zippin JH, Kamenetsky M, Buck J, Levin LR (2008) Glucose and GLP-1 stimulate cAMP production via distinct adenylyl cyclases in INS-1E insulinoma cells. J Gen Physiol 132:329–338

Rehmann H (2012) Epac2: a sulfonylurea receptor? Biochem Soc Trans 40:6–10

Renström E, Eliasson L, Rorsman P (1997) Protein kinase A-dependent and independent stimulation of exocytosis by cAMP in mouse pancreatic β-cells. J Physiol 502:105–118

Roger B, Papin J, Vacher P, Raoux M, Mulot A, Dubois M, Kerr-Conte J, Voy BH, Pattou F, Charpentier G, Jonas JC, Moustaïd-Moussa N, Lang J (2011) Adenylyl cyclase 8 is central to glucagon-like peptide 1 signalling and effects of chronically elevated glucose in rat and human pancreatic β cells. Diabetologia 54:390–402

Scott JD, Santana LF (2010) A-kinase anchoring proteins: getting to the heart of the matter. Circulation 121:1264–1271

Seino S, Shibasaki T (2005) PKA-dependent and PKA-independent pathways for cAMP-regulated exocytosis. Physiol Rev 85:1303–1342

Seino S, Takahashi H, Fujimoto W, Shibasaki T (2009) Roles of cAMP signalling in insulin granule exocytosis. Diabetes Obes Metab 11(Suppl 4):180–188

Seino S, Shibasaki T, Minami K (2011) Dynamics of insulin secretion and the clinical implications for obesity and diabetes. J Clin Invest 121:2118–2125

Shah U, Kowalski TJ (2010) GPR119 agonists for the potential treatment of type 2 diabetes and related metabolic disorders. Vitam Horm 84:415–448

Shao W, Yu Z, Fantus IG, Jin T (2010) Cyclic AMP signaling stimulates proteasome degradation of thioredoxin interacting protein (TxNIP) in pancreatic β-cells. Cell Signal 22:1240–1246

Shao W, Wang Z, Ip W, Chiang YT, Xiong X, Chai T, Xu C, Wang Q, Jin T (2013) GLP-1(28–36) improves β-cell mass and glucose disposal in streptozotocin induced diabetes mice and activates PKA-β-catenin signaling in β-cells in vitro. Am J Physiol Endocrinol Metab 304: E1263–E1272

Sharp GW (1996) Mechanisms of inhibition of insulin release. Am J Physiol 271:C1781–C1799

Shibasaki T, Sunaga Y, Seino S (2004) Integration of ATP, cAMP, and Ca^{2+} signals in insulin granule exocytosis. Diabetes 53(Suppl 3):S59–S62

Shibasaki T, Takahashi H, Miki T, Sunaga Y, Matsumura K, Yamanaka M, Zhang C, Tamamoto A, Satoh T, Miyazaki J, Seino S (2007) Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP. Proc Natl Acad Sci USA 104:19333–19338

Shiota C, Larsson O, Shelton KD, Shiota M, Efanov AM, Hoy M, Lindner J, Kooptiwut S, Juntti-Berggren L, Gromada J, Berggren PO, Magnuson MA (2002) Sulfonylurea receptor type 1 knock-out mice have intact feeding-stimulated insulin secretion despite marked impairment in their response to glucose. J Biol Chem 277:37176–37183

Singh S, Chang HY, Richards TM, Weiner JP, Clark JM, Segal JB (2013) Glucagonlike peptide 1-based therapies and risk of hospitalization for acute pancreatitis in type 2 diabetes mellitus: a population-based matched case–control study. JAMA Intern Med 173:534–539

Skelin M, Rupnik M (2011) cAMP increases the sensitivity of exocytosis to Ca^{2+} primary through protein kinase A in mouse pancreatic β cells. Cell Calcium 49:89–99

Skoglund G, Hussain MA, Holz GG (2000) Glucagon-like peptide 1 stimulates insulin gene promoter activity by protein kinase A-independent activation of the rat insulin I gene cAMP response element. Diabetes 49:1156–1164

Smrcka AV, Brown JH, Holz GG (2012) Role of phospholipase Cϵ in physiological phosphoinositide signaling networks. Cell Signal 24:1333–1343

Soga T, Ohishi T, Matsui T, Saito T, Matsumoto M, Takasaki J, Kamohara M, Hiyama H, Yoshida S, Momose K, Ueda Y, Matsushime H, Kobori M, Furuichi K (2005) Lysophosphatidylcholine enhances glucose-dependent insulin secretion via an orphan G-protein-coupled receptor. Biochem Biophys Res Commun 326:744–751

Song WJ, Schreiber WE, Zhong E, Liu FF, Kornfeld BD, Wondisford FE, Hussain MA (2008) Exendin-4 stimulation of cyclin A2 in β-cell proliferation. Diabetes 57:2371–2381

Song WJ, Seshadri M, Ashraf U, Mdluli T, Mondal P, Keil M, Azevedo M, Kirschner LS, Stratakis CA, Hussain MA (2011) Snapin mediates incretin action and augments glucose-dependent insulin secretion. Cell Metab 13:308–319

Song WJ, Mondal P, Li Y, Lee SE, Hussain MA (2013) Pancreatic β-cell response to increased metabolic demand and to pharmacologic secretagogues requires EPAC2A. Diabetes 62:2796–2807

Sonoda N, Imamura T, Yoshizaki T, Babendure JL, Lu JC, Olefsky JM (2008) β-arrestin-1 mediates glucagon-like peptide-1 signaling to insulin secretion in cultured pancreatic β cells. Proc Natl Acad Sci USA 105:6614–6619

Straub SG, Sharp GW (2012) Evolving insights regarding mechanisms for the inhibition of insulin release by norepinephrine and heterotrimeric G proteins. Am J Physiol Cell Physiol 302: C1687–C1698

Suga S, Kanno T, Ogawa Y, Takeo T, Kamimura N, Wakui M (2000) cAMP-independent decrease of ATP-sensitive K^+ channel activity by GLP-1 in rat pancreatic β-cells. Pflugers Arch 440:566–572

Takahashi N, Kadowaki T, Yazaki Y, Ellis-Davies GC, Miyashita Y, Kasai H (1999) Post-priming actions of ATP on Ca^{2+}-dependent exocytosis in pancreatic β cells. Proc Natl Acad Sci USA 96:760–765

Takeda Y, Amano A, Noma A, Nakamura Y, Fujimoto S, Inagaki N (2011) Systems analysis of GLP-1 receptor signaling in pancreatic β-cells. Am J Physiol Cell Physiol 301: C792–C803

Talbot J, Joly E, Prentki M, Buteau J (2012) β-arrestin 1-mediated recruitment of c-Src underlies the proliferative action of glucagon-like peptide-1 in pancreatic β INS832/13 cells. Mol Cell Endocrinol 364:65–70

Tarasov AI, Semplici F, Li D, Rizzuto R, Ravier MA, Gilon P, Rutter GA (2013) Frequency-dependent mitochondrial Ca^{2+} accumulation regulates ATP synthesis in pancreatic β-cells. Pflugers Arch 465:543–554

Taylor SS, Kim C, Cheng CY, Brown SH, Wu J, Kannan N (2008) Signaling through cAMP and cAMP-dependent protein kinase: diverse strategies for drug design. Biochim Biophys Acta 1784:16–26

Tengholm A (2012) Cyclic AMP dynamics in the pancreatic β-cell. Ups J Med Sci 117:365–369

Thams P, Anwar MR, Capito K (2005) Glucose triggers protein kinase A-dependent insulin secretion in mouse pancreatic islets through activation of the K-ATP channel-dependent pathway. Eur J Endocrinol 152:671–677

Thorens B (1992) Expression cloning of the pancreatic β cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc Natl Acad Sci USA 89:8641–8645

Tian G, Sandler S, Gylfe E, Tengholm A (2011) Glucose- and hormone-induced cAMP oscillations in α- and β-cells within intact pancreatic islets. Diabetes 60:1535–1543

Tian G, Tepikin AV, Tengholm A, Gylfe E (2012) cAMP induces stromal interaction molecule 1 (STIM1) puncta but neither Orai1 protein clustering nor store-operated Ca^{2+} entry (SOCE) in islet cells. J Biol Chem 287:9862–9872

Tourrel C, Bailbe D, Meile MJ, Kergoat M, Portha B (2001) Glucagon-like peptide-1 and exendin-4 stimulate β-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. Diabetes 50:1562–1570

Tsalkova T, Gribenko AV, Cheng X (2011) Exchange protein directly activated by cyclic AMP isoform 2 is not a direct target of sulfonylurea drugs. Assay Drug Dev Technol 9:88–91

Tsalkova T, Mei FC, Li S, Chepurny OG, Leech CA, Liu T, Holz GG, Woods VL, Cheng X (2012) Isoform-specific antagonists of exchange proteins directly activated by cAMP. Proc Natl Acad Sci USA 109:18613–18618

Tschen SI, Georgia S, Dhawan S, Bhushan A (2011) Skp2 is required for incretin hormone-mediated β-cell proliferation. Mol Endocrinol 25:2134–2143

Tsuboi T, da Silva Xavier G, Holz GG, Jouaville LS, Thomas AP, Rutter GA (2003) Glucagon-like peptide-1 mobilizes intracellular Ca^{2+} and stimulates mitochondrial ATP synthesis in pancreatic MIN6 β-cells. Biochem J 369:287–299

Vikman J, Svensson H, Huang YC, Kang Y, Andersson SA, Gaisano HY, Eliasson L (2009) Truncation of SNAP-25 reduces the stimulatory action of cAMP on rapid exocytosis in insulin-secreting cells. Am J Physiol Endocrinol Metab 297:E452–E461

Vliem MJ, Ponsioen B, Schwede F, Pannekoek WJ, Riedl J, Jalink K, Genieser HG, Bos JL, Rehmann H (2008) 8-pCPT-2′-*O*-Me-cAMP-AM: an improved Epac-selective cAMP analogue. Chembiochem 9:2052–2054

Wan QF, Dong Y, Yang H, Lou X, Ding J, Xu T (2004) Protein kinase activation increases insulin secretion by sensitizing the secretory machinery to Ca^{2+}. J Gen Physiol 124: 653–662

Wang X, Zhou J, Doyle ME, Egan JM (2001) Glucagon-like peptide-1 causes pancreatic duodenal homeobox-1 protein translocation from the cytoplasm to the nucleus of pancreatic β-cells by a cyclic adenosine monophosphate/protein kinase A-dependent mechanism. Endocrinology 142:1820–1827

Wang Q, Li L, Xu E, Wong V, Rhodes C, Brubaker PL (2004) Glucagon-like peptide-1 regulates proliferation and apoptosis via activation of protein kinase B in pancreatic INS-1 β cells. Diabetologia 47:478–487

Welch EJ, Jones BW, Scott JD (2010) Networking with AKAPs: context-dependent regulation of anchored enzymes. Mol Interv 10:86–97

Wice BM, Wang S, Crimmins DL, Diggs-Anrews KA, Althage MC, Ford EL, Tran H, Ohlendorf M, Griest TA, Wang Q, Fisher SJ, Ladenson JH, Polonsky KS (2010) Xenin-25 potentiates glucose-dependent insulinotropic polypeptide action via a novel cholinergic relay mechanism. J Biol Chem 285:19842–19853

Wice BM, Reeds DN, Tran H, Crimmins DL, Patterson BW, Dunai J, Wallendorf MJ, Ladenson JH, Villareal DT, Polonsky KS (2012) Xenin-25 amplifies GIP-mediated insulin secretion in humans with normal and impaired glucose tolerance but not type 2 diabetes. Diabetes 61:1793–1800

Wiederkehr A, Wollheim CB (2008) Impact of mitochondrial calcium on the coupling of metabolism to insulin secretion in the pancreatic β-cell. Cell Calcium 44:64–76

Winzell MS, Ahrén B (2004) The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. Diabetes 53(Suppl 3): S215–S219

Winzell MS, Ahrén B (2007) G-protein-coupled receptors and islet functions – implications for treatment of type 2 diabetes. Pharmacol Ther 116:437–448

Xu G, Stoffers DA, Habener JF, Bonner-Weir S (1999) Exendin-4 stimulates both β-cell replication and neogenesis, resulting in increased β-cell mass and improved glucose tolerance in diabetic rats. Diabetes 48:2270–2276

Yada T, Itoh K, Nakata M (1993) Glucagon-like peptide-1-(7–36)amide and a rise in cyclic adenosine 3′,5′-monophosphate increase cytosolic free Ca^{2+} in rat pancreatic β-cells by enhancing Ca^{2+} channel activity. Endocrinology 133:1685–1692

Yaekura K, Yada T (1998) $[Ca^{2+}]_i$-reducing action of cAMP in rat pancreatic β-cells: involvement of thapsigargin-sensitive stores. Am J Physiol 274(Cell Physiol 43):C513–C521

Yang Y, Gillis KD (2004) A highly Ca^{2+}-sensitive pool of granules is regulated by glucose and protein kinases in insulin-secreting INS-1 cells. J Gen Physiol 124:641–651

Yang Y, Craig TJ, Chen X, Ciufo LF, Takahashi M, Morgan A, Gillis KD (2007) Phosphomimetic mutation of Ser-187 of SNAP-25 increases both syntaxin binding and highly Ca^{2+}-sensitive exocytosis. J Gen Physiol 129:233–244

Yang Y, Shu X, Liu D, Shang Y, Wu Y, Pei L, Xu X, Tian Q, Zhang J, Qian K, Wang YX, Petralia RS, Tu W, Zhu LQ, Wang JZ, Lu Y (2012) EPAC null mutation impairs learning and social interactions via aberrant regulation of miR-124 and Zif268 translation. Neuron 73:774–788

Yi P, Park JC, Melton DA (2013) Betatrophin: a hormone that controls pancreatic β cell proliferation. Cell 153:747–758

Ying Y, Li L, Cao W, Yan D, Zeng Q, Kong X, Lu L, Yan M, Xu X, Qu J, Su Q, Ma X (2012) The microtubule associated protein syntabulin is required for glucose-stimulated and cAMP-potentiated insulin secretion. FEBS Lett 586:3674–3680

Yoshida M, Yamato S, Dezaki K, Nakata M, Kawakami M, Yada T, Kakei M (2012) Activation of nonselective cation channels via cAMP-EPAC2 pathway is involved in exendin-4 potentiated insulin secretion. European Association for the Study of Diabetes (EASD), annual meeting, Berlin. Abstract #488

Yusta B, Baggio LL, Estall JL, Koehler JA, Holland DP, Li H, Pipeleers D, Ling Z, Drucker DJ (2006) GLP-1 receptor activation improves β cell function and survival following induction of endoplasmic reticulum stress. Cell Metab 4:391–406

Zhang CL, Katoh M, Shibasaki T, Minami K, Sunaga Y, Takahashi H, Yokoi N, Iwasaki M, Miki T, Seino S (2009) The cAMP sensor Epac2 is a direct target of antidiabetic sulfonylurea drugs. Science 325:607–610

Zhao AZ, Bornfeldt KE, Beavo JA (1998) Leptin inhibits insulin secretion by activation of phosphodiesterase 3B. J Clin Invest 102:869–873

Zippin JH, Chen Y, Straub SG, Hess KC, Diaz A, Lee D, Tso P, Holz GG, Sharp GW, Levin LR, Buck J (2013) CO_2/HCO_3^- and calcium regulated soluble adenylyl cyclase as a physiological ATP sensor. J Biol Chem 288:33283–33291

Calcium Signaling in the Islets

21

Md. Shahidul Islam

Contents

Abstract

Easy access to rodent insulinoma cells and rodent islets and the ease of measuring Ca^{2+} by fluorescent indicators have resulted in an overflow of data that have clarified details of Ca^{2+} signaling in the rodent islets. Our understanding of the mechanisms and the roles of Ca^{2+} signaling in the human islets, under

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_9,

physiological conditions, has been influenced by extrapolation of the rodent data obtained under suboptimal experimental conditions. More recently, electrophysiological and Ca^{2+} studies have elucidated the ion channel repertoire relevant for Ca^{2+} signaling in the human islets and have examined their relative contributions. Several channels belonging to the transient receptor potential (TRP) family are present in the β-cells. Intracellular Ca^{2+} channels and Ca^{2+}-induced Ca^{2+} release (CICR) add new dimension to the complexity of Ca^{2+} signaling in the human β-cells. While a lot more remains to be learnt about the mechanisms of generation and decoding of Ca^{2+} signals, much de-learning will also be needed. Human β-cells do not have a resting state in the normal human body even under physiological fasting conditions. Their membrane potential under physiologically relevant resting conditions is ~ −50 mV. Biphasic insulin secretion is an experimental epiphenomenon unrelated to the physiological pulsatile insulin secretion into the portal vein in the human body. Human islets show a wide variety of electrical activities, and patterns of $[Ca^{2+}]_i$ changes, whose roles in mediating pulsatile secretion of insulin remain unclear. Future studies need to be directed toward a better understanding of Ca^{2+} signaling in the human islet cells in the context of the pathogenesis, prevention, and treatment of human diabetes.

Keywords

CICR • Transient receptor potential channels • Calcium oscillation • Depolarization • TRP channels • TRPV1 • Ryanodine receptor • TRPV4 • Basal calcium • RyR1 • TRPM2 • TRPV2 • TRPM3 • RyR2 • K_{ATP} channel • RyR3 • Membrane potential • Calcium-induced calcium release

Introduction

Changes in the concentration of the free Ca^{2+} in the cytoplasm ($[Ca^{2+}]_i$) or in subcellular compartments can act as signals for many cellular processes. Increase in $[Ca^{2+}]_i$ may be local (e.g., Ca^{2+} "sparks"), which may give rise to global $[Ca^{2+}]_i$ changes (Pinton et al. 2002). $[Ca^{2+}]_i$ changes take the forms of oscillations and propagating waves (Stozer et al. 2013). Generation and shaping of the Ca^{2+} signals require participation of different membranes, channels, pumps, stores, other organelle, as well as many Ca^{2+}-binding proteins (Schwaller 2012). $[Ca^{2+}]_i$ changes are often loosely termed "Ca^{2+} signals," although it is likely that all $[Ca^{2+}]_i$ changes do not have a signaling role. Ca^{2+} signals control events such as exocytosis that take place in seconds and events like gene transcription that take place over minutes to hours. In this review, I shall not compile a catalog of all of the molecules and phenomena that are known in connection with Ca^{2+} signaling in the islets; instead, I shall depict some emerging and intriguing areas and give my views. The review is structured to deliver selected messages rather than to dilute them by writing a complete treatise on Ca^{2+} signaling.

When it comes to Ca^{2+} signaling in the islets, the literature is dominated by data obtained from in vitro experiments that have used islets or insulinoma cells from rodents. We understand how rodent islets behave in Petri dishes or in in vitro perfusion systems, better than we understand how human islets behave in their native environment in the pancreas in the normal human body. If we want to learn how mountain gorillas behave, we can do that by poking a monkey in a cage or by watching mountain gorillas in their social and natural environments in Rwanda. We tend to draw far-reaching conclusions not only about the function of normal human islets, but also about the dysfunctions of human islets in diabetes, from in vitro studies done on rodent islets. It is important that we examine what key experiments were done, what conditions were used in those experiments, and what results were obtained. This may enable us to reinterpret the existing data and draw our own conclusions about some fundamental issues, some of which are illustrated in the following paragraphs.

The Human β-Cells as a Group Are Never Resting

The notion that β-cells have a "resting" state is a myth arising from in vitro experimental protocols. In vitro experimentalists find it convenient to work with a stable low rate of insulin secretion and a stable low basal $[Ca^{2+}]_i$ at the beginning of an experiment. They want to ensure that the $[Ca^{2+}]_i$ or insulin curves show a stable baseline, which reviewers like to see. To achieve this, investigators expose islets to low concentrations of glucose (often 2–3 mM, sometimes 0–1 mM), and no other nutrients are included in the solution. Human islets are incubated in zero glucose for as long as an hour to force them to rest (Henquin et al. 2006). Under such conditions, β-cells are largely depleted of energy, and consequently, a high proportion of K_{ATP} channels are open. β-Cells in the human body, however, even after overnight fast, are bathed in ~4–6 mM glucose, other nutrients like the amino acids, and the hormone glucagon that is present in high concentration in the fasting plasma. The availability of these nutrients ensures that the human β-cells, even under fasting conditions, are not energy depleted. The K_{ATP} channels of many β-cells in the human islets are thus mostly in a closed state even under fasting conditions. In in vitro experiments, human β-cells secrete insulin even when they are exposed to only 3–5 mM glucose as the only nutrient (Henquin et al. 2006). When human β-cells are exposed to only 5–6 mM glucose, as the sole nutrient, they keep firing action potentials from a baseline membrane potential of ~ −50 to ~ −45 mV, at rates ranging from one every 4 s to one every 2 s (Misler et al. 2005). Complex patterns of membrane potential oscillations are seen in human islets even when they are exposed to only 2.8 mM glucose (and no other nutrients) and even when the experiments are performed at 34 °C (Manning Fox et al. 2006). (To the cell biologists 34 °C or even 21 °C is okay; to the clinicians, a patient with 34 °C body temperature poses a real emergency.) Thus, in the normal human body, β-cells are not resting even after overnight fast. Under fasting conditions, the concentration of insulin in the portal vein of human is 440 ± 25 pmol/L (Song et al. 2000). Under

such conditions, islets secrete not only insulin but also glucagon, which protects against hypoglycemia. This is evident from the observations that total pancreatectomy in human leads not only to diabetes but also to a rather more difficult complication, namely, hypoglycemia, due to the lack of glucagon (Kahl and Malfertheiner 2004).

It is accepted that the $[Ca^{2+}]_i$ of "resting" β-cells is ~25–100 nM and that the membrane potential of "resting" β-cells is ~ −70 mV. These values are obtained from experiments where β-cells are forced to artificial "resting conditions" that are different from the physiological resting conditions. If β-cells are, instead, kept in a solution that mimics the human plasma after an overnight fast (i.e., physiologically relevant resting condition), then their resting membrane potentials will be different (perhaps ~ −50 to ~ −45 mV). Consequently, their resting $[Ca^{2+}]_i$ will also be different (perhaps ~300 nM and perhaps in the form of oscillations). In other words, β-cells in the normal human body spend most of their lifetime with a much higher $[Ca^{2+}]_i$ and secretory activity than can be guessed from conventional in vitro experiments.

Biphasic Insulin Secretion Is an Experimental Epiphenomenon

In experiments where β-cells are first forced to rest (often by incubating in ~2–3 mM glucose, as the only nutrient), and then suddenly exposed to a high concentration of glucose (often >10 mM, but sometimes 30 mM!), continuously for a prolonged period, then one sees a pattern of insulin secretion that has been called "biphasic insulin secretion," over the past decades. Biphasic refers to two phases of insulin secretion: the first phase consists of the initial large insulin secretion that peaks at 5–6 min after increasing the concentration of glucose and the second phase consists of the subsequent lower rate of insulin secretion that remains stable or slowly rises as long as the glucose concentration remains high (over a period of 1–2 h or more) (see the chapter "► (Dys)Regulation of Insulin Secretion by Macronutrients"). (Electrophysiologists have a different definition of "biphasic," their first phase peaking in <500 ms! (Rorsman et al. 2000).) Human β-cells in normal human body encounter conditions of stimulations that are substantially different from the experimental conditions that are used to demonstrate the biphasic nature of insulin secretion in in vitro studies. As mentioned before, normal human β-cells are not in a resting state even under fasting conditions. They are seldom subjected to a sudden increase of glucose to a very high concentration (or sudden increase of$[Ca^{2+}]_i$ to 30 μM by ultra violet flash (Rorsman et al. 2000)). They are usually triggered by lower concentrations of glucose (usually by ~7–<10 mM glucose after a mixed meal), and normally glucose concentrations in the plasma do oscillate. The result is that normal insulin secretion in the human portal vein is oscillatory and not biphasic as elicited by artificial experimental conditions. Experiments that are designed to demonstrate the biphasic nature of insulin secretion are not usually designed to detect oscillations of insulin secretion (e.g., samples for insulin assay are not collected at 1 min or more frequent intervals).

Thus, normal insulin secretion in normal human being during fasting states, and after mixed meals, may employ a set of molecular mechanisms that may be substantially different from those involved in mediating biphasic insulin secretion elicited by experimental protocols described above.

Glucose Increases Insulin Secretion by Increasing $[Ca^{2+}]_i$, and by Providing ATP in the Face of Energy-Consuming Processes Triggered by Ca^{2+} Influx Through the Voltage-Gated Ca^{2+} Channels (VGCC)

When 30 mM KCl is applied to islets in the presence of low concentration of glucose (or zero glucose [8], and no other nutrients are included in the solution), there is an increase of both $[Ca^{2+}]_i$ and insulin secretion with a biphasic time course (Henquin et al. 2006; Gembal et al. 1993). A large and persistent increase of $[Ca^{2+}]_i$ in a cell that is kept at 1 mM glucose (and no other nutrients) reduces cytoplasmic [ATP] (Maechler et al. 1999). This is due to the fact that plasma membrane Ca^{2+}-ATPase and other Ca^{2+}-sensitive biochemical cascades that link Ca^{2+} influx to insulin secretion consume ATP of the cell, which is kept in only1 mM glucose (and which has a high-K_m glucokinase to phosphorylate the sugar) (Jung et al. 2009). In fact, in the later part of the second phase, $[Ca^{2+}]_i$ increases slowly since the cell can no longer pump out Ca^{2+} adequately because of energy deficiency (Gembal et al. 1993). Consequently, Ca^{2+}-mediated insulin secretion (which is an energy-consuming process) is progressively reduced in the second phase of prolonged $[Ca^{2+}]_i$ increase by KCl (Gembal et al. 1993). If one now applies 15 mM glucose (and thereby improves energy status of the cells) to these "$[Ca^{2+}]_i$-clamped" islets, a larger amount of secretion is obtained (Henquin et al. 2006; Gembal et al. 1993). So, to recapitulate, in the first scenario, insulin secretion increases because of an increase of $[Ca^{2+}]_i$, but the magnitude of the increase is low, and it declines further over time because of inadequate energy availability to support secretion. In the second scenario, glucose does what it is supposed to do, i.e., it performs its universal fuel function by supplying energy to the cells, and thereby it increases insulin secretion further. Of course, glucose metabolism produces many other molecules too, e.g., cAMP (via ATP), which can increase insulin secretion (Idevall-Hagren et al. 2013).

That glucose can stimulate insulin secretion from human β-cells in vivo, without inducing further closure of K_{ATP} channels, is evident from cases of severe poisoning with sulfonylureas. In these patients, the K_{ATP} channels are presumably completely closed, and $[Ca^{2+}]_i$ of β-cells is certainly high. However, when glucose is infused into such patients (as an attempt to correct hypoglycemia), the β-cells secrete even more insulin, making the hypoglycemia recurrent and difficult to treat (Lheureux et al. 2005). Similarly, people with $SUR1^{-/-}$, who do not respond to tolbutamide, do respond to glucose by insulin secretion (Grimberg et al. 2001).

Thus, while investigating signaling roles of glucose, the more universal role of glucose as a fuel needs to be considered explicitly. In experimental conditions

where glucose is the only nutrient, its role as a fuel becomes even more critical. If concentration of glucose in the human plasma is reduced to less than 3 mM (and all other nutrients are kept normal), one will become unconscious within seconds, a vivid example of the role of glucose as a fuel in the central neurons. Similarly, if there is no glucose or only very low glucose in the perfusion medium (and no other nutrients are present), muscle cells will eventually fail to contract, heart will stop beating, and, not surprisingly, islets will fail to secrete insulin properly.

Mechanism of Initial Depolarization of β-Cells by Glucose

Initial depolarization of plasma membrane to the thresholds for activation of voltage-gated Ca^{2+} channels is one of the most critical signaling events leading to Ca^{2+} signaling and insulin secretion. The most important function of β-cells is to prevent death due to hypoglycemia. If your fasting plasma glucose concentration is raised from 5 mM to 8 mM (i.e., you have diabetes), you will not die immediately. You may not even feel for years that the concentration of glucose in your blood is high. On the other hand, if your fasting plasma glucose drops from 5 mM to 3 mM, you will have hypoglycemic symptoms and you may become unconscious and die. Other hormones in the body are not like insulin; if your pituitary or adrenal hormones are acutely low, it will not kill you immediately. β-Cells, thus, secrete a hormone that is potentially a killer. Nature has equipped β-cells with powerful brakes to immediately stop insulin secretion, when glucose concentration is inappropriately low. Key elements of this brake system are the high-K_m glucokinase and the K_{ATP} channels. When plasma glucose concentration is reduced to near hypoglycemic levels, there is less glucose metabolism via glucokinase, leading to a reduced cytoplasmic MgATP/MgADP, opening of the K_{ATP} channels, and repolarization of plasma membrane potential (see chapter "► ATP-Sensitive Potassium Channels in Health and Disease"). Thus, K_{ATP} channels play a crucial role in stopping insulin secretion quickly, and its main function is to mediate quick repolarization of plasma membrane potential. Defects in these two brake systems, namely, inactivating mutations of the K_{ATP} channels or activating mutations of glucokinase, lead to hypoglycemia (Cuesta-Munoz et al. 2004).

At low glucose concentration (provided that no other nutrients are present), a high proportion of the K_{ATP} channels are in the open state. This situation occurs only in in vitro experiments that are often done at ~21 °C (Tarasov et al. 2006) and by using cells or tissues that are to a variable degree "metabolically stunned." It has no resemblance to any in vivo situation in any living human being, where β-cells are at 37 °C and are constantly bathed in a variety of nutrients including 20 different amino acids and fatty acids, even under normal fasting conditions, when plasma glucose concentration is ~4–6 mM. In vivo, a healthy β-cell, thus, has enough ATP to keep almost 100 % of the K_{ATP} channels closed. In the normal human body, where plasma glucose concentration changes between only ~4 mM in the fasting conditions to ~8 mM after meals, further closure of the K_{ATP} channels is thus not the likely mechanism for bringing about depolarization to the threshold for the

activation of VGCCs. Glucose depolarizes β-cells in Sur1 or Kir6.2 knocked-out mice (Ravier et al. 2009; Szollosi et al. 2007). Thus, under normal fasting conditions, the input resistance of β-cells is high and depolarization to the thresholds for the activation of VGCCs is brought about by various inward depolarizing currents mainly carried by Na^+. Here we are talking about small currents, which are difficult to measure in native β-cells. Thus, mere anticipation, sight, or smell of food will depolarize β-cells and stimulate insulin secretion by vagus-mediated acetylcholine-induced depolarizing Na^+ current (Gilon and Henquin 2001; Mears and Zimliki 2004). Similarly, after a mixed meal, the incretin hormone GLP-1 depolarizes β-cells by triggering a cAMP-activated Na^+ current (Holz et al. 1995). It is important to elucidate the molecular identity of the channels that mediate inward depolarizing currents in the β-cells. In this respect, there is currently considerable interest in the transient receptor potential (TRP) channels, which is the topic of the next paragraphs.

TRP Channels

Several TRP channels have been identified in the β-cells (Islam 2011). It is thought that these channels may account for the background depolarizing current (sometimes called the "leak" current) carried mostly by Na^+. Activation of some of these channels leads to an increase of $[Ca^{2+}]_i$ directly or by way of membrane depolarization. Examples of Ca^{2+}-permeable TRP channels in different insulin-secreting cells are TRPC1, TRPC4, TRPV1, TRPV2, TRPV4, TRPV5, TRPM2, TRPM3, and TRPA1. TRPs are tetrameric ion channels, and many form heterotetramers giving rise to a variety of ion channels with a variety of regulatory mechanisms. Expression of some TRP channels in the native cells is often low, and their regulation is often studied in heterologous systems, where the channels are overexpressed. In the following paragraphs, I will write a few lines about each of the TRP channels that have been described in the β-cells.

Examination of formalin-fixed paraffin-embedded tissue shows strong TRPC1immunoreactivity in the human islets (www.hpr.se). By RT-PCR, TRPC1 mRNA can be readily detected in mouse islets, MIN6 cells, INS-1 cells, and rat β-cells (Li and Zhang 2009; Sakura and Ashcroft 1997). TRPC1 is the only TRPC channel that is expressed at high level in MIN6 cells and mouse islets (Sakura and Ashcroft 1997). In contrast, another mouse insulinoma cell line βTC3 does not express TRPC1 mRNA. The only TRPC channel that can be detected by Northern blot in βTC3 cells is TRPC4 (Roe et al. 1998). TRPC4 is also abundant in INS-1 cells and rat β-cells (Li and Zhang 2009). TRPC4 has two abundant splice variants: the full-length TRPC4α and a shorter TRPC4β that lacks 84 amino acids in the C-terminus. In INS-1 cells, TRPC4α is the dominant isoform, whereas in rat β-cells, TRPC4β dominates (Li and Zhang 2009). TRPC4α is inhibited by phosphatidylinositol 4,5-bisphosphate (PIP2) (Otsuguro et al. 2008). TRPC5, which is closely related to TRPC4, is not expressed in mouse islets (Roe et al. 1998). TRPC1and TRPC4 are nonspecific cation channels with about equal permeability to Na^+

and Ca^{2+}. As alluded to earlier, it is possible that TRPC1 and other TRP channels mediate the inward depolarizing currents in β-cells. TRPC1 and TRPC4 are also molecular candidates for nonselective cation currents activated by Gq-/PLC-coupled receptors or by store depletion (Cheng et al. 2013). From studies in other cells, it appears that TRPC1 together with STIM1 and Orai1 can mediate store-operated Ca^{2+} entry (SOCE), but the issue is complex and controversial (Kim et al. 2009).

In the islets, TRPV1 is present in a subset of sensory nerve fibers (Gram et al. 2007). In 2006, one group published in Cell, a paper claiming that TRPV1 positive nerve fibers are involved in mediating local islet inflammation in autoimmune diabetes, but so far no one has reproduced those findings. The TRPV1-expressing fibers secrete calcitonin gene-related peptide, which inhibits insulin secretion. In Zucker diabetic rats, it has been demonstrated that ablation of the TRPV1-expressing fibers by capsaicin treatment improves insulin secretion (Gram et al. 2007). Insulinoma cell lines RIN and INS-1 express TRPV1 (Jabin et al. 2012). TRPV1 has been demonstrated in the primary β-cells of Sprague Dawley rats (Akiba et al. 2004), but not in those of Zucker diabetic rats (Gram et al. 2007). Human β-cells do not express functional TRPV1 (Jabin et al. 2012).

TRPV2 channel of β-cells is constitutively active (Hisanaga et al. 2009), and it may be one of the channels that mediate the background depolarizing current. Insulin and insulin-like growth factors translocate the TRPV2 channel from the cytoplasm to the plasma membrane (Hisanaga et al. 2009) resulting in increase in Ca^{2+} entry, insulin secretion, and β-cell growth. This observation implies that hyperinsulinemia, which is common in type 2 diabetes, may act as a positive feedback to increase insulin secretion. High concentration of glucose also induces translocation of TRPV2 to the plasma membrane. It appears that while glucose closes K_{ATP} channel, it, at the same time, increases inward depolarizing current through the TRPV2 channel by inducing translocation of the channel to the plasma membrane. The antiaging gene Klotho increases insulin secretion by upregulating TRPV2 in the plasma membrane (Lin and Sun 2012).

Immunohistochemistry of formalin-fixed paraffin-embedded tissues shows that the TRPV4 protein is highly expressed in the human islets, in contrast to the pancreatic acinar cells, where it is almost absent (www.hpr.se). Even though TRPV4 is known to be a plasma membrane channel, the immunoreactivity is mostly in the cytoplasm, a situation apparently similar to that of TRPV2 in the β-cells. TRPV4 acts as a mechano-sensor and osmo-sensor, but it can be activated by various ligands including 4α-Phorbol 12,13-didecanoate, anandamide, arachidonic acid, and epoxyeicosatrienoic acids. Aggregated human islet amyloid polypeptide (hIAPP) induces changes in the plasma membrane leading to the activation of TRPV4, membrane depolarization, increase in $[Ca^{2+}]_i$, induction of ER stress, and apoptosis. hIAPP-induced $[Ca^{2+}]_i$ changes and β-cell death are reduced by siRNA against TRPV4 (Casas et al. 2008).

By using immunohistochemistry, one study showed TRPV5 protein in the β-cells, but the data, apparently, cannot be reproduced. Native TRPV5 current has

not been demonstrated in the β-cells. TRPV5 protein has not been demonstrated in human β-cells.

TRPA1 is expressed in the β-cells where it mediates Ca^{2+} influx and plays a role in insulin secretion (Cao et al. 2012). It is activated by inflammatory mediators like 15-deoxy-Delta (12,14)-prostaglandin J(2) (15d-PGJ(2), nitric oxide (NO), H_2O_2, and H^+ (Takahashi et al. 2008). Glibenclamide activates the channel, which could possibly mediate failure of β-cells to secrete insulin after long-term use of this antidiabetic drug (Babes et al. 2013).

The presence of TRPM2 channels in the β-cells is well established (Bari et al. 2009). In human islets, there are at least two main isoforms of the channel: the full-length form (TRPM2-L) and a short form (TRPM2-S), where the four C-terminal transmembrane domains, the putative pore region, and the entire C-terminus are deleted (Zhang et al. 2003). TRPM2-S does not form a functional channel. There are other splice variants of TRPM2 which form channels and are differentially regulated (Eisfeld and Luckhoff 2007). TRPM2 is activated by intracellular ADP ribose, β-NAD^+, nitric oxide, H_2O_2, free radicals, and Ca^{2+}. ADP ribose formed by the degradation of NAD^+ by poly(ADP ribose) polymerase is an important activator of the TRPM2 channel. The nonselective cation channel activated by the diabetogenic agent alloxan is probably TRPM2 (Herson 1997). The channel can be gated also by warm temperature (>35 °C). Arachidonic acid, which is produced on stimulation of β-cells by glucose, is a positive modulator of TRPM2 channel (Jones and Persaud 1993; Hara et al. 2002). Cyclic ADP ribose potentiates activation of the channel (Togashi et al. 2006), but this is not a universal observation (Heiner et al. 2006). All of the splice forms of TRPM2 that form a channel are activated by Ca^{2+}. Ca^{2+} released from the intracellular stores can activate the channel (Du et al. 2009). TRPM2 is located also on the lysosomal membranes, and activation of the intracellular TRPM2 releases $Ca^{2+}$from the lysosomes (Lange et al. 2009). Insulin secretion and $[Ca^{2+}]_i$ response are impaired in the β-cells of TRPM2 knockout mice (Uchida et al. 2011; Zhang et al. 2012). The channel may provide a mechanism for eliminating β-cells that have been severely damaged by oxidative stress.

The TRPM3 channel has many splice variants that differ in their functional properties including their permeabilities for divalent cations (Oberwinkler et al. 2005; Fruhwald et al. 2012). The channel is activated by nifedipine, commonly used as a blocker of L-type VGCCs, and is inhibited by mefenamic acid and progesterone (Majeed et al. 2012; Klose et al. 2011). Micromolar concentrations of the steroid pregnenolone directly activate TRPM3 channel of β-cells leading to increase of $[Ca^{2+}]_i$ and augmentation of glucose-stimulated insulin secretion (Wagner et al. 2008).

TRPM4 is permeable to monovalent cations but not to Ca^{2+} (Launay et al. 2002). It is activated by elevated $[Ca^{2+}]_i$ and its activity is regulated by voltage. Immunohistochemistry shows that TRPM4 protein is present in human β-cells (Marigo et al. 2009). In rodent insulinoma cells, increased $[Ca^{2+}]_i$ activates TRPM4 and generates a large depolarizing membrane current (Cheng et al. 2007). An increase in $[Ca^{2+}]_i$ in β-cells upon stimulation by glucose or activation of PLC-linked

receptors activates TRPM4 channel (Marigo et al. 2009). Another regulator of TRPM4 is PIP2, which sensitizes the channel to the activation by $[Ca^{2+}]_i$, whereas depletion of PIP2 inhibits the channel (Nilius et al. 2006). Glucose, by increasing cytoplasmic MgATP/MgADP ratio, increases the concentration of PIP2 in the plasma membrane of β-cells (Thore et al. 2007). This is a potential mechanism by which glucose may sensitize TRPM4 channel. On the other hand, glucose increases cytoplasmic [ATP], which has inhibitory effect on the TRPM4 channel (Ullrich et al. 2005). Amino acid sequence of TRPM4 shows two motifs that look like the ABC transporter signature motif (Nilius et al. 2005). Consistent with this, TRPM4 is inhibited by glibenclamide (Demion et al. 2007). TRPM4 is also present in the α-cells where it plays a role in stimulating glucagon secretion (Nelson et al. 2011).

Another voltage-modulated intracellular Ca^{2+}-activated monovalent-specific cation channel, which is closely related to the TRPM4 channel, is the TRPM5 channel (Prawitt et al. 2003). Compared withTRPM4, TRPM5 is even more sensitive to activation by $[Ca^{2+}]_i$, but in contrast to TRPM4, it is not inhibited by ATP (Ullrich et al. 2005). TRPM5 mRNA is present in MIN6 cells, INS-1 cells, and in whole human islets (Prawitt et al. 2003). TRPM4 and TRPM5 may mediate Na^+ entry into the β-cells activated by glucose, sulfonylureas, and muscarinic agonists and thereby depolarize membrane potential. The frequency of glucose-induced oscillations of $[Ca^{2+}]_i$ and the glucose-induced insulin secretion is reduced in the TRPM5 knockout mice obtained from one particular source (Colsoul et al. 2010; Brixel et al. 2010). The channel is also involved in the taste-receptor mediated of potentiation of insulin secretion by fructose (Kyriazis et al. 2012). In rat islets, we found that triphenylphosphine oxide, an inhibitor of TRPM5 (Palmer et al. 2010), inhibits insulin secretion by glucose, and arginine, but not fructose (Krishnan et al. 2014).

Store-Operated Ca^{2+} Entry (SOCE)

The filling state of the ER Ca^{2+} store may trigger Ca^{2+} entry across the plasma membrane in β-cells as in many other cells (Dyachok and Gylfe 2001). Thus, depletion of ER Ca^{2+} pools by SERCA inhibitors induces Ca^{2+} entry and depolarizes the plasma membrane potential of β-cells (Gilon and Henquin 1992). The ER Ca^{2+} store thus plays a role in the regulation of membrane potential (Worley et al. 1994; Haspel et al. 2005). Two important molecular players involved in SOCE are stromal interaction molecule (STIM) and Orai1. STIM1 has an intraluminal EF-hand domain which enables it to act as a sensor of $[Ca^{2+}]$ in the ER lumen. STIM1, by its association with Orai1 or TRPC, regulates SOCE in some cells.

Depletion of the ER Ca^{2+} stores in the α-cells and the β-cells of mice causes subplasmalemmal accumulation of STIM1-YFP. In the human β-cells, glucose-induced alterations in the filling state of the ER Ca^{2+} stores are reflected by the translocation of STIM1-YFP from the plasma membrane to the ER or from the ER

to the plasma membrane (Tian et al. 2012). It is not known whether STIM1 interacts with Orai1 or TRPC channels in β-cells. The roles of TRPCs and the roles of STIM1and Orai1 in mediating SOCE remain unsettled. Some results support the view that STIM1-Orai1-TRPC1 complex provides an important mechanism for SOCE (Kim et al. 2009); others demonstrate that TRPC channels operate by mechanisms that do not involve STIM1 (Dehaven et al. 2009). It should be noted that in β-cells, activation of muscarinic receptors leads to the activation of nonselective cation currents that have a store-operated and a store-independent component (Mears and Zimliki 2004). We found that activation of RyRs of β-cells leads to Ca^{2+} entry through TRP-like channels by mechanisms that apparently do not involve store depletion (Gustafsson et al. 2005). For a detailed description of SOCE read the chapter "► β Cell Store-Operated Ion Channels".

Voltage-Gated Ca^{2+} Channels of β-Cells

In β-cells, the most robust mechanism for the entry of extracellular Ca^{2+} is the Ca^{2+} entry through the VGCCs. Opening of VGCCs leads to a large increase of $[Ca^{2+}]_i$ in microdomains near the plasma membrane and triggers exocytosis of insulin (Bokvist et al. 1995). Both high-voltage-activated (HVA) and low-voltage-activated (LVA) Ca^{2+} currents are detected in human β-cells (Barnett et al. 1995; Davalli et al. 1996). The major component of the HVA current is L type that is blocked by dihydropyridine antagonists and enhanced by BAYK8644. A second component of HVA current is resistant to inhibition by dihydropyridines and ω-conotoxin GVIA, an inhibitor of N-type Ca^{2+} channel, but is blocked by P/Q channel blocker ω-agatoxin IVA. Consistent with this, 80–100 % of glucose-induced insulin secretion from human islets is blocked by saturating concentration of dihydropyridine antagonists (Davalli et al. 1996; Braun et al. 2008). Such dramatic inhibition is thought to be due to the fact that the L-type channels play an essential role in the generation of electrical activity. In contrast, their roles in mediating exocytosis are less pronounced (Braun et al. 2008). The L-type Ca^{2+} current in rat and human β-cells is mediated mainly by $Ca_v1.3(\alpha_{1D})$ channel and to a lesser extent by $Ca_v1.2(\alpha_{1C})$. Human β-cells express 60-fold more mRNA for $Ca_v1.3$ compared to that for $Ca_v1.2$ (Reinbothe et al. 2013). Compared to $Ca_v1.2$, the $Ca_v1.3$ channels activate at lower membrane potential (~−55 mV),which suggests that the latter may be the more important isoform in human β-cells. This is in contrast to mouse β-cells where $Ca_v1.2$ plays a central role in insulin secretion (Schulla et al. 2003). Compared to the $Ca_v1.2$ channels, the $Ca_v1.3$ channels are less sensitive to the dihydropyridine antagonists (Xu and Lipscombe 2001). Identical de novo mutation (G406R) in $Ca_v1.2$ channel causes prolonged inward Ca^{2+} currents and causes episodic hypoglycemia (Splawski et al. 2004). Polymorphisms in the CACNA1D gene that encodes $Ca_v1.3$ are associated with human type 2 diabetes (Reinbothe et al. 2013).

The P-/Q-type Ca^{2+} channels ($Ca_v2.1,\alpha_{1A}$) account for 45 % of integrated whole-cell Ca^{2+} current in human β-cells. These channels are blocked by ω-agatoxin IVA.

Compared to the L-type Ca^{2+} channels, the P-/Q-type Ca^{2+} channels are more tightly coupled to exocytosis.

The LVA current is of T type which is activated at −50 mV and reaches a peak between −40 and −30. It inactivates within less than 1 s of sustained depolarization to −40 mV. T-type current is blocked by NNC 55–0396. The T-type current in human β-cells is mediated by $Ca_V3.2(\alpha_{1G})$. T-type channels are involved in insulin release induced by 6 mM, but not by 20 mM glucose (Braun et al. 2008).

If all of these ion channels are present in a given β-cell, one can envisage that closure of the K_{ATP} channels depolarizes membrane potential to above −55 mV, which then leads to the activation of T-type Ca^{2+} channels (which open at voltage above −60 mV), and then to the activation of the L-type Ca^{2+} channels (which open at voltage above −50 mV), which generates the action potential. Further depolarization occurs due to the activation of the voltage-gated Na^+ channels (which open at above −40 mV) leading finally to the activation of the P-/Q-type Ca^{2+} channels (which opens at above −20 mV) (Braun et al. 2008).

R-type Ca^{2+} channels ($Ca_v2.3,\alpha_{1E}$) are not present in human β-cells (Braun et al. 2008). Mice lacking the R-type Ca^{2+} channels exhibit impaired insulin secretion. In this context, it is noteworthy that polymorphisms and common variability in the gene encoding the R-type Ca^{2+} channels $Ca_v2.3$ (CACNA1E) are associated with impaired insulin secretion and type 2 diabetes in human too (Holmkvist et al. 2007; Trombetta et al. 2012). It is possible that, in human, R-type Ca^{2+} channels are involved in insulin secretion by operating other glucose-sensing cells like central neurons or GLP-1-producing L-cells in the gut.

Intracellular Ca^{2+} Channels of β-Cells

Among the channels that release Ca^{2+} from the ER or the secretory vesicles, the roles of the inositol 1,4,5-trisphosphate receptors (IP_3R) in the β-cells are well known. From immunohistochemistry pictures of paraffin-embedded formalin-fixed human tissues in the human protein atlas (www.hpr.se), it is evident that human islets express mainly the IP_3R2 and to a lesser extent the IP_3R3 but not IP_3R1. INS-1and rat β-cells express predominantly IP_3R3 and IP_3R2 and to a lesser extent IP_3R1 (Li and Zhang 2009). It is evident from the same atlas that the tissue distribution of RyRs is wider than that of the IP_3Rs. In fact, all of the three RyRs (i.e., RyR1, RyR2, and RyR3) are expressed to a variable degree, in almost all human tissues examined. All of the three RyRs are present also in the human islets. By RT-PCR, the mRNAs of the three types of RyRs can be detected in whole human islets (Dror et al. 2008). β-Cells certainly express the RyR2 and probably also the RyR1 isoform (Dror et al. 2008; Islam et al. 1998; Mitchell et al. 2003). By RT-PCR, mRNA for RyR1 was not detectable in INS-1 cells and rat islets, whereas mRNA for RyR2 was readily detected (Li and Zhang 2009). Takasawa et al. have identified a novel splicing subtype of RyR in human islets, and this needs to be followed up (Takasawa et al. 2010). By immunofluorescence using a monoclonal antibody that detects RyR1 and RyR2, Johnson et al. show that RyRs are present

in ~80 % of β-cells in dispersed human islets (Johnson et al. 2004a, b). Earlier studies on the RyRs in the β-cells and regulation of these channels have been reviewed (Islam 2002).

In MIN6 cells, it has been shown that RyR1 is located mainly on the insulin-containing dense-core secretory vesicles, whereas RyR2 is located mainly on the ER (Mitchell et al. 2003). Dantrolene, a blocker of RyR1, inhibits Ca^{2+} release from the vesicles and inhibits insulin secretion (Mitchell et al. 2003). By using a variety of approaches, including siRNA technology, Rosker et al. show that RINm5F cells express RyR2 also on the plasma membrane (Rosker et al. 2009). These putative plasma membrane RyR channels have conductance properties that are different from those reported for RyR2 in the literature, which makes one speculate that it could be a different nonspecific cation channel.

Low concentration of ryanodine (e.g., 1 nM) increases $[Ca^{2+}]_i$ and stimulates insulin secretion from human β-cells (Johnson et al. 2004b). Another activator of RyR, 9-Methyl-7-bromoeudistomin D increases insulin secretion in a glucose-dependent manner (Bruton et al. 2003). Four molecules of FKBP12.6 are tightly associated with the four RyR2 protomers, whereby it stabilizes and modulates activity of the channel. In FKBP12.6 knockout mice, glucose-induced insulin secretion is impaired (Noguchi et al. 2008). Among the glycolytic intermediates, fructose 1,6 diphosphate activates RyR2 (Kermode et al. 1998). Stimulation of β-cells by glucose increases the concentration of arachidonic acid which can activate RyRs (Jones and Persaud 1993). Other molecules that can link glucose metabolism to the RyRs are long-chain Acyl CoA, cADPR, and of course ATP (Islam 2002).

A mathematical model to explain mechanism of glucose-induced changes in membrane potential of β-cells postulates that RyR stimulation changes the pattern from "bursting" to "complex bursting" (Zhan et al. 2008). The term "complex" or "compound" bursting refers to cyclic variations in the duration of the slow waves of depolarization and repolarization intervals observed in some islets, when they are stimulated by glucose (Henquin et al. 1982; Beauvois et al. 2006). In mouse islets, compound bursting gives rise to mixed $[Ca^{2+}]_i$ oscillations (i.e., rapid $[Ca^{2+}]_i$ oscillations superimposed on slow ones) (Beauvois et al. 2006). If Ca^{2+} release from the ER (through RyRs or IP_3Rs) is responsible for compound bursting and consequent mixed $[Ca^{2+}]_i$ oscillations, then both of them should be abolished when the ER Ca^{2+} pool is empty. In fact, that is exactly what happens. Thus, if the ER Ca^{2+} pool is emptied by thapsigargin in the normal mice, or by knocking out SERCA3, then there is no compound bursting and no mixed $[Ca^{2+}]_i$ oscillations (Beauvois et al. 2006). Analysis of electrical activity shows a higher percentage of active phases in SERCA3$^{-/-}$mice (Beauvois et al. 2006), which suggests that Ca^{2+} release (through RyRs or IP_3Rs) from SERCA3-equipped ER Ca^{2+} pool terminates the active phase (for instance, by activating the K_{Ca} channels).

Glinides are a group of drugs used to stimulate insulin secretion in the treatment of type 2 diabetes. These drugs stimulate exocytosis even in SUR1 knockout mice (Nagamatsu et al. 2006). One of the mechanisms by which glinides induce insulin secretion is activation of the RyRs (Shigeto et al. 2007). GLP-1 stimulates insulin

secretion by cAMP-dependent mechanisms that include sensitization of RyR-mediated CICR (Kang et al. 2005).

In human type 2 diabetes, there is increased phosphorylation of the RyR2 of the β-cells by Ca^{2+}-/calmodulin-dependent protein kinase II (CAMKII). This leads to leaky RyR2, futile Ca^{2+} cycling, lower $[Ca^{2+}]_i$ transients, basal hyperinsulinemia, and impaired glucose-stimulated insulin secretion (Dixit et al. 2013).

Cyclic ADP Ribose (cADPR) and Nicotinic Acid Adenine Dinucleotide Phosphate (NAADP)

These two intracellular messengers are formed from β-NAD^+ and $NADP^+$ by several ADP ribosyl cyclases including CD38 (Lee 2012). These messengers release Ca^{2+} from the intracellular stores. While cADPR releases Ca^{2+} from the ER, NAADP releases Ca^{2+} from acidic Ca^{2+} stores like lysosomes and even from insulin secretory vesicles (Mitchell et al. 2003). Several groups have reported the roles for cADPR and NAADP in the regulation of Ca^{2+} signaling and insulin secretion. In β-cells, cADPR not only releases Ca^{2+} from the ER but also triggers Ca^{2+} entry across the plasma membrane by activating the TRPM2 channel (Togashi et al. 2006). High concentrations of glucose increase cADPR level in the β-cells. PKA phosphorylation activates CD38 and thereby increases formation of cADPR (Kim et al. 2008). Thus, incretins like GLP-1 lead to an increased formation of cADPR (Kim et al. 2008). Abscisic acid is a proinflammatory cytokine released by β-cells upon stimulation by glucose. It acts in an autocrine/paracrine fashion on a putative receptor that is coupled to a pertussis toxin-sensitive G protein, and it increases cAMP level, which via PKA phosphorylation of CD38 increases formation of cADPR. Nanomolar concentration of abscisic acid increases glucose-stimulated insulin secretion from human islets (Bruzzone et al. 2008).

Glucose increases NAADP level in MIN6 cells and uncaging of microinjected caged NAADP increases $[Ca^{2+}]_i$ in these cells by releasing Ca^{2+} from a thapsigargin-insensitive pool (Masgrau et al. 2003). NAADP-induced Ca^{2+} release is blocked by nifedipine and some other blockers of the L-type VGCCs. One of the organelles that constitutes the NAADP-sensitive Ca^{2+} stores in the cells is the dense-core insulin secretory vesicles (Mitchell et al. 2003). Microinjection of NAADP into human β-cells induces Ca^{2+} release from the intracellular stores in an oscillatory manner (Johnson and Misler 2002). Insulin increases $[Ca^{2+}]_i$ in about 30 % of human β-cells by a NAADP-dependent mechanism (Johnson and Misler 2002). It is not known whether insulin increases NAADP level in human β-cells. It does not increase NAADP in mouse β-cells (Kim et al. 2008).

A widely held view is that NAADP releases Ca^{2+} by activating a group of voltage-gated ion channels called the "two-pore channels" (TPCs also termed TPCNs) (Calcraft et al. 2009). TPC2 is located on the lysosomal membranes, and it releases Ca^{2+} when activated by low nanomolar concentration of NAADP. Micromolar concentration of NAADP inhibits the channel. In the TPC2 knockout mice, NAADP fails to release Ca^{2+} from the intracellular stores of β-cells

(Calcraft et al. 2009). However, direct recording of TPCs shows that these are actually Na^+-selective channels, activated by phosphatidylinositol 3,5-biphosphate and not by NAADP (Wang et al. 2012).

The most well-known enzyme that synthesizes cADPR and NAADP is CD38. However, studies using CD38 knockout mice suggest that CD38 does not play an essential role in glucose stimulation of Ca^{2+} signals or insulin secretion. In CD38 knockout mice, the islets are more susceptible to apoptosis suggesting that CD38/cADPR/NAADP system may instead be important for β-cell survival (Johnson et al. 2006).

Ca^{2+}-Induced Ca^{2+} Release (CICR)

Just as there are voltage-gated Ca^{2+} channels (VGCC) in the plasma membrane, there are Ca^{2+}-gated Ca^{2+} channels (CGCC) on the intracellular Ca^{2+} stores. Both IP_3Rs and RyRs are CGCCs (Swatton et al. 1999; Bezprozvanny et al. 1991), and both can mediate CICR, making the process a universal one (Dyachok et al. 2004). It is easy to study VGCCs on the plasma membrane by patch clamp. Nevertheless, to activate a given VGCC, one has to carefully choose the holding potential, the voltage jump, and its duration depending on which VGCC one is looking for. Availability of potent and specific inhibitors of VGCCs has made it further easier to study these channels. This is why the literature on Ca^{2+} signaling in the islets is hugely dominated by VGCCs. The situation is far more difficult when it comes to the study of CGCCs. In analogy with VGCCs, for triggering CGCCs by Ca^{2+}, one has to carefully choose the magnitude and the duration of the Ca^{2+} trigger (Fabiato 1985). In practice, this is not easy. Activation of CGCCs is further dependent on the filling state of the Ca^{2+} store, phosphorylation status, and co-agonists, e.g., IP_3 and cADPR. The pharmacology of CGCCs is also more complex than that of VGCCs. Thus, low nanomolar concentration of ryanodine activates RyRs, and high concentration of ryanodine irreversibly locks the RyRs in a subconductance state. Inhibition of Ca^{2+} release by ryanodine is a use-dependent process and needs attention to appropriate protocols (Woolcott et al. 2006).

Measurement of spatially averaged $[Ca^{2+}]_i$ by using nonlinear Ca^{2+} indicators like fura-2 and indo-1 is not particularly suitable for quantitative studies of CICR, which takes the form of transient rises of $[Ca^{2+}]_i$ in discrete locations in the cytoplasm (Yue and Wier 1985). Moreover, some of these indicators act as mobile buffers that bind the triggering Ca^{2+} with high affinity and snatch it away from the site of action (Neher 1995). In this respect, lower affinity brighter indicators like fluo-3, which can be used at lower concentrations, are less of a problem. The global increase of $[Ca^{2+}]_i$ that one sees in a β-cell upon stimulation by glucose plus incretin hormones (e.g., GLP-1) is a net result of Ca^{2+} that enters through the plasma membrane and Ca^{2+} that is released from the stores by CICR (provided the conditions for engaging CICR mechanism are in place). However, direct visualization of the CICR component may be difficult because of cell-wide increase of $[Ca^{2+}]_i$. One trick we employed was to use Sr^{2+} instead of Ca^{2+} as the

trigger and exploit the differences in the fluorescence properties of Ca^{2+}- and Sr^{2+}-bound fluo-3. By this way one can show Sr^{2+}-induced Ca^{2+} release and assume that it is equivalent to CICR (Lemmens et al. 2001). Another trick is to use verapamil, which reduces the probability of opening of the L-type VGCCs, and thereby reduces their contribution to the $[Ca^{2+}]_i$ increase. This enables better visualization of the $[Ca^{2+}]_i$ increase that is attributable to CICR. The rationale of such approach is based on the facts that verapamil does not reduce the amplitude of the single channel current; it reduces only the frequency of the triggering events, but not their effectiveness in eliciting CICR (Lopez-Lopez et al. 1995). In the experiment illustrated in Fig. 1, we stimulated a human β-cell first by 30 mM KCl, which resulted in an increase of $[Ca^{2+}]_i$ to ~400 nM. We then applied verapamil, which reduced the $[Ca^{2+}]_i$ to the baseline. We then washed away KCl and added, instead, glucose plus GLP-1. Glucose depolarized the β-cells, but the expected sustained $[Ca^{2+}]_i$ increase was absent because of verapamil. Nevertheless, the L-type VGCC-mediated trigger events (which were now less frequent because of verapamil) did elicit large $[Ca^{2+}]_i$ transients by activating CICR. These $[Ca^{2+}]_i$ transients are too large to be explained by Ca^{2+} entry through the L-type VGCCs per se. These are due to synchronous activation of RyRs in clusters. In this protocol, glucose facilitates CICR presumably by increasing the ER Ca^{2+} content and by providing ATP, and fructose 1,6 diphosphate, all of which sensitize the RyRs. GLP-1 was included in this protocol since it facilitates CICR by PKA-dependent phosphorylation of the RyRs (Islam et al. 1998; Holz et al. 1999).

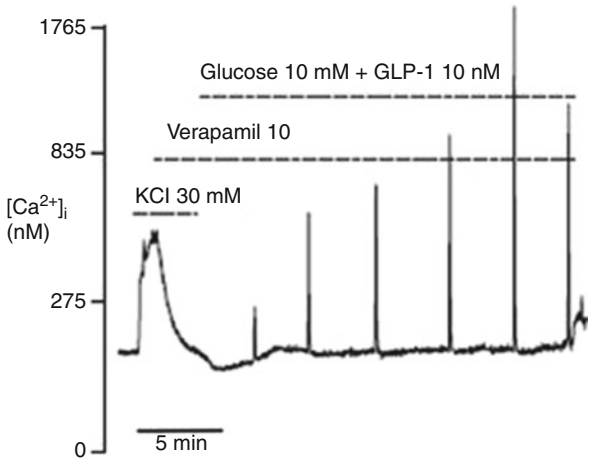

Fig. 1 CICR in human β-cells. $[Ca^{2+}]_i$ was measured by microfluorometry in fura-2-loaded single human β-cells. The cell was depolarized by KCl (30 mM) which increased $[Ca^{2+}]_i$. Verapamil (10 μM) was then added and it lowered $[Ca^{2+}]_i$ to the baseline. (For rationale of using verapamil, please see the text and the references.) KCl was then removed, and the cell was activated by glucose (10 mM) plus GLP-1 (10 nM). This protocol allowed visualization of CICR in the form of large Ca^{2+} transients

In addition, cAMP-regulated guanine nucleotide exchange factors (Epac) can also activate CICR via RyRs in human β-cells (Kang et al. 2003).

One important function of CICR in the β-cells is that it amplifies Ca^{2+}-dependent exocytosis (Kang and Holz 2003; Dyachok and Gylfe 2004). In addition RyRs associated with the secretory vesicles are thought to play a role in the exocytosis by increasing local Ca^{2+} concentration (Mitchell et al. 2003). It may be noted that stimulation of β-cells by glucose alone (without cAMP-elevating agents) does not engage RyRs, and thus glucose-induced insulin secretion from human β-cells is not sensitive to inhibition or stimulation by ryanodine, especially when protocols for use-dependent inhibition of RyRs by ryanodine are not used (Johnson et al. 2004b). CICR takes the form of large local Ca^{2+} transients, and their function depends on the subcellular location of the transients. One possibility is that a large Ca^{2+} transient caused by CICR repolarizes plasma membrane potential by activating K_{Ca} channels. Thus, a CICR event can end a burst of electrical activity and bring back the membrane potential from the plateau depolarization to the baseline repolarized state and thereby increase the frequency of membrane potential oscillations. This view is supported by the observations that β-cells of SERCA3$^{-/-}$ mice as well as thapsigargin-treated β-cells (both of which would be unable to trigger CICR) spend a higher proportion of time in depolarized state and have lower frequency of membrane potential oscillation (Beauvois et al. 2006). One may speculate that at early stages of development of type 2 diabetes, β-cell failure can be predominantly a depolarization failure or a repolarization failure. This view is a kin to two forms of heart failure where one can have predominantly systolic failure or predominantly diastolic failure. Repolarization failure of β-cells (failure of β-cells to "relax") will lead to hyperinsulinemia and disturb the pulsatility of insulin secretion, all too well-known features of early stages of diabetes. In terms of Ca^{2+} signaling, such repolarization failure can be attributed to failure of CICR, which can in principle be corrected by GLP-1, an established therapeutic agent for type 2 diabetes.

$[Ca^{2+}]_i$ Oscillation in the β-Cells

In the normal human body, β-cells are stimulated by glucose, the concentration of which oscillates at ~4 min interval. However, in most in vitro experiments, β-cells are stimulated by a constantly elevated concentration of glucose. In the normal human body, β-cells are supplied with glucose (and other nutrients, hormones) through a rich network of capillaries; in most in vitro experiments, glucose is not delivered to the islet cells through capillaries. As mentioned earlier, human islets secrete insulin in the form of pulses at ~5 min intervals both in the fasting and in the fed states. One would expect that $[Ca^{2+}]_i$ in the human islets would change in the form of oscillations with one $[Ca^{2+}]_i$ peak every ~5 min; $[Ca^{2+}]_i$ would return to the baseline in between the peaks. This expectation is based on the observations made in isolated and cultured mouse islets, where glucose induces baseline $[Ca^{2+}]_i$ oscillations and corresponding pulses of insulin secretion (Ravier et al. 2005).

However, stimulation of human islets by glucose shows many types of $[Ca^{2+}]_i$ responses (Martin and Soria 1996). In many islets, $[Ca^{2+}]_i$ is increased and remains persistently elevated, and in others there are some high-frequency sinusoidal oscillations of $[Ca^{2+}]_i$ on top of the $[Ca^{2+}]_i$ plateau (Martin and Soria 1996; Kindmark et al. 1991, 1994; Hellman et al. 1994). Such sinusoidal oscillations of $[Ca^{2+}]_i$ on top of a $[Ca^{2+}]_i$ plateau have been described also in islets obtained from a subject with impaired glucose tolerance (Kindmark et al. 1994). As early as in 1992, Misleret al. wrote: "four of 11 islets showed little or no response to 10 mM glucose while still responding to 20 μM tolbutamide. The pattern of glucose response of glucose-sensitive islets was also variable. Four islets displayed glucose-induced oscillations superimposed on a plateau. Two islets displayed a slow rise to a plateau without oscillations. The remaining islets showed an increasing frequency of short transients on an unchanging baseline; these transients ultimately coalesced into a prominent spike-like rise" (Misler et al. 1992). Note that these are not bad islets; in fact these are islets of such good quality that they could be used for transplantation into human body for the cure of diabetes. Investigators know that stimulation of human islets by glucose often leads to persistent elevations of $[Ca^{2+}]_i$, rather than baseline oscillations of $[Ca^{2+}]_i$. To increase chances of obtaining oscillatory changes in $[Ca^{2+}]_i$, some investigators replace extracellular Ca^{2+} by Sr^{2+} (Hellman et al. 1997). This maneuver yields nicer oscillatory changes in $[Sr^{2+}]_i$ and pulsatile insulin secretion from human islets (Hellman et al. 1997). But again, nature has chosen Ca^{2+} and not Sr^{2+} for signaling.

Some islet researchers assume that normal human islets should respond by $[Ca^{2+}]_i$ increase in the form of baseline $[Ca^{2+}]_i$ oscillations and that persistent $[Ca^{2+}]_i$ elevation is a sign of subtle damage to the islets or suboptimal experimental conditions (Hellman et al. 1997). At first sight, this seems to be a fair argument: for instance, some Ca^{2+} laboratories receive islets from a human islet isolation facility located next door; others receive islets via transatlantic flights. Ca^{2+} measurement techniques that use UV light and fura-2 acetoxymethyl esters (or similar probes) can damage islets whose metabolism is often stunned and whose microcirculation and neural connections are lost. In fact, many individual islets obtained from normal subjects do not show any Ca^{2+} response at all to any stimulus (Kindmark et al. 1991). Investigators select, consciously or subconsciously, the experiments that show nice $[Ca^{2+}]_i$ oscillations (because the islets that do not show oscillations are presumed to be the bad ones). In fact, they select the very islet that they choose to examine. There are several millions of islets in a human pancreas, and they differ in their sizes, structures, and cellular makeup (see chapter "► Microscopic Anatomy of the Human Islet of Langerhans"). They look different even to the naked eyes and under the microscope. Some look like "nice" encapsulated islets, and others look like small aggregates of loosely associated cells, both types being normal. Investigators choose the "nice" ones for their experiments, but still get different kinds of $[Ca^{2+}]_i$ responses. It is noteworthy that most such studies did not employ any cAMP-elevating agents, making CICR impossible.

$[Ca^{2+}]_i$ responses of single human β-cells to glucose are also extremely heterogeneous. Nevertheless, when single human β-cells are stimulated by glucose (in the

absence of other nutrients, hormones, or neurotransmitters), many of them do respond by $[Ca^{2+}]_i$ changes in the form of slow oscillations, whereby $[Ca^{2+}]_i$ reaches to peaks every 2–5 min and then returns to the baseline. Some investigators show that when $[Ca^{2+}]_i$ oscillations occur in one human β-cell, the neighboring β-cells in an aggregate or in an islet show $[Ca^{2+}]_i$ oscillation in a synchronized manner (Martin and Soria 1996; Hellman et al. 1997). This is due to coupling between β-cells via gap junctions made of connexin36 (Ravier et al. 2005; Serre-Beinier et al. 2009). Other investigators report that synchrony of $[Ca^{2+}]_i$ oscillation between groups of β-cells occurs in mouse islets, but not in human islets (Manning Fox et al. 2006; Cabrera et al. 2006). Experiments using expressed fluorescent vesicle cargo proteins, and total internal reflection fluorescence microscopy, show that stimulation of single human β-cells by glucose gives rise to bursts of insulin vesicle secretion (at intervals of 15–45 s) that coincides with transient increase of $[Ca^{2+}]_i$ (Michael et al. 2007). However, it needs to be pointed out that glucose-induced baseline $[Ca^{2+}]_i$ oscillations in single β-cells that we are talking about occur only in Petri dishes and are unlikely to occur in vivo. In vivo, hormones (e.g., glucagon and incretins) and amino acids (e.g., glycine and many others) are likely to transform the oscillatory $[Ca^{2+}]_i$ changes to a persistent elevation of $[Ca^{2+}]_i$ (Hellman et al. 1994). Thus, in the human β-cells and islets, persistent increase of $[Ca^{2+}]_i$ in response to glucose is a rule rather than exception. The underlying cause of glucose-induced baseline $[Ca^{2+}]_i$ oscillations in β-cells is thought to be the electrical bursts (clusters of large amplitude brief action potentials; one burst accounting for one episode of $[Ca^{2+}]_i$ increase). Study of β-cells from large mammals (e.g., dogs), however, shows that bursts occur only during the initial period of stimulation by glucose. In the later part of stimulation, bursts disappear; instead, there is sustained plateau depolarization to −35 to −20 mV and sustained increase of $[Ca^{2+}]_i$ to 500–1,000 nM, which causes tonic exocytosis (Misler et al. 2009). Furthermore, at least some studies claim that insulin secretion is pulsatile even when $[Ca^{2+}]_i$ is stably elevated (Bergsten and Hellman 1993; Kjems et al. 2002). It should be noted that stimulation by glucose increases concentration of many molecules in the β-cells in an oscillatory manner (e.g., ATP (Ainscow and Rutter 2002) and cAMP (Dyachok et al. 2008)). Of these, oscillations of $[Ca^{2+}]_i$ are the easiest one to record and have, therefore, been adopted for modeling studies. It is thus not surprising that pulsatility of insulin secretion from human islets in vivo has been modeled based on data obtained from in vitro experiments done on mice islets (read the chapter "▶ Electrical, Calcium, and Metabolic Oscillations in Pancreatic Islets"). This is in spite of the fact that the kind of electrical bursts and baseline $[Ca^{2+}]_i$ oscillations that occur in mouse islets has not been reproducibly demonstrated in human islets. This is not because of scarcity of human islets. In fact, during recent years, it has become easier to obtain human islets for basic researches (Kaddis et al. 2009). At present, it appears that human islets show a wide variety of electrical activities and patterns of $[Ca^{2+}]_i$ changes, which cannot explain the pulsatile insulin secretion into the portal vein. Other less obvious factors that are unrelated to $[Ca^{2+}]_i$ oscillations, e.g., islet-liver interaction, may well constitute part of the mechanisms that determine pulsatile insulin secretion into the portal vein under normal conditions (Goodner et al. 1982).

Concluding Remarks

The literature on the Ca^{2+} signaling in the islet cells and a variety of other insulin secreting cells is huge. The number of players that participate in the generation and the decoding processes of various Ca^{2+} signals is also increasing. While interpreting results of experiments designed to elucidate any aspects of Ca^{2+} signaling in these cells, it is important to scrutinize what experimental models and protocols have been used, and how the use of different molecular or pharmacological tools might have led to adaptive changes for ensuring Ca^{2+} homeostasis. Many key experiments must be repeated by more than one group to examine how reproducible the results are. Reproducibility factor is more important than citation factor. Emphasis on the study of the human islet cells and the study of Ca^{2+} signaling in these cells in the context of understanding the pathogenesis of islet failure, or β-cell death, is likely to lead to a clearer understanding of the pathogenesis of human diabetes and eventually to the discovery of new ways and means for the prevention and treatment of the disease.

Acknowledgements Research in the author's lab was supported by the funds from Karolinska Institutet and Uppsala County Council.

Cross-References

- ▶ ATP-Sensitive Potassium Channels in Health and Disease
- ▶ β Cell Store-Operated Ion Channels
- ▶ (Dys)Regulation of Insulin secretion by Macronutrients
- ▶ Electrical, Calcium, and Metabolic Oscillations in Pancreatic Islets
- ▶ Electrophysiology of Islet Cells
- ▶ Exocytosis in Islet β-Cells
- ▶ Molecular Basis of cAMP Signaling in Pancreatic β Cells
- ▶ Role of Mitochondria in β-Cell Function and Dysfunction

References

Ainscow EK, Rutter GA (2002) Glucose-stimulated oscillations in free cytosolic ATP concentration imaged in single islet β-cells: evidence for a Ca^{2+}-dependent mechanism. Diabetes 51(Suppl 1):S162–S170

Akiba Y, Kato S, Katsube KI, Nakamura M, Takeuchi K, Ishii H, Hibi T (2004) Transient receptor potential vanilloid subfamily 1 expressed in pancreatic islet β cells modulates insulin secretion in rats. Biochem Biophys Res Commun 321:219–225

Babes A, Fischer MJ, Filipovic M, Engel MA, Flonta ML, Reeh PW (2013) The anti-diabetic drug glibenclamide is an agonist of the transient receptor potential Ankyrin 1 (TRPA1) ion channel. Eur J Pharmacol 704:15–22

Bari MR, Akbar S, Eweida M, Kuhn FJ, Gustafsson AJ, Luckhoff A, Islam MS (2009) H2O2-induced Ca^{2+} influx and its inhibition by N-(p-amylcinnamoyl) anthranilic acid in the β-cells: involvement of TRPM2 channels. J Cell Mol Med 13:3260–3267

Barnett DW, Pressel DM, Misler S (1995) Voltage-dependent Na^+ and Ca^{2+} currents in human pancreatic islet β-cells: evidence for roles in the generation of action potentials and insulin secretion. Pflugers Arch 431:272–282

Beauvois MC, Merezak C, Jonas JC, Ravier MA, Henquin JC, Gilon P (2006) Glucose-induced mixed $[Ca^{2+}]c$ oscillations in mouse β-cells are controlled by the membrane potential and the SERCA3 Ca^{2+}-ATPase of the endoplasmic reticulum. Am J Physiol Cell Physiol 290:C1503–C1511

Bergsten P, Hellman B (1993) Glucose-induced amplitude regulation of pulsatile insulin secretion from individual pancreatic islets. Diabetes 42:670–674

Bezprozvanny I, Watras J, Ehrlich BE (1991) Bell-shaped calcium-response curves of Ins(1,4,5) P*3*- and calcium gated channels from endoplasmic reticulum of cerebellum. Nature 351:751–754

Bokvist K, Eliasson L, Ämmälä C, Renström E, Rorsman P (1995) Co-localization of L-type Ca^{2+} channels and insulin-containing secretory granules and its significance for the initiation of exocytosis in mouse pancreatic β-cells. EMBO J 14:50–57

Braun M, Ramracheya R, Bengtsson M, Zhang Q, Karanauskaite J, Partridge C, Johnson PR, Rorsman P (2008) Voltage-gated ion channels in human pancreatic β-cells: electrophysiological characterization and role in insulin secretion. Diabetes 57:1618–1628

Brixel LR, Monteilh-Zoller MK, Ingenbrandt CS, Fleig A, Penner R, Enklaar T, Zabel BU, Prawitt D (2010) TRPM5 regulates glucose-stimulated insulin secretion. Pflugers Arch 460:69–76

Bruton JD, Lemmens R, Shi CL, Persson-Sjögren S, Westerblad H, Ahmed M, Pyne NJ, Frame M, Furman BL, Islam MS (2003) Ryanodine receptors of pancreatic β-cells mediate a distinct context-dependent signal for insulin secretion. FASEB J 17:301–303

Bruzzone S, Bodrato N, Usai C, Guida L, Moreschi I, Nano R, Antonioli B, Fruscione F, Magnone M, Scarfi S, De Flora A, Zocchi E (2008) Abscisic acid is an endogenous stimulator of insulin release from human pancreatic islets with cyclic ADP ribose as second messenger. J Biol Chem 283:32188–32197

Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A (2006) The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proc Natl Acad Sci USA 103:2334–2339

Calcraft PJ, Ruas M, Pan Z, Cheng X, Arredouani A, Hao X, Tang J, Rietdorf K, Teboul L, Chuang KT, Lin P, Xiao R, Wang C, Zhu Y, Lin Y, Wyatt CN, Parrington J, Ma J, Evans AM, Galione A, Zhu MX (2009) NAADP mobilizes calcium from acidic organelles through two-pore channels. Nature 459:596–600

Cao DS, Zhong L, Hsieh TH, Abooj M, Bishnoi M, Hughes L, Premkumar LS (2012) Expression of Transient Receptor Potential Ankyrin 1 (TRPA1) and its role in insulin release from rat pancreatic β cells. PLoS One 7:e38005

Casas S, Novials A, Reimann F, Gomis R, Gribble FM (2008) Calcium elevation in mouse pancreatic β cells evoked by extracellular human islet amyloid polypeptide involves activation of the mechanosensitive ion channel TRPV4. Diabetologia 51:2252–2262

Cheng H, Beck A, Launay P, Gross SA, Stokes AJ, Kinet JP, Fleig A, Penner R (2007) TRPM4 controls insulin secretion in pancreatic β-cells. Cell Calcium 41:51–61

Cheng KT, Ong HL, Liu X, Ambudkar IS (2013) Contribution and regulation of TRPC channels in store-operated Ca^{2+} entry. Curr Top Membr 71:149–179

Colsoul B, Schraenen A, Lemaire K, Quintens R, Van Lommel L, Segal A, Owsianik G, Talavera K, Voets T, Margolskee RF, Kokrashvili Z, Gilon P, Nilius B, Schuit FC, Vennekens R (2010) Loss of high-frequency glucose-induced Ca^{2+} oscillations in pancreatic islets correlates with impaired glucose tolerance in $Trpm5^{-/-}$ mice. Proc Natl Acad Sci USA 107:5208–5213

Cuesta-Munoz AL, Huopio H, Otonkoski T, Gomez-Zumaquero JM, Nanto-Salonen K, Rahier J, Lopez-Enriquez S, Garcia-Gimeno MA, Sanz P, Soriguer FC, Laakso M (2004) Severe persistent hyperinsulinemic hypoglycemia due to a de novo glucokinase mutation. Diabetes 53:2164–2168

Davalli AM, Biancardi E, Pollo A, Socci C, Pontiroli AE, Pozza G, Clementi F, Sher E, Carbone E (1996) Dihydropyridine-sensitive and -insensitive voltage-operated calcium channels participate in the control of glucose-induced insulin release from human pancreatic β cells. J Endocrinol 150:195–203

Dehaven WI, Jones BF, Petranka JG, Smyth JT, Tomita T, Bird GS, Putney JW Jr (2009) TRPC channels function independently of STIM1 and Orai1. J Physiol 587:2275–2298

Demion M, Bois P, Launay P, Guinamard R (2007) TRPM4, a Ca^{2+}-activated nonselective cation channel in mouse sino-atrial node cells. Cardiovasc Res 73:531–538

Dixit SS, Wang T, Manzano EJ, Yoo S, Lee J, Chiang DY, Ryan N, Respress JL, Yechoor VK, Wehrens XH (2013) Effects of CaMKII-mediated phosphorylation of ryanodine receptor type 2 on islet calcium handling, insulin secretion, and glucose tolerance. PLoS One 8:e58655

Dror V, Kalynyak TB, Bychkivska Y, Frey MH, Tee M, Jeffrey KD, Nguyen V, Luciani DS, Johnson JD (2008) Glucose and endoplasmic reticulum calcium channels regulate HIF-1β via presenilin in pancreatic β-cells. J Biol Chem 283:9909–9916

Du J, Xie J, Yue L (2009) Intracellular calcium activates TRPM2 and its alternative spliced isoforms. Proc Natl Acad Sci USA 106:7239–7244

Dyachok O, Gylfe E (2001) Store-operated influx of Ca^{2+} in pancreatic β-cells exhibits graded dependence on the filling of the endoplasmic reticulum. J Cell Sci 114:2179–2186

Dyachok O, Gylfe E (2004) Ca^{2+}-induced Ca^{2+} release via inositol 1,4,5-trisphosphate receptors is amplified by protein kinase A and triggers exocytosis in pancreatic β-cells. J Biol Chem 279:45455–45461

Dyachok O, Tufveson G, Gylfe E (2004) Ca^{2+}-induced Ca^{2+} release by activation of inositol 1,4,5-trisphosphate receptors in primary pancreatic β-cells. Cell Calcium 36:1–9

Dyachok O, Idevall-Hagren O, Sagetorp J, Tian G, Wuttke A, Arrieumerlou C, Akusjarvi G, Gylfe E, Tengholm A (2008) Glucose-induced cyclic AMP oscillations regulate pulsatile insulin secretion. Cell Metab 8:26–37

Eisfeld J, Luckhoff A (2007) TRPM2. Handb Exp Pharmacol 179:237–252

Fabiato A (1985) Time and calcium dependence of activation and inactivation of calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. J Gen Physiol 85:247–289

Fruhwald J, Camacho LJ, Dembla S, Mannebach S, Lis A, Drews A, Wissenbach U, Oberwinkler J, Philipp SE (2012) Alternative splicing of a protein domain indispensable for function of transient receptor potential melastatin 3 (TRPM3) ion channels. J Biol Chem 287:36663–36672

Gembal M, Detimary P, Gilon P, Gao ZY, Henquin JC (1993) Mechanisms by which glucose can control insulin release independently from its action on adenosine triphosphate-sensitive K^+ channels in mouse B cells. J Clin Invest 91:871–880

Gilon P, Henquin JC (1992) Influence of membrane potential changes on cytoplasmic Ca^{2+} concentration in an electrically excitable cell, the insulin-secreting pancreatic β-cell. J Biol Chem 267:20713–20720

Gilon P, Henquin JC (2001) Mechanisms and physiological significance of the cholinergic control of pancreatic β-cell function. Endocr Rev 22:565–604

Goodner CJ, Hom FG, Koerker DJ (1982) Hepatic glucose production oscillates in synchrony with the islet secretory cycle in fasting rhesus monkeys. Science 215:1257–1260

Gram DX, Ahren B, Nagy I, Olsen UB, Brand CL, Sundler F, Tabanera R, Svendsen O, Carr RD, Santha P, Wierup N, Hansen AJ (2007) Capsaicin-sensitive sensory fibers in the islets of Langerhans contribute to defective insulin secretion in Zucker diabetic rat, an animal model for some aspects of human type 2 diabetes. Eur J Neurosci 25:213–223

Grimberg A, Ferry RJ Jr, Kelly A, Koo-McCoy S, Polonsky K, Glaser B, Permutt MA, Aguilar-Bryan L, Stafford D, Thornton PS, Baker L, Stanley CA (2001) Dysregulation of insulin secretion in children with congenital hyperinsulinism due to sulfonylurea receptor mutations. Diabetes 50:322–328

Gustafsson AJ, Ingelman-Sundberg H, Dzabic M, Awasum J, Nguyen KH, Östenson CG, Pierro C, Tedeschi P, Woolcott O, Chiounan S, Lund PE, Larsson O, Islam MS (2005) Ryanodine receptor-operated activation of TRP-like channels can trigger critical Ca^{2+} signaling events in pancreatic β-cells. FASEB J 19:301–303

Hara Y, Wakamori M, Ishii M, Maeno E, Nishida M, Yoshida T, Yamada H, Shimizu S, Mori E, Kudoh J, Shimizu N, Kurose H, Okada Y, Imoto K, Mori Y (2002) LTRPC2 Ca^{2+}-permeable channel activated by changes in redox status confers susceptibility to cell death. Mol Cell 9:163–173

Haspel D, Krippeit-Drews P, Aguilar-Bryan L, Bryan J, Drews G, Dufer M (2005) Crosstalk between membrane potential and cytosolic Ca^{2+} concentration in β cells from $Sur1^{-/-}$ mice. Diabetologia 48:913–921

Heiner I, Eisfeld J, Warnstedt M, Radukina N, Jungling E, Lückhoff A (2006) Endogenous ADP-ribose enables calcium-regulated cation currents through TRPM2 channels in neutrophil granulocytes. Biochem J 398:225–232

Hellman B, Gylfe E, Bergsten P, Grapengiesser E, Lund PE, Berts A, Tengholm A, Pipeleers DG, Ling Z (1994) Glucose induces oscillatory Ca^{2+} signalling and insulin release in human pancreatic β cells. Diabetologia 37(Suppl 2):S11–S20

Hellman B, Gylfe E, Bergsten P, Grapengiesser E, Berts A, Liu YJ, Tengholm A, Westerlund J (1997) Oscillatory signaling and insulin release in human pancreatic β-cells exposed to strontium. Endocrinology 138:3161–3165

Henquin JC, Meissner HP, Schmeer W (1982) Cyclic variations of glucose-induced electrical activity in pancreatic B cells. Pflugers Arch 393:322–327

Henquin JC, Dufrane D, Nenquin M (2006) Nutrient control of insulin secretion in isolated normal human islets. Diabetes 55:3470–3477

Herson PS, Ashford ML (1997) Activation of a novel non-selective cation channel by alloxan and H2O2 in the rat insulin-secreting cell line CRI-G1. J Physiol 501(Pt 1):59–66

Hisanaga E, Nagasawa M, Ueki K, Kulkarni RN, Mori M, Kojima I (2009) Regulation of calcium-permeable TRPV2 channel by insulin in pancreatic β-cells. Diabetes 58:174–184

Holmkvist J, Tojjar D, Almgren P, Lyssenko V, Lindgren CM, Isomaa B, Tuomi T, Berglund G, Renstrom E, Groop L (2007) Polymorphisms in the gene encoding the voltage-dependent Ca^{2+} channel Ca (V)2.3 (CACNA1E) are associated with type 2 diabetes and impaired insulin secretion. Diabetologia 50:2467–2475

Holz GG, Leech CA, Habener JF (1995) Activation of a cAMP-regulated Ca^{2+}-signaling pathway in pancreatic β-cells by the insulinotropic hormone glucagon-like peptide-1. J Biol Chem 270:17749–17757

Holz GG, Leech CA, Heller RS, Castonguay M, Habener JF (1999) cAMP-dependent mobilization of intracellular Ca^{2+} stores by activation of ryanodine receptors in pancreatic β-cells. A Ca^{2+} signalling system stimulated by the insulinotropic hormone glucagon-like peptide-1-(7-37). J Biol Chem 274:14147–14156

Idevall-Hagren O, Jakobsson I, Xu Y, Tengholm A (2013) Spatial control of Epac2 activity by cAMP and Ca^{2+}-mediated activation of Ras in pancreatic β cells. Sci Signal 6:ra29-6

Islam MS (2002) The ryanodine receptor calcium channel of β-cells: molecular regulation and physiological significance. Diabetes 51:1299–1309

Islam MS (2011) TRP channels of islets. Adv Exp Med Biol 704:811–830

Islam MS, Leibiger I, Leibiger B, Rossi D, Sorrentino V, Ekström TJ, Westerblad H, Andrade FH, Berggren PO (1998) *In situ* activation of the type 2 ryanodine receptor in pancreatic β cells requires cAMP-dependent phosphorylation. Proc Natl Acad Sci USA 95:6145–6150

Jabin FA, Kannisto K, Bostrom A, Hadrovic B, Farre C, Eweida M, Wester K, Islam MS (2012) Insulin-secreting INS-1E cells express functional TRPV1 channels. Islets 4:56–63

Johnson JD, Misler S (2002) Nicotinic acid-adenine dinucleotide phosphate-sensitive calcium stores initiate insulin signaling in human β cells. Proc Natl Acad Sci USA 99:14566–14571

Johnson JD, Han Z, Otani K, Ye H, Zhang Y, Wu H, Horikawa Y, Misler S, Bell GI, Polonsky KS (2004a) RyR2 and calpain-10 delineate a novel apoptosis pathway in pancreatic islets. J Biol Chem 279:24794–24802

Johnson JD, Kuang S, Misler S, Polonsky KS (2004b) Ryanodine receptors in human pancreatic β cells: localization and effects on insulin secretion. FASEB J 18:878–880

Johnson JD, Ford EL, Bernal-Mizrachi E, Kusser KL, Luciani DS, Han Z, Tran H, Randall TD, Lund FE, Polonsky KS (2006) Suppressed insulin signaling and increased apoptosis in CD38-null islets. Diabetes 55:2737–2746

Jones PM, Persaud SJ (1993) Arachidonic acid as a second messenger in glucose-induced insulin secretion from pancreatic β-cells. J Endocrinol 137:7–14

Jung SR, Reed BJ, Sweet IR (2009) A highly energetic process couples calcium influx through L-type calcium channels to insulin secretion in pancreatic β-cells. Am J Physiol Endocrinol Metab 297:E717–E727

Kaddis JS, Olack BJ, Sowinski J, Cravens J, Contreras JL, Niland JC (2009) Human pancreatic islets and diabetes research. JAMA 301:1580–1587

Kahl S, Malfertheiner P (2004) Exocrine and endocrine pancreatic insufficiency after pancreatic surgery. Best Pract Res Clin Gastroenterol 18:947–955

Kang G, Holz GG (2003) Amplification of exocytosis by Ca^{2+}-induced Ca^{2+} release in INS-1 pancreatic β cells. J Physiol 546:175–189

Kang G, Joseph JW, Chepurny OG, Monaco M, Wheeler MB, Bos JL, Schwede F, Genieser HG, Holz GG (2003) Epac-selective cAMP analog 8-pCPT-2′-O-Me-cAMP as a stimulus for Ca^{2+}-induced Ca^{2+} release and exocytosis in pancreatic β-cells. J Biol Chem 278:8279–8285

Kang G, Chepurny OG, Rindler MJ, Collis L, Chepurny Z, Li WH, Harbeck M, Roe MW, Holz GG (2005) A cAMP and Ca^{2+} coincidence detector in support of Ca^{2+}-induced Ca^{2+} release in mouse pancreatic β cells. J Physiol 566:173–188

Kermode H, Chan WM, Williams AJ, Sitsapesan R (1998) Glycolytic pathway intermediates activate cardiac ryanodine receptors. FEBS Lett 431:59–62

Kim BJ, Park KH, Yim CY, Takasawa S, Okamoto H, Im MJ, Kim UH (2008) Generation of nicotinic acid adenine dinucleotide phosphate and cyclic ADP-ribose by glucagon-like peptide-1 evokes Ca^{2+} signal that is essential for insulin secretion in mouse pancreatic islets. Diabetes 57:868–878

Kim MS, Zeng W, Yuan JP, Shin DM, Worley PF, Muallem S (2009) Native store-operated Ca^{2+} influx requires the channel function of Orai1 and TRPC1. J Biol Chem 284:9733–9741

Kindmark H, Köhler M, Nilsson T, Arkhammar P, Wiechel K-L, Rorsman P, Efendic S (1991) Measurements of cytoplasmic free Ca^{2+} concentration in human pancreatic islets and insulinoma cells. FEBS Lett 291:310–314

Kindmark H, Kohler M, Arkhammar P, Efendic S, Larsson O, Linder S, Nilsson T, Berggren PO (1994) Oscillations in cytoplasmic free calcium concentration in human pancreatic islets from subjects with normal and impaired glucose tolerance. Diabetologia 37:1121–1131

Kjems LL, Ravier MA, Jonas JC, Henquin JC (2002) Do oscillations of insulin secretion occur in the absence of cytoplasmic Ca^{2+} oscillations in β-cells? Diabetes 51(Suppl 1):S177–S182

Klose C, Straub I, Riehle M, Ranta F, Krautwurst D, Ullrich S, Meyerhof W, Harteneck C (2011) Fenamates as TRP channel blockers: mefenamic acid selectively blocks TRPM3. Br J Pharmacol 162:1757–1769

Krishnan K, Ma Z, Björklund A, Islam MS (2014) Role of Transient Receptor Potential Melastatin-like Subtype 5 Channel in Insulin Secretion From Rat β-Cells. Pancreas 43:597–604

Kyriazis GA, Soundarapandian MM, Tyrberg B (2012) Sweet taste receptor signaling in β cells mediates fructose-induced potentiation of glucose-stimulated insulin secretion. Proc Natl Acad Sci USA 109:E524–E532

Lange I, Yamamoto S, Partida-Sanchez S, Mori Y, Fleig A, Penner R (2009) TRPM2 functions as a lysosomal Ca^{2+}-release channel in β cells. Sci Signal 2:ra23

Launay P, Fleig A, Perraud AL, Scharenberg AM, Penner R, Kinet JP (2002) TRPM4 is a Ca^{2+}-activated nonselective cation channel mediating cell membrane depolarization. Cell 109:397–407

Lee HC (2012) Cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate (NAADP) as messengers for calcium mobilization. J Biol Chem 287:31633–31640

Lemmens R, Larsson O, Berggren PO, Islam MS (2001) Ca^{2+}-induced Ca^{2+} release from the endoplasmic reticulum amplifies the Ca^{2+} signal mediated by activation of voltage-gated L-type Ca^{2+} channels in pancreatic β-cells. J Biol Chem 276:9971–9977

Lheureux PE, Zahir S, Penaloza A, Gris M (2005) Bench-to-bedside review: antidotal treatment of sulfonylurea-induced hypoglycaemia with octreotide. Crit Care 9:543–549

Li F, Zhang ZM (2009) Comparative identification of Ca^{2+} channel expression in INS-1 and rat pancreatic β cells. World J Gastroenterol 15:3046–3050

Lin Y, Sun Z (2012) Antiaging gene Klotho enhances glucose-induced insulin secretion by up-regulating plasma membrane levels of TRPV2 in MIN6 β-cells. Endocrinology 153:3029–3039

Lopez-Lopez JR, Shacklock PS, Balke CW, Wier WG (1995) Local calcium transients triggered by single L-type calcium channel currents in cardiac cells. Science 268:1042–1045

Maechler P, Kennedy ED, Sebo E, Valeva A, Pozzan T, Wollheim CB (1999) Secretagogues modulate the calcium concentration in the endoplasmic reticulum of insulin-secreting cells. Studies in aequorin-expressing intact and permeabilized INS-1 cells. J Biol Chem 274:12583–12592

Majeed Y, Tumova S, Green BL, Seymour VA, Woods DM, Agarwal AK, Naylor J, Jiang S, Picton HM, Porter KE, O'Regan DJ, Muraki K, Fishwick CW, Beech DJ (2012) Pregnenolone sulphate-independent inhibition of TRPM3 channels by progesterone. Cell Calcium 51:1–11

Manning Fox JE, Gyulkhandanyan AV, Satin LS, Wheeler MB (2006) Oscillatory membrane potential response to glucose in islet β-cells: a comparison of islet-cell electrical activity in mouse and rat. Endocrinology 147:4655–4663

Marigo V, Courville K, Hsu WH, Feng JM, Cheng H (2009) TRPM4 impacts on Ca^{2+} signals during agonist-induced insulin secretion in pancreatic β-cells. Mol Cell Endocrinol 299:194–203

Martin F, Soria B (1996) Glucose-induced $[Ca^{2+}]_i$ oscillations in single human pancreatic islets. Cell Calcium 20:409–414

Masgrau R, Churchill GC, Morgan AJ, Ashcroft SJ, Galione A (2003) NAADP. A new second messenger for glucose-induced Ca^{2+} responses in clonal pancreatic β cells. Curr Biol 13:247–251

Mears D, Zimliki CL (2004) Muscarinic agonists activate Ca^{2+} store-operated and -independent ionic currents in insulin-secreting HIT-T15 cells and mouse pancreatic β-cells. J Membr Biol 197:59–70

Michael DJ, Xiong W, Geng X, Drain P, Chow RH (2007) Human insulin vesicle dynamics during pulsatile secretion. Diabetes 56:1277–1288

Misler S, Barnett DW, Pressel DM, Gillis KD, Scharp DW, Falke LC (1992) Stimulus-secretion coupling in β-cells of transplantable human islets of Langerhans. Evidence for a critical role for Ca^{2+} entry. Diabetes 41:662–670

Misler S, Dickey A, Barnett DW (2005) Maintenance of stimulus-secretion coupling and single β-cell function in cryopreserved-thawed human islets of Langerhans. Pflugers Arch 450:395–404

Misler S, Zhou Z, Dickey AS, Silva AM, Pressel DM, Barnett DW (2009) Electrical activity and exocytotic correlates of biphasic insulin secretion from β-cells of canine islets of Langerhans: contribution of tuning two modes of Ca^{2+} entry-dependent exocytosis to two modes of glucose-induced electrical activity. Channels (Austin) 3:181–193

Mitchell KJ, Lai FA, Rutter GA (2003) Ryanodine receptor type I and nicotinic acid adenine dinucleotide phosphate receptors mediate Ca^{2+} release from insulin-containing vesicles in living pancreatic β-cells (MIN6). J Biol Chem 278:11057–11064

Nagamatsu S, Ohara-Imaizumi M, Nakamichi Y, Kikuta T, Nishiwaki C (2006) Imaging docking and fusion of insulin granules induced by antidiabetes agents: sulfonylurea and glinide drugs preferentially mediate the fusion of newcomer, but not previously docked, insulin granules. Diabetes 55:2819–2825

Neher E (1995) The use of fura-2 for estimating Ca buffers and Ca fluxes. Neuropharmacology 34:1423–1442

Nelson PL, Zolochevska O, Figueiredo ML, Soliman A, Hsu WH, Feng JM, Zhang H, Cheng H (2011) Regulation of Ca^{2+}-entry in pancreatic α-cell line by transient receptor potential melastatin 4 plays a vital role in glucagon release. Mol Cell Endocrinol 335:126–134

Nilius B, Prenen J, Tang J, Wang C, Owsianik G, Janssens A, Voets T, Zhu MX (2005) Regulation of the Ca^{2+} sensitivity of the nonselective cation channel TRPM4. J Biol Chem 280:6423–6433

Nilius B, Mahieu F, Prenen J, Janssens A, Owsianik G, Vennekens R, Voets T (2006) The Ca^{2+}-activated cation channel TRPM4 is regulated by phosphatidylinositol 4,5-biphosphate. EMBO J 25:467–478

Noguchi N, Yoshikawa T, Ikeda T, Takahashi I, Shervani NJ, Uruno A, Yamauchi A, Nata K, Takasawa S, Okamoto H, Sugawara A (2008) FKBP12.6 disruption impairs glucose-induced insulin secretion. Biochem Biophys Res Commun 371:735–740

Oberwinkler J, Lis A, Giehl KM, Flockerzi V, Philipp SE (2005) Alternative splicing switches the divalent cation selectivity of TRPM3 channels. J Biol Chem 280:22540–22548

Otsuguro K, Tang J, Tang Y, Xiao R, Freichel M, Tsvilovskyy V, Ito S, Flockerzi V, Zhu MX, Zholos AV (2008) Isoform-specific inhibition of TRPC4 channel by phosphatidylinositol 4,5-bisphosphate. J Biol Chem 283:10026–10036

Palmer RK, Atwal K, Bakaj I, Carlucci-Derbyshire S, Buber MT, Cerne R, Cortes RY, Devantier HR, Jorgensen V, Pawlyk A, Lee SP, Sprous DG, Zhang Z, Bryant R (2010) Triphenylphosphine oxide is a potent and selective inhibitor of the transient receptor potential melastatin-5 ion channel. Assay Drug Dev Technol 8:703–713

Pinton P, Tsuboi T, Ainscow EK, Pozzan T, Rizzuto R, Rutter GA (2002) Dynamics of glucose-induced membrane recruitment of protein kinase C β II in living pancreatic islet β-cells. J Biol Chem 277:37702–37710

Prawitt D, Monteilh-Zoller MK, Brixel L, Spangenberg C, Zabel B, Fleig A, Penner R (2003) TRPM5 is a transient Ca^{2+}-activated cation channel responding to rapid changes in $[Ca^{2+}]_i$. Proc Natl Acad Sci USA 100:15166–15171

Ravier MA, Guldenagel M, Charollais A, Gjinovci A, Caille D, Sohl G, Wollheim CB, Willecke K, Henquin JC, Meda P (2005) Loss of connexin36 channels alters β-cell coupling, islet synchronization of glucose-induced Ca^{2+} and insulin oscillations, and basal insulin release. Diabetes 54:1798–1807

Ravier MA, Nenquin M, Miki T, Seino S, Henquin JC (2009) Glucose controls cytosolic Ca^{2+} and insulin secretion in mouse islets lacking adenosine triphosphate-sensitive K^+ channels owing to a knockout of the pore-forming subunit Kir6.2. Endocrinology 150:33–45

Reinbothe TM, Alkayyali S, Ahlqvist E, Tuomi T, Isomaa B, Lyssenko V, Renstrom E (2013) The human L-type calcium channel $Ca_v1.3$ regulates insulin release and polymorphisms in CACNA1D associate with type 2 diabetes. Diabetologia 56:340–349

Roe MW, Worley JF III, Qian F, Tamarina N, Mittal AA, Dralyuk F, Blair NT, Mertz RJ, Philipson LH, Dukes ID (1998) Characterization of a Ca^{2+} release-activated nonselective cation current regulating membrane potential and $[Ca^{2+}]_i$ oscillations in transgenically derived β-cells. J Biol Chem 273:10402–10410

Rorsman P, Eliasson L, Renstrom E, Gromada J, Barg S, Gopel S (2000) The cell physiology of biphasic insulin secretion. News Physiol Sci 15:72–77

Rosker C, Meur G, Taylor EJ, Taylor CW (2009) Functional ryanodine receptors in the plasma membrane of RINm5F pancreatic β-cells. J Biol Chem 284:5186–5194

Sakura H, Ashcroft FM (1997) Identification of four trp1 gene variants murine pancreatic β-cells. Diabetologia 40:528–532

Schulla V, Renstrom E, Feil R, Feil S, Franklin I, Gjinovci A, Jing XJ, Laux D, Lundquist I, Magnuson MA, Obermuller S, Olofsson CS, Salehi A, Wendt A, Klugbauer N, Wollheim CB, Rorsman P, Hofmann F (2003) Impaired insulin secretion and glucose tolerance in β cell-selective $Ca_v1.2$ Ca^{2+} channel null mice. EMBO J 22:3844–3854

Schwaller B (2012) The regulation of a cell's Ca^{2+} signaling toolkit: the Ca^{2+} homeostasome. Adv Exp Med Biol 740:1–25

Serre-Beinier V, Bosco D, Zulianello L, Charollais A, Caille D, Charpantier E, Gauthier BR, Diaferia GR, Giepmans BN, Lupi R, Marchetti P, Deng S, Buhler L, Berney T, Cirulli V, Meda P (2009) Cx36 makes channels coupling human pancreatic β-cells, and correlates with insulin expression. Hum Mol Genet 18:428–439

Shigeto M, Katsura M, Matsuda M, Ohkuma S, Kaku K (2007) Nateglinide and mitiglinide, but not sulfonylureas, induce insulin secretion through a mechanism mediated by calcium release from endoplasmic reticulum. J Pharmacol Exp Ther 322:1–7

Song SH, McIntyre SS, Shah H, Veldhuis JD, Hayes PC, Butler PC (2000) Direct measurement of pulsatile insulin secretion from the portal vein in human subjects. J Clin Endocrinol Metab 85:4491–4499

Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R, Napolitano C, Schwartz PJ, Joseph RM, Condouris K, Tager-Flusberg H, Priori SG, Sanguinetti MC, Keating MT (2004) $Ca_v1.2$ calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. Cell 119:19–31

Stozer A, Dolensek J, Rupnik MS (2013) Glucose-stimulated calcium dynamics in islets of Langerhans in acute mouse pancreas tissue slices. PLoS One 8:e54638

Swatton JE, Morris SA, Cardy TJ, Taylor CW (1999) Type 3 inositol trisphosphate receptors in RINm5F cells are biphasically regulated by cytosolic Ca^{2+} and mediate quantal Ca^{2+} mobilization. Biochem J 344(Pt 1):55–60

Szollosi A, Nenquin M, Aguilar-Bryan L, Bryan J, Henquin JC (2007) Glucose stimulates Ca^{2+} influx and insulin secretion in 2-week-old β-cells lacking ATP-sensitive K^+ channels. J Biol Chem 282:1747–1756

Takahashi N, Mizuno Y, Kozai D, Yamamoto S, Kiyonaka S, Shibata T, Uchida K, Mori Y (2008) Molecular characterization of TRPA1 channel activation by cysteine-reactive inflammatory mediators. Channels (Austin) 2:287–298

Takasawa S, Kuroki M, Nata K, Noguchi N, Ikeda T, Yamauchi A, Ota H, Itaya-Hironaka A, Sakuramoto-Tsuchida S, Takahashi I, Yoshikawa T, Shimosegawa T, Okamoto H (2010) A novel ryanodine receptor expressed in pancreatic islets by alternative splicing from type 2 ryanodine receptor gene. Biochem Biophys Res Commun 397:140–145

Tarasov AI, Girard CA, Ashcroft FM (2006) ATP sensitivity of the ATP-sensitive K^+ channel in intact and permeabilized pancreatic β-cells. Diabetes 55:2446–2454

Thore S, Wuttke A, Tengholm A (2007) Rapid turnover of phosphatidylinositol-4,5-bisphosphate in insulin-secreting cells mediated by Ca^{2+} and the ATP-to-ADP ratio. Diabetes 56:818–826

Tian G, Tepikin AV, Tengholm A, Gylfe E (2012) cAMP induces stromal interaction molecule 1 (STIM1) puncta but neither Orai1 protein clustering nor store-operated Ca^{2+} entry (SOCE) in islet cells. J Biol Chem 287:9862–9872

Togashi K, Hara Y, Tominaga T, Higashi T, Konishi Y, Mori Y, Tominaga M (2006) TRPM2 activation by cyclic ADP-ribose at body temperature is involved in insulin secretion. EMBO J 25:1804–1815

Trombetta M, Bonetti S, Boselli M, Turrini F, Malerba G, Trabetti E, Pignatti P, Bonora E, Bonadonna RC (2012) CACNA1E variants affect β cell function in patients with newly diagnosed type 2 diabetes. The Verona newly diagnosed type 2 diabetes study (VNDS) 3. PLoS One 7:e32755

Uchida K, Dezaki K, Damdindorj B, Inada H, Shiuchi T, Mori Y, Yada T, Minokoshi Y, Tominaga M (2011) Lack of TRPM2 impaired insulin secretion and glucose metabolisms in mice. Diabetes 60:119–126

Ullrich ND, Voets T, Prenen J, Vennekens R, Talavera K, Droogmans G, Nilius B (2005) Comparison of functional properties of the Ca^{2+}-activated cation channels TRPM4 and TRPM5 from mice. Cell Calcium 37:267–278

Wagner TF, Loch S, Lambert S, Straub I, Mannebach S, Mathar I, Dufer M, Lis A, Flockerzi V, Philipp SE, Oberwinkler J (2008) Transient receptor potential M3 channels are ionotropic steroid receptors in pancreatic β cells. Nat Cell Biol 10:1421–1430

Wang X, Zhang X, Dong XP, Samie M, Li X, Cheng X, Goschka A, Shen D, Zhou Y, Harlow J, Zhu MX, Clapham DE, Ren D, Xu H (2012) TPC proteins are phosphoinositide- activated sodium-selective ion channels in endosomes and lysosomes. Cell 151:372–383

Woolcott OO, Gustafsson AJ, Dzabic M, Pierro C, Tedeschi P, Sandgren J, Bari MR, Hoa NK, Bianchi M, Rakonjac M, Radmark O, Ostenson CG, Islam MS (2006) Arachidonic acid is a physiological activator of the ryanodine receptor in pancreatic β-cells. Cell Calcium 39:529–537

Worley JF III, McIntyre MS, Spencer B, Mertz RJ, Roe MW, Dukes ID (1994) Endoplasmic reticulum calcium store regulates membrane potential in mouse islet β-cells. J Biol Chem 269:14359–14362

Xu W, Lipscombe D (2001) Neuronal $Ca_V.3\alpha_1$ L-type channels activate at relatively hyperpolarized membrane potentials and are incompletely inhibited by dihydropyridines. J Neurosci 21:5944–5951

Yue DT, Wier WG (1985) Estimation of intracellular [Ca^{2+}] by nonlinear indicators. A quantitative analysis. Biophys J 48:533–537

Zhan X, Yang L, Yi M, Jia Y (2008) RyR channels and glucose-regulated pancreatic β-cells. Eur Biophys J 37:773–782

Zhang W, Chu X, Tong Q, Cheung JY, Conrad K, Masker K, Miller BA (2003) A novel TRPM2 isoform inhibits calcium influx and susceptibility to cell death. J Biol Chem 278:16222–16229

Zhang Z, Zhang W, Jung DY, Ko HJ, Lee Y, Friedline RH, Lee E, Jun J, Ma Z, Kim F, Tsitsilianos N, Chapman K, Morrison A, Cooper MP, Miller BA, Kim JK (2012) TRPM2 Ca^{2+} channel regulates energy balance and glucose metabolism. Am J Physiol Endocrinol Metab 302:E807–E816

Role of Mitochondria in β-Cell Function and Dysfunction

22

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Contents

Abstract

Pancreatic β-cells are poised to sense glucose and other nutrient secretagogues to regulate insulin exocytosis, thereby maintaining glucose homeostasis. This process requires translation of metabolic substrates into intracellular messengers recognized by the exocytotic machinery. Central to this metabolism-secretion coupling, mitochondria integrate and generate metabolic signals, thereby connecting glucose

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_7,

recognition to insulin exocytosis. In response to a glucose rise, nucleotides and metabolites are generated by mitochondria and participate, together with cytosolic calcium, to the stimulation of insulin release. This review describes the mitochondrion-dependent pathways of regulated insulin secretion. Mitochondrial defects, such as mutations and reactive oxygen species production, are discussed in the context of β-cell failure that may participate to the etiology of diabetes.

Keywords
Pancreatic β-cell • Insulin secretion • Diabetes • Mitochondria • Amplifying pathway • Glutamate • Reactive oxygen species

Introduction

The primary stimulus for pancreatic β-cells is in fact the most common nutrient for all cell types, i.e., glucose. Tight coupling between glucose metabolism and insulin exocytosis is required to physiologically modulate the secretory response. Accordingly, pancreatic β-cells function as glucose sensors with the crucial task of perfectly adjusting insulin release to blood glucose levels. Homeostasis depends on the normal regulation of insulin secretion from the β-cells and the action of insulin on its target tissues. The initial stages of type 1 diabetes, before β-cell destruction, are characterized by impaired glucose-stimulated insulin secretion. The large majority of diabetic patients are classified as type 2 diabetes or non-insulin-dependent diabetes mellitus. The patients display dysregulation of insulin secretion that may be associated with insulin resistance of the liver, muscle, and fat.

The exocytotic process is tightly controlled by signals generated by nutrient metabolism, as well as by neurotransmitters and circulating hormones. Through its particular gene expression profile, the β-cell is poised to rapidly adapt the rate of insulin secretion to fluctuation in the blood glucose concentration. This chapter describes the molecular basis of metabolism-secretion coupling in general and in particular how mitochondria function both as sensors and as generators of metabolic signals. Finally, we will describe mitochondrial damages associated with β-cell dysfunction.

Overview of Metabolism-Secretion Coupling

Glucose entry within the β-cell initiates the cascade of metabolism-secretion coupling (Fig. 1). Glucose follows its concentration gradient by facilitative diffusion through specific transporters. Then, glucose is phosphorylated by glucokinase, thereby initiating glycolysis (Iynedjian 2009). Subsequently, mitochondrial metabolism generates ATP, which promotes the closure of ATP-sensitive K^+ channels (K_{ATP} channel) and, as a consequence, depolarization of the plasma membrane (Ashcroft 2006). This leads to Ca^{2+} influx through voltage-gated Ca^{2+} channels and a rise in cytosolic Ca^{2+} concentrations triggering insulin exocytosis (Eliasson et al. 2008).

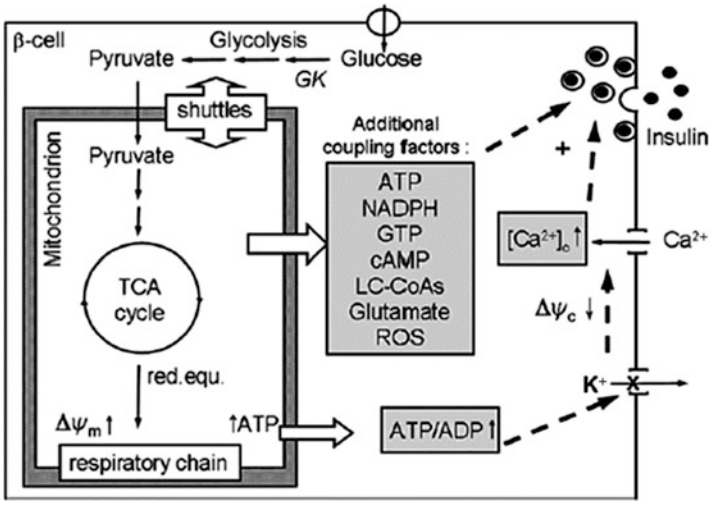

Fig. 1 Model for coupling of glucose metabolism to insulin secretion in the β-cell. Glucose equilibrates across the plasma membrane and is phosphorylated by glucokinase (*GK*). Further, glycolysis produces pyruvate, which preferentially enters the mitochondria and is metabolized by the TCA cycle. The TCA cycle generates reducing equivalents (*red. equ.*), which are transferred to the electron transport chain, leading to hyperpolarization of the mitochondrial membrane ($\Delta\Psi_m$) and generation of ATP. ATP is then transferred to the cytosol, raising the ATP/ADP ratio. Subsequently, closure of K_{ATP} channels depolarizes the cell membrane ($\Delta\Psi_c$). This opens voltage-dependent Ca^{2+} channels, increasing cytosolic Ca^{2+} concentration ($[Ca^{2+}]c$), which triggers insulin exocytosis. Additive signals participate to the amplifying pathway of metabolism-secretion coupling

Additional signals are necessary to reproduce the sustained secretion elicited by glucose. They participate in the amplifying pathway (Henquin 2000) formerly referred to as the K_{ATP}-channel-independent stimulation of insulin secretion. Efficient coupling of glucose recognition to insulin secretion is ensured by the mitochondrion, an organelle that integrates and generates metabolic signals. This crucial role goes far beyond the sole generation of ATP necessary for the elevation of cytosolic Ca^{2+} (Maechler et al. 1997). The additional coupling factors amplifying the action of Ca^{2+} (Fig. 1) will be discussed in this chapter.

Mitochondrial NADH Shuttles

In the course of glycolysis, i.e., upstream of pyruvate production, mitochondria are already implicated in the necessary reoxidation of NADH to NAD^+, thereby enabling maintenance of glycolytic flux. In most tissues, lactate dehydrogenase ensures NADH oxidation to avoid inhibition of glycolysis secondary to the lack of NAD^+ (Fig. 2). In β-cells, according to low lactate dehydrogenase activity (Sekine et al. 1994), high rates of glycolysis are maintained through the activity of mitochondrial NADH shuttles, thereby transferring glycolysis-derived electrons to

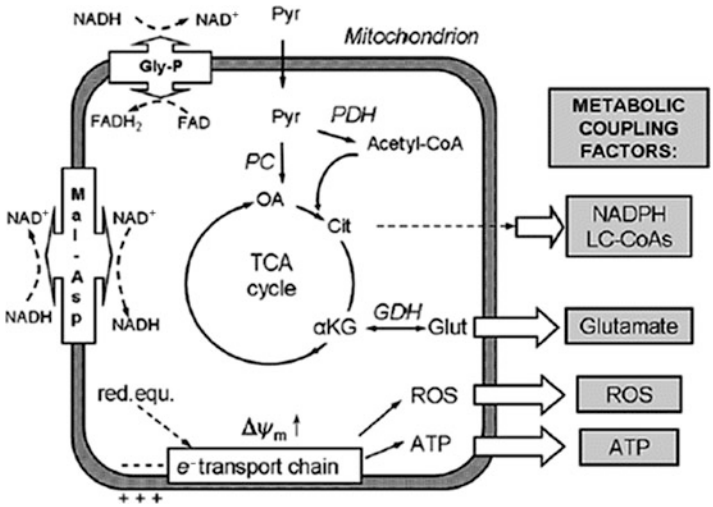

Fig. 2 In the mitochondria, pyruvate (*Pyr*) is a substrate for both pyruvate dehydrogenase (*PDH*) and pyruvate carboxylase (*PC*), forming, respectively, acetyl-CoA and oxaloacetate (*OA*). Condensation of acetyl-CoA with OA generates citrate (Cit) that is either processed by the TCA cycle or exported out of the mitochondrion as a precursor for long-chain acyl-CoA (*LC-CoA*) synthesis. Glycerophosphate (*Gly-P*) and malate/aspartate (*Mal-Asp*) shuttles as well as the TCA cycle generate reducing equivalents (*red. equ.*) in the form of NADH and $FADH_2$, which are transferred to the electron transport chain resulting in hyperpolarization of the mitochondrial membrane ($\Delta\Psi_m$) and ATP synthesis. As a by-product of electron transport chain activity, reactive oxygen species (*ROS*) are generated. Upon glucose stimulation, glutamate (Glu) can be produced from α-ketoglutarate (α-KG) by glutamate dehydrogenase (*GDH*)

mitochondria (Bender et al. 2006). Early evidence for tight coupling between glycolysis and mitochondrial activation came from studies showing that anoxia inhibits glycolytic flux in pancreatic islets (Hellman et al. 1975). Therefore, NADH shuttle systems are necessary to couple glycolysis to the activation of mitochondrial energy metabolism, leading to insulin secretion.

The NADH shuttle system is composed essentially of the glycerophosphate and the malate/aspartate shuttles (MacDonald 1982), with its respective key members mitochondrial glycerol phosphate dehydrogenase and aspartate-glutamate carrier (AGC). Mice lacking mitochondrial glycerol phosphate dehydrogenase exhibit a normal phenotype (Eto et al. 1999), whereas general abrogation of AGC results in severe growth retardation, attributed to the observed impaired central nervous system function (Jalil et al. 2005). Islets isolated from mitochondrial glycerol phosphate dehydrogenase knockout mice respond normally to glucose regarding metabolic parameters and insulin secretion (Eto et al. 1999). Additional inhibition of transaminases with aminooxyacetate, to nonspecifically inhibit the malate/aspartate shuttle in these islets, strongly impairs the secretory response to glucose (Eto et al. 1999). The respective importance of these shuttles is indicated in islets of mice with abrogation of NADH shuttle activities, pointing to the malate/aspartate shuttle as essential for both mitochondrial metabolism and cytosolic redox state.

Aralar1 (or aspartate-glutamate carrier 1, AGC1) is a Ca^{2+}-sensitive member of the malate/aspartate shuttle (del Arco and Satrustegui 1998). Aralar1/AGC1 and citrin/AGC2 are members of the subfamily of Ca^{2+}-binding mitochondrial carriers and correspond to two isoforms of the mitochondrial aspartate-glutamate carrier. These proteins are activated by Ca^{2+} acting on the external side of the inner mitochondrial membrane (del Arco and Satrustegui 1998; Palmieri et al. 2001). We showed that adenoviral-mediated overexpression of Aralar1/AGC1 in insulin-secreting cells increases glucose-induced mitochondrial activation and secretory response (Rubi et al. 2004). This is accompanied by enhanced glucose oxidation and reduced lactate production. Conversely, silencing AGC1 in INS-1E β-cells reduces glucose oxidation and the secretory response, while primary rat β-cells are not sensitive to such a maneuver (Casimir et al. 2009b). Therefore, aspartate-glutamate carrier capacity appears to set a limit for NADH shuttle function and mitochondrial metabolism. The importance of the NADH shuttle system also illustrates the tight coupling between glucose metabolism and the control of insulin secretion.

Mitochondria as Metabolic Sensors

Downstream of the NADH shuttles, pyruvate produced by glycolysis is preferentially transferred to mitochondria. Import of pyruvate into the mitochondrial matrix through the recently identified pyruvate carrier (Herzig et al. 2012) is associated with a futile cycle that transiently depolarizes the mitochondrial membrane (de Andrade et al. 2004). After its entry into the mitochondria, the pyruvate is converted to acetyl-CoA by pyruvate dehydrogenase or to oxaloacetate by pyruvate carboxylase (Fig. 2). The pyruvate carboxylase pathway ensures the provision of carbon skeleton (i.e., anaplerosis) to the tricarboxylic acid (TCA) cycle, a key pathway in β-cells (Brun et al. 1996; Schuit et al. 1997; Brennan et al. 2002; Lu et al. 2002). The importance of this pathway is highlighted in a study showing that inhibition of the pyruvate carboxylase reduces glucose-stimulated insulin secretion in rat islets (Fransson et al. 2006). The high anaplerotic activity suggests the loss of TCA cycle intermediates (i.e., cataplerosis), compensated for by oxaloacetate. In the control of glucose-stimulated insulin secretion, such TCA cycle derivates might potentially operate as mitochondrion-derived coupling factors (Maechler et al. 1997).

Importance of mitochondrial metabolism for β-cell function is illustrated by stimulation with substrates bypassing glycolysis. This is the case for the TCA cycle intermediates succinate or cell permeant methyl derivatives that have been shown to efficiently promote insulin secretion in pancreatic islets (Maechler et al. 1998; Zawalich et al. 1993; Mukala-Nsengu et al. 2004). Succinate induces hyperpolarization of the mitochondrial membrane, resulting in elevation of mitochondrial Ca^{2+} and ATP generation, while its catabolism is Ca^{2+} dependent (Maechler et al. 1998).

Besides its importance for ATP generation, the mitochondrion in general, and the TCA cycle in particular, is the key metabolic crossroad enabling fuel oxidation as well as provision of building blocks, or cataplerosis, for lipids and proteins

(Owen et al. 2002). In β-cells, approximately 50 % of pyruvate is oxidized to acetyl-CoA by pyruvate dehydrogenase (Schuit et al. 1997). Pyruvate dehydrogenase is an important site of regulation as, among other effectors, the enzyme is activated by elevation of mitochondrial Ca^{2+} (McCormack et al. 1990; Rutter et al. 1996) and, conversely, its activity is reduced upon exposures to either excess fatty acids (Randle et al. 1994) or chronic high glucose (Liu et al. 2004). Pyruvate dehydrogenase is also regulated by reversible phosphorylation, activity of the PDH kinases inhibiting the enzyme. Silencing of PDH kinase 1 in INS-1 832/13 cells increases the secretory response to glucose (Krus et al. 2010), whereas downregulation of PDH kinases 1 and 3 in INS-1E cells does not change metabolism-secretion coupling (Akhmedov et al. 2012). Therefore, the importance of the phosphorylation state of pyruvate dehydrogenase for the regulation of insulin secretion remains unclear.

Oxaloacetate, produced by the anaplerotic enzyme pyruvate carboxylase, condenses with acetyl-CoA forming citrate, which undergoes stepwise oxidation and decarboxylation yielding α-ketoglutarate. The TCA cycle is completed via succinate, fumarate, and malate, in turn producing oxaloacetate (Fig. 2). The fate of α-ketoglutarate is influenced by the redox state of mitochondria. Low NADH-to-NAD^+ ratio would favor further oxidative decarboxylation to succinyl-CoA as NAD^+ is required as cofactor for this pathway. Conversely, high NADH-to-NAD^+ ratio would promote NADH-dependent reductive transamination forming glutamate, a spin-off product of the TCA cycle (Owen et al. 2002). The latter situation, i.e., high NADH-to-NAD^+ ratio, is observed following glucose stimulation.

Although the TCA cycle oxidizes also fatty acids and amino acids, carbohydrates are the most important fuel under physiological conditions for the β-cell. Upon glucose exposure, mitochondrial NADH elevations reach a plateau after approximately 2 min (Rocheleau et al. 2004). In order to maintain pyruvate input into the TCA cycle, this new redox steady state requires continuous reoxidation of mitochondrial NADH to NAD^+ primarily by complex I on the electron transport chain. However, as complex I activity is limited by the inherent thermodynamic constraints of proton gradient formation (Antinozzi et al. 2002), additional NADH contributed by this high TCA cycle activity must be reoxidized by other dehydrogenases, i.e., through cataplerotic functions. Significant cataplerotic function in β-cells was suggested by the quantitative importance of anaplerotic pathway through pyruvate carboxylase (Brun et al. 1996; Schuit et al. 1997), as confirmed by the use of NMR spectroscopy (Brennan et al. 2002; Lu et al. 2002; Cline et al. 2004).

A Focus on Glutamate Dehydrogenase

The enzyme glutamate dehydrogenase (GDH) has been proposed to participate in the development of the secretory response (Fig. 2). GDH is a homohexamer located in the mitochondrial matrix and catalyzes the reversible reaction α-ketoglutarate + NH_3 + NADH ↔ glutamate + NAD^+, inhibited by GTP and activated by ADP (Hudson and Daniel 1993; Frigerio et al. 2008). Regarding β-cell, allosteric activation of GDH has

triggered most of the attention over the last three decades (Sener and Malaisse 1980). Numerous studies have used the GDH allosteric activator L-leucine or its nonmetabolized analog β-2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH) to question the role of GDH in the control of insulin secretion (Sener and Malaisse 1980; Sener et al. 1981; Panten et al. 1984; Fahien et al. 1988). Alternatively, one can increase GDH activity by means of overexpression, an approach that we combined with allosteric activation of the enzyme (Carobbio et al. 2004). To date, the role of GDH in β-cell function remains unclear and debated. Specifically, GDH might play a role in glucose-induced amplifying pathway through generation of glutamate (Maechler and Wollheim 1999; Hoy et al. 2002; Broca et al. 2003). GDH is also an amino acid sensor triggering insulin release upon glutamine stimulation in conditions of GDH allosteric activation (Sener et al. 1981; Fahien et al. 1988; Li et al. 2006).

Recently, the importance of GDH has been further highlighted by studies showing that SIRT4, a mitochondrial ADP-ribosyltransferase, downregulates GDH activity and thereby modulates insulin secretion (Haigis et al. 2006; Ahuja et al. 2007). Clinical data and associated genetic studies also revealed GDH as a key enzyme for the control of insulin secretion. Indeed, mutations rendering GDH more active are responsible for a hyperinsulinism syndrome (Stanley et al. 1998). Mutations producing a less-active, or even nonactive, GDH enzyme have not been reported, leaving open the question if such mutations would be either lethal or asymptomatic. We recently generated and characterized transgenic mice (named $\beta Glud1^{-/-}$) with conditional β-cell-specific deletion of GDH (Carobbio et al. 2009). Data show that GDH accounts for about 40 % of glucose-stimulated insulin secretion and that GDH pathway lacks redundant mechanisms. In $\beta Glud1^{-/-}$ mice, the reduced secretory capacity resulted in lower plasma insulin levels in response to both feeding and glucose load, while body weight gain and glucose homeostasis were preserved (Carobbio et al. 2009). This demonstrates that GDH is essential for the full development of the secretory response in β-cells, being sensitive in the upper range of physiological glucose concentrations. In particular, the amplifying pathway of the glucose response fails to develop in the absence of GDH, as demonstrated in $\beta Glud1^{-/-}$ islets (Vetterli et al. 2012).

Mitochondrial Activation Results in ATP Generation

TCA cycle activation induces transfer of electrons to the respiratory chain resulting in hyperpolarization of the mitochondrial membrane and generation of ATP (Fig. 2). The electrons are transferred by the pyridine nucleotide NADH and the flavin adenine nucleotide FADH2. In the mitochondrial matrix, NADH is formed by several dehydrogenases, some of which being activated by Ca^{2+} (McCormack et al. 1990), and FADH2 is generated in the succinate dehydrogenase reaction.

Electron transport chain activity promotes proton export from the mitochondrial matrix across the inner membrane, establishing a strong mitochondrial membrane potential, negative inside. The respiratory chain comprises five complexes, the subunits of which are encoded by both the nuclear and the mitochondrial genomes (Wallace 1999). Complex I is the only acceptor of electrons from NADH in the inner mitochondrial membrane, and its blockade abolishes glucose-induced insulin secretion (Antinozzi et al. 2002). Complex II (succinate dehydrogenase) transfers electrons to coenzyme-Q from $FADH_2$, the latter being generated by both the oxidative activity of the TCA cycle and the glycerophosphate shuttle. Complex V (ATP synthase) promotes ATP formation from ADP and inorganic phosphate. The synthesized ATP is translocated to the cytosol in exchange for ADP by the adenine nucleotide translocator (ANT). Thus, the work of the separate complexes of the electron transport chain and the adenine nucleotide translocator couples respiration to ATP supply.

NADH electrons are transferred to the electron transport chain, which in turn supplies the energy necessary to create a proton electrochemical gradient that drives ATP synthesis. In addition to ATP generation, mitochondrial membrane potential drives the transport of metabolites between mitochondrial and cytosolic compartments, including the transfer of mitochondrial factors participating in insulin secretion. Hyperpolarization of the mitochondrial membrane relates to the proton export from the mitochondrial matrix and directly correlates with insulin secretion stimulated by different secretagogues (Antinozzi et al. 2002).

Accordingly, potentiation of glucose-stimulated insulin secretion by enhanced mitochondrial NADH generation is accompanied by increased glucose metabolism and mitochondrial hyperpolarization (Rubi et al. 2004).

Mitochondrial activity can be modulated according to nutrient nature, although glucose is the chief secretagogue as compared to amino acid catabolism (Newsholme et al. 2005) and fatty acid β-oxidation (Rubi et al. 2002). Additional factors regulating ATP generation include mitochondrial Ca^{2+} levels (McCormack et al. 1990; Duchen 1999), mitochondrial protein tyrosine phosphatase (Pagliarini et al. 2005), mitochondrial GTP (Kibbey et al. 2007), and matrix alkalinization (Wiederkehr et al. 2009).

Mitochondrial function is also modulated by their morphology and contacts. Mitochondria form dynamic networks, continuously modified by fission and fusion events under the control of specific mitochondrial membrane anchor proteins (Westermann 2008). Mitochondrial fission/fusion state was recently investigated in insulin-secreting cells. Altering fission by downregulation of fission-promoting Fis1 protein impairs respiratory function and glucose-stimulated insulin secretion (Twig et al. 2008). The reverse experiment, consisting in overexpression of Fis1 causing mitochondrial fragmentation, results in a similar phenotype, i.e., reduced energy metabolism and secretory defects (Park et al. 2008). Fragmented pattern obtained by dominant-negative expression of fusion-promoting Mfn1 protein does not affect metabolism-secretion coupling (Park et al. 2008). Therefore, mitochondrial fragmentation per se seems not to alter insulin-secreting cells at least in vitro.

The Amplifying Pathway of Insulin Secretion

The Ca^{2+} signal in the cytosol is necessary but not sufficient for the full development of sustained insulin secretion. Nutrient secretagogues, in particular glucose, evoke a long-lasting second phase of insulin secretion. In contrast to the transient secretion induced by Ca^{2+}-raising agents, the sustained insulin release depends on the generation of metabolic factors (Fig. 1). The elevation of cytosolic Ca^{2+} is a prerequisite also for this phase of secretion, as evidenced among others by the inhibitory action of voltage-sensitive Ca^{2+} channel blockers. Glucose evokes K_{ATP}-channel-independent stimulation of insulin secretion or amplifying pathway (Henquin 2000), which is unmasked by glucose stimulation when cytosolic Ca^{2+} is clamped at permissive levels (Panten et al. 1988; Gembal et al. 1992; Sato et al. 1992). This suggests the existence of metabolic coupling factors generated by glucose.

Mitochondria Promote the Generation of Nucleotides Acting as Metabolic Coupling Factors

ATP is the primary metabolic factor implicated in K_{ATP}-channel regulation (Miki et al. 1999), secretory granule movement (Yu et al. 2000; Varadi et al. 2002), and the process of insulin exocytosis (Vallar et al. 1987; Rorsman et al. 2000).

Among other putative nucleotide messengers, NADH and NADPH are generated by glucose metabolism (Prentki 1996). Single β-cell measurements of NAD(P)H fluorescence have demonstrated that the rise in pyridine nucleotides precedes the rise in cytosolic Ca^{2+} concentrations (Pralong et al. 1990; Gilon and Henquin 1992) and that the elevation in the cytosol is reached more rapidly than in the mitochondria (Patterson et al. 2000). Cytosolic NADPH is generated by glucose metabolism via the pentose phosphate shunt (Verspohl et al. 1979), although mitochondrial shuttles being the main contributors in β-cells (Farfari et al. 2000). The pyruvate/citrate shuttle has triggered attention over the last years and has been postulated as the key cycle responsible for the elevation of cytosolic NADPH (Farfari et al. 2000). As a consequence of mitochondrial activation, cytosolic NADPH is generated by NADP-dependent malic enzyme, and suppression of its activity was shown to inhibit glucose-stimulated insulin secretion in insulinoma cells (Guay et al. 2007; Joseph et al. 2007). However, such effects have not been reproduced in primary cells in the form of rodent islets (Ronnebaum et al. 2008), leaving the question open.

Regarding the action of NADPH, it was proposed as a coupling factor in glucose-stimulated insulin secretion based on experiments using toadfish islets (Watkins et al. 1968). A direct effect of NADPH was reported on the release of insulin from isolated secretory granules (Watkins 1972), NADPH being possibly bound or taken up by granules (Watkins and Moore 1977). More recently, the putative role of NADPH, as a signaling molecule in β-cells, has been substantiated by experiments showing direct stimulation of insulin exocytosis upon intracellular addition of NADPH (Ivarsson et al. 2005).

Glucose also promotes the elevation of GTP (Detimary et al. 1996), which could trigger insulin exocytosis via GTPases (Vallar et al. 1987; Lang 1999). In the cytosol, GTP is mainly formed through the action of nucleoside diphosphate kinase from GDP and ATP. In contrast to ATP, GTP is capable of inducing insulin exocytosis in a Ca^{2+}-independent manner (Vallar et al. 1987). An action of mitochondrial GTP as positive regulator of the TCA cycle has been mentioned above (Kibbey et al. 2007).

The universal second messenger cAMP, generated at the plasma membrane from ATP, potentiates glucose-stimulated insulin secretion (Ahren 2000). Many neurotransmitters and hormones, including glucagon as well as the intestinal hormones glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide, increase cAMP levels in the β-cell by activating adenyl cyclase (Schuit et al. 2001). In human β-cells, activation of glucagon receptors synergistically amplifies the secretory response to glucose (Huypens et al. 2000). Glucose itself promotes cAMP elevation (Charles et al. 1975), and oscillations in cellular cAMP concentrations are related to the magnitude of pulsatile insulin secretion (Dyachok et al. 2008). Moreover, GLP-1 might preserve β-cell mass, by both induction of cell proliferation and inhibition of apoptosis (Drucker 2003). According to all these actions, GLP-1 and biologically active-related molecules are of interest for the treatment of diabetes (Drucker and Nauck 2006).

Fatty Acid Pathways and the Metabolic Coupling Factors

Metabolic profiling of mitochondria is modulated by the relative contribution of glucose and lipid products for oxidative catabolism. Carnitine palmitoyltransferase I, which is expressed in the pancreas as the liver isoform (LCPTI), catalyzes the rate-limiting step in the transport of fatty acids into the mitochondria for their oxidation. In glucose-stimulated β-cells, citrate exported from the mitochondria (Fig. 2) to the cytosol reacts with coenzyme-A (CoA) to form cytosolic acetyl-CoA that is necessary for malonyl-CoA synthesis. Then, malonyl-CoA derived from glucose metabolism regulates fatty acid oxidation by inhibiting LCPTI. The malonyl-CoA/long-chain acyl-CoA hypothesis of glucose-stimulated insulin release postulates that malonyl-CoA derived from glucose metabolism inhibits fatty acid oxidation, thereby increasing the availability of long-chain acyl-CoA for lipid signals implicated in exocytosis (Brun et al. 1996). In the cytosol, this process promotes the accumulation of long-chain acyl-CoAs such as palmitoyl-CoA (Liang and Matschinsky 1991; Prentki et al. 1992), which enhances Ca^{2+}-evoked insulin exocytosis (Deeney et al. 2000).

In agreement with the malonyl-CoA/long-chain acyl-CoA model, overexpression of native LCPTI in clonal INS-1E β-cells was shown to increase β-oxidation of fatty acids and to decrease insulin secretion at high glucose (Rubi et al. 2002), although glucose-derived malonyl-CoA was still able to inhibit LCPTI in these conditions. When the malonyl-CoA/CPTI interaction is altered in cells expressing a malonyl-CoA-insensitive CPTI, glucose-induced insulin release is impaired (Herrero et al. 2005).

Over the last years, the malonyl-CoA/long-chain acyl-CoA model has been challenged, essentially by modulating cellular levels of malonyl-CoA, either up or down. Each way resulted in contradictory conclusions, according to the respective laboratories performing such experiments. First, malonyl-CoA decarboxylase was overexpressed to reduce malonyl-CoA levels in the cytosol. In disagreement with the malonyl-CoA/long-chain acyl-CoA model, abrogation of malonyl-CoA accumulation during glucose stimulation does not attenuate the secretory response (Antinozzi et al. 1998). However, overexpression of malonyl-CoA decarboxylase in the cytosol in the presence of exogenous free fatty acids, but not in their absence, reduces glucose-stimulated insulin release (Roduit et al. 2004). The second approach was to silence ATP-citrate lyase, the enzyme that forms cytosolic acetyl-CoA leading to malonyl-CoA synthesis. Again, one study observed that such maneuver reduces glucose-stimulated insulin secretion (Guay et al. 2007), whereas another group concluded that metabolic flux through malonyl-CoA is not required for the secretory response to glucose (Joseph et al. 2007).

The role of long-chain acyl-CoA derivatives remains a matter of debate, although several studies indicate that malonyl-CoA could act as a coupling factor regulating the partitioning of fatty acids into effector molecules in the insulin secretory pathway (Prentki et al. 2002). Moreover, fatty acids stimulate the G-protein-coupled receptor GPR40/FFAR1 that is highly expressed in β-cells (Itoh et al. 2003). Activation of GPR40 receptor results in enhancement of glucose-induced elevation of cytosolic Ca^{2+} and consequently insulin secretion (Nolan et al. 2006).

Mitochondrial Metabolites as Coupling Factors

Acetyl-CoA carboxylase catalyzes the formation of malonyl-CoA, a precursor in the biosynthesis of long-chain fatty acids. Interestingly, glutamate-sensitive protein phosphatase 2A-like protein activates acetyl-CoA carboxylase in β-cells (Kowluru et al. 2001). This observation might link two metabolites proposed to participate in the control of insulin secretion. Indeed, the amino acid glutamate is another discussed metabolic factor proposed to participate in the amplifying pathway (Maechler and Wollheim 1999, 2000; Hoy et al. 2002). Glutamate can be produced from the TCA cycle intermediate α-ketoglutarate or by transamination reactions (Frigerio et al. 2008; Newsholme et al. 2005; Maechler et al. 2000). During glucose stimulation, total cellular glutamate levels have been shown to increase in human, mouse, and rat islets as well as in clonal β-cells (Brennan et al. 2002; Carobbio et al. 2004; Maechler and Wollheim 1999; Broca et al. 2003; Rubi et al. 2001; Bertrand et al. 2002; Lehtihet et al. 2005), whereas one study reported no change (MacDonald and Fahien 2000).

The finding that mitochondrial activation in permeabilized β-cells directly stimulates insulin exocytosis (Maechler et al. 1997) initiated investigations that identified glutamate as a putative intracellular messenger (Maechler and Wollheim 1999; Hoy et al. 2002). In the in situ pancreatic perfusion, increased provision of glutamate using a cell permeant precursor results in augmentation of the sustained

phase of insulin release (Maechler et al. 2002). The glutamate hypothesis was challenged by the overexpression of glutamate decarboxylase (GAD) in β-cells to reduce cytosolic glutamate levels (Rubi et al. 2001). In control cells, stimulatory glucose concentrations increased glutamate concentrations, whereas the glutamate response was significantly reduced in GAD overexpressing cells. GAD overexpression also blunted insulin secretion induced by high glucose, showing direct correlation between the glutamate changes and the secretory response (Rubi et al. 2001). In contrast, it was reported by others that the glutamate changes may be dissociated from the amplification of insulin secretion elicited by glucose (Bertrand et al. 2002). Recently, we abrogated GDH, the enzyme responsible for glutamate formation, specifically in the β-cells of transgenic mice. This resulted in a 40 % reduction of glucose-stimulated insulin secretion (Carobbio et al. 2009). Measurements of carbon fluxes in mouse islets revealed that, upon glucose stimulation, GDH contributes to the net synthesis of glutamate from the TCA cycle intermediate α-ketoglutarate (Vetterli et al. 2012). Moreover, silencing of the mitochondrial glutamate carrier GC1 in β-cells inhibits insulin exocytosis evoked by glucose stimulation, an effect rescued by the provision of exogenous glutamate to the cell (Casimir et al. 2009a).

The use of selective inhibitors led to a model where glutamate, downstream of mitochondria, would be taken up by secretory granules, thereby promoting Ca^{2+}-dependent exocytosis (Maechler and Wollheim 1999; Hoy et al. 2002). Such a model was strengthened by the demonstration that clonal β-cells express two vesicular glutamate transporters (VGLUT1 and VGLUT2) and that glutamate transport characteristics are similar to neuronal transporters (Bai et al. 2003). The mechanism of action inside the granule could possibly be explained by glutamate-induced pH changes, as observed in secretory vesicles from pancreatic β-cells (Eto et al. 2003). An alternative mechanism of action at the secretory vesicle level implicates glutamate receptors. Indeed, clonal β-cells have been shown to express the metabotropic glutamate receptor mGlu5 in insulin-containing granules, thereby mediating insulin secretion (Storto et al. 2006). Recent studies have further substantiated the functional link between intracellular glutamate and secretory granules. It has been reported that the flux of glutamate through the secretory granules leads to the acidification of vesicles, thereby favoring insulin release (Gammelsaeter et al. 2011). Collectively, data favor a model for necessary permissive levels of intracellular glutamate rendering insulin granules exocytosis competent.

Another action of glutamate has been proposed. In insulin-secreting cells, rapidly reversible protein phosphorylation/dephosphorylation cycles have been shown to play a role in the rate of insulin exocytosis (Jones and Persaud 1998). It has also been reported that glutamate, generated upon glucose stimulation, might sustain glucose-induced insulin secretion through inhibition of protein phosphatase enzymatic activities (Lehtihet et al. 2005). An alternative or additive mechanism of action would be the activation of acetyl-CoA carboxylase (Kowluru et al. 2001) as mentioned above. Finally, glutamate might serve as a precursor for related pathways, such as GABA (gamma-aminobutyric acid) metabolism that could then contribute to the stimulation of insulin secretion through the so-called GABA shunt (Pizarro-Delgado et al. 2009).

Several mechanisms of action have been proposed for glutamate as a metabolic factor playing a role in the control of insulin secretion. However, we lack a consensus model, and further studies should dissect these complex pathways that might be either additive or cooperative.

Among mitochondrial metabolites, succinate has been proposed to control insulin production. Indeed, it was reported that succinate and/or succinyl-CoA is a metabolic stimulus coupling factor for glucose-induced proinsulin biosynthesis (Alarcon et al. 2002). Later, an alternative mechanism has been postulated regarding succinate stimulation of insulin production. Authors showed that such stimulation was dependent on succinate metabolism via succinate dehydrogenase, rather than being the consequence of a direct effect of succinate itself (Leibowitz et al. 2005).

Citrate export out of the mitochondria has been described as a signal of fuel abundance that contributes to β-cell stimulation in both the mitochondrial and the cytosolic compartments (Farfari et al. 2000). In the cytosol, citrate contributes to the formation of NADPH and malonyl-CoA, both proposed as metabolic coupling factors as discussed in this review.

Reactive Oxygen Species Participate to β-Cell Function

Reactive oxygen species (ROS) include superoxide $O_2^{-}\bullet$ hydroxyl radical (OH•) and hydrogen peroxide (H_2O_2). Superoxide can be converted to less-reactive H_2O_2 by superoxide dismutase (SOD) and then to oxygen and water by catalase (CAT), glutathione peroxidase (GPx), and peroxiredoxin, which constitute antioxidant defenses. Increased oxidative stress and free radical-induced damages have been proposed to be implicated in diabetic state (Yu 1994). However, metabolism of physiological nutrient increases ROS without causing deleterious effects on cell function. Recently, the concept emerged that ROS might participate to cell signaling (Rhee 2006). In insulin-secreting cells, it has been reported that ROS, and probably H_2O_2 in particular, is one of the metabolic coupling factors in glucose-induced insulin secretion (Pi et al. 2007). Therefore, ROS fluctuations may also contribute to physiological control of β-cell functions. However, uncontrolled increase of oxidants, or reduction of their detoxification, may lead to free radical-mediated chain reactions ultimately triggering pathogenic events (Li et al. 2008).

Mitochondria Can Generate ROS

Mitochondrial electron transport chain is the major site of ROS production within the cell. Electrons from sugar, fatty acid, and amino acid catabolism accumulate on the electron carriers NADH and $FADH_2$ and are subsequently transferred through the electron transport chain to oxygen, promoting ATP synthesis. ROS formation is coupled to this electron transportation as a by-product of normal mitochondrial respiration through the one-electron reduction of molecular oxygen (Chance et al. 1979; Raha and Robinson 2000). The main submitochondrial

localization of ROS formation is the inner mitochondrial membrane, i.e., NADH dehydrogenase at complex I and the interface between ubiquinone and complex III (Nishikawa et al. 2000). Increased mitochondrial free radical production has been regarded as a result of diminished electron transport occurring when ATP demand declines or under certain stress conditions impairing specific respiratory chain complexes (Ambrosio et al. 1993; Turrens and Boveris 1980). This is consistent with the observation that inhibition of mitochondrial electron transport chain by mitochondrial complex blockers, antimycin A and rotenone, leads to increased ROS production in INS-1 β-cells (Pi et al. 2007).

Mitochondria Are Sensitive to ROS

Mitochondria not only produce ROS but are also the primary target of ROS attacks. The mitochondrial genome is more vulnerable to oxidative stress, and consecutive damages are more extensive than those in nuclear DNA due to the lack of protective histones and low repair mechanisms (Croteau et al. 1997; Yakes and Van Houten 1997). Being in close proximity to the site of free radical generation, mitochondrial inner membrane components are at a high risk for oxidative injuries, eventually resulting in depolarized mitochondrial membrane and impaired ATP production. Such sensitivity has been shown for mitochondrial membrane proteins such as the adenine nucleotide transporter and ATP synthase (Yan and Sohal 1998; Lippe et al. 1991). In the mitochondrial matrix, aconitase was also reported to be modified in an oxidative environment (Yan et al. 1997).

Furthermore, mitochondrial membrane lipids are highly susceptible to oxidants, in particular the long-chain polyunsaturated fatty acids. ROS may directly lead to lipid peroxidation, and the production of highly reactive aldehyde species exerts further detrimental effects (Chen and Yu 1994). The mitochondrion membrane-specific phospholipid cardiolipin is particularly vulnerable to oxidative damages, altering the activities of adenine nucleotide transporter and cytochrome c oxidase (Hoch 1992).

ROS May Trigger β-Cell Dysfunction

ROS may have different actions according to cellular concentrations being either below or above a specific threshold, i.e., signaling or toxic effects, respectively. Robust oxidative stress caused by either direct exposure to oxidants or secondary to gluco-lipotoxicity has been shown to impair β-cell functions (Maechler et al. 1999; Robertson 2006; Robertson et al. 2004). In type 1 diabetes , ROS participate in β-cell dysfunction initiated by autoimmune reactions and inflammatory cytokines (Rabinovitch 1998). In type 2 diabetes, excessive ROS impair insulin synthesis (Evans et al. 2002, 2003; Robertson et al. 2003) and activate β-cell apoptotic pathways (Evans et al. 2002; Mandrup-Poulsen 2001).

Hyperglycemia induces generation of superoxide at the mitochondrial level in endothelial cells and triggers a vicious cycle of oxidative reactions implicated in the

development of diabetic complications (Nishikawa et al. 2000). In the rat Zucker diabetic fatty model of type 2 diabetes, direct measurements of superoxide in isolated pancreatic islets revealed ROS generation coupled to mitochondrial metabolism and perturbed mitochondrial function (Bindokas et al. 2003).

Short transient exposure to oxidative stress is sufficient to impair glucose-stimulated insulin secretion in pancreatic islets (Maechler et al. 1999). Specifically, ROS attacks in insulin-secreting cells result in mitochondrial inactivation, thereby interrupting transduction of signals normally coupling glucose metabolism to insulin secretion (Maechler et al. 1999). Recently, we observed that one single acute oxidative stress induces β-cell dysfunction lasting over days, explained by persistent damages in mitochondrial components accompanied by subsequent generation of endogenous ROS of mitochondrial origin (Li et al. 2009).

The degree of oxidative damages also depends on protective capability of ROS scavengers. Mitochondria have a large set of defense strategies against oxidative injuries. Superoxide is enzymatically converted to H_2O_2 by the mitochondrion-specific manganese SOD (Fridovich 1995). Other antioxidants like mitochondrial GPx, peroxiredoxin, vitamin E, and coenzymes Q and various repair mechanisms contribute to maintain redox homeostasis in mitochondria (Beckman and Ames 1998; Costa et al. 2003). However, β-cells are characterized by relatively weak expression of free radical-quenching enzymes SOD, CAT, and GPx (Tiedge et al. 1997). Overexpression of such enzymes in insulin-secreting cells inactivates ROS attacks (Lortz and Tiedge 2003). Besides ROS inactivation, the uncoupling protein (UCP) 2 was shown to reduce cytokine-induced ROS production, an effect independent of mitochondrial uncoupling (Produit-Zengaffinen et al. 2007).

Mitochondrial DNA Mutations and β-Cell Dysfunction

Mitochondrial DNA (mtDNA) carries only 37 genes (16,569 bp) encoding 13 polypeptides, 22 tRNAs, and 2 ribosomal RNAs (Wallace 1999). Mitochondrial protein biogenesis is determined by both nuclear and mitochondrial genomes, and the few polypeptides encoded by the mtDNA are all subunits of the electron transport chain (Buchet and Godinot 1998). Transgenic mice lacking expression of the mitochondrial genome specifically in the β-cells are diabetic, and their islets exhibit impaired glucose-stimulated insulin secretion (Silva et al. 2000). Moreover, mtDNA-deficient β-cell lines are glucose unresponsive and carry defective mitochondria, although they still exhibit secretory responses to Ca^{2+}-raising agents (Soejima et al. 1996; Kennedy et al. 1998; Tsuruzoe et al. 1998).

Mitochondrial inherited diabetes and deafness (MIDD) is often associated with mtDNA A3243G point mutation on the tRNA (Leu) gene (Ballinger et al. 1992; van den Ouweland et al. 1992), usually in the heteroplasmic form, i.e., a mixture of wild-type and mutant mtDNA in patient cells. Mitochondrial diabetes usually appears during adulthood with maternal transmission and often in combination with bilateral hearing impairment (Maassen et al. 2005). The etiology of diabetes may not be primarily associated with β-cells, rendering the putative link between

mtDNA mutations and β-cell dysfunction still hypothetical (Lowell and Shulman 2005). Moreover, pancreatic islets of such patients may carry low heteroplasmy percentage of the mutation (Lynn et al. 2003), and, accordingly, the pathogenicity of this mutation is hardly detectable in the endocrine pancreas (Lynn et al. 2003; Maassen et al. 2001).

Some clinical studies strongly suggest a direct link between mtDNA mutations and β-cell dysfunction. Diabetic patients carrying mtDNA mutations exhibit marked reduction in insulin release upon intravenous glucose tolerance tests and hyperglycemic clamps compared to noncarriers (Velho et al. 1996; Brandle et al. 2001; Maassen et al. 2004). It is hypothesized that mtDNA mutations could result in mitochondrial impairment associated with β-cell dysfunction as a primary abnormality in carriers of the mutation (Velho et al. 1996). Alternatively, impaired mitochondrial metabolism in cells of individuals carrying mtDNA mutations might rather predispose for β-cell dysfunction, explaining late onset of the disease. Due to technical limitation of β-cell accessibility in individuals, the putative impact of mtDNA mutations on insulin secretion still lacks direct demonstration.

In cellular models, direct investigation of β-cell functions carrying specific mtDNA mutations also faces technical obstacles. Indeed, as opposed to genomic DNA, specific mtDNA manipulations are not feasible. The alternative commonly used is to introduce patient-derived mitochondria into cell lines by fusing enucleated cells carrying mitochondria of interest with cells depleted of mtDNA (ρ° cells), resulting in cytosolic hybrids, namely, cybrids.

Mitochondria derived from patients with mtDNA A3243G mutation were introduced into a human ρ° osteosarcoma cell line. The resulting clonal cell lines contained either exclusively mutated mtDNA or wild-type mtDNA from the same patient (van den Ouweland et al. 1999). The study shows that mitochondrial A3243G mutation is responsible for defective mitochondrial metabolism associated with impaired Ca^{2+} homeostasis (de Andrade et al. 2006). The A3243G mutation induces a shift to dominantly glycolytic metabolism, while glucose oxidation is reduced (de Andrade et al. 2006). The levels of reducing equivalents in the form of NAD(P)H are not efficiently elevated upon glucose stimulation in mtDNA-mutant cells, reflecting the impact of this mutation on the electron transport chain activity (van den Ouweland et al. 1999). As a metabolic consequence, we observed a switch to anaerobic glucose utilization accompanied by increased lactate generation (de Andrade et al. 2006). Accordingly, ATP supply is totally dependent on high glycolytic rates, enabling the mtDNA-mutant cells to only reach basal normal ATP levels at the expense of stimulatory glucose concentrations. Such a phenotype is well known to dramatically impair glucose-stimulated insulin secretion in β-cells.

Conclusion

Mitochondria are key organelles that generate the largest part of cellular ATP and represent the central crossroad of metabolic pathways. Metabolic profiling of β-cell function identified mitochondria as sensors and generators of metabolic

signals controlling insulin secretion. Recent molecular tools available for cell biology studies shed light on new mechanisms regarding the coupling of glucose recognition to insulin exocytosis. Delineation of metabolic signals required for β-cell function will be instrumental in therapeutic approaches for the management of diabetes.

Acknowledgments We thank the long-standing support of the Swiss National Science Foundation and the State of Geneva.

References

Ahren B (2000) Autonomic regulation of islet hormone secretion – implications for health and disease. Diabetologia 43:393–410

Ahuja N, Schwer B, Carobbio S, Waltregny D, North BJ, Castronovo V, Maechler P, Verdin E (2007) Regulation of insulin secretion by SIRT4, a mitochondrial ADP-ribosyltransferase. J Biol Chem 282:33583–33592

Akhmedov D, De Marchi U, Wollheim CB, Wiederkehr A (2012) Pyruvate dehydrogenase E1α phosphorylation is induced by glucose but does not control metabolism-secretion coupling in INS-1E clonal β-cells. Biochim Biophys Acta 1823:1815–1824

Alarcon C, Wicksteed B, Prentki M, Corkey BE, Rhodes CJ (2002) Succinate is a preferential metabolic stimulus-coupling signal for glucose-induced proinsulin biosynthesis translation. Diabetes 51:2496–2504

Ambrosio G, Zweier JL, Duilio C, Kuppusamy P, Santoro G, Elia PP, Tritto I, Cirillo P, Condorelli M, Chiariello M et al (1993) Evidence that mitochondrial respiration is a source of potentially toxic oxygen free radicals in intact rabbit hearts subjected to ischemia and reflow. J Biol Chem 268:18532–18541

Antinozzi PA, Segall L, Prentki M, McGarry JD, Newgard CB (1998) Molecular or pharmacologic perturbation of the link between glucose and lipid metabolism is without effect on glucose-stimulated insulin secretion. A re-evaluation of the long-chain acyl-CoA hypothesis. J Biol Chem 273:16146–16154

Antinozzi PA, Ishihara H, Newgard CB, Wollheim CB (2002) Mitochondrial metabolism sets the maximal limit of fuel-stimulated insulin secretion in a model pancreatic β cell: a survey of four fuel secretagogues. J Biol Chem 277:11746–11755

Ashcroft FM (2006) K_{ATP} channels and insulin secretion: a key role in health and disease. Biochem Soc Trans 34:243–246

Bai L, Zhang X, Ghishan FK (2003) Characterization of vesicular glutamate transporter in pancreatic α- and β-cells and its regulation by glucose. Am J Physiol Gastrointest Liver Physiol 284:G808–G814

Ballinger SW, Shoffner JM, Hedaya EV, Trounce I, Polak MA, Koontz DA, Wallace DC (1992) Maternally transmitted diabetes and deafness associated with a 10.4 kb mitochondrial DNA deletion. Nat Genet 1:11–15

Beckman KB, Ames BN (1998) The free radical theory of aging matures. Physiol Rev 78:547–581

Bender K, Newsholme P, Brennan L, Maechler P (2006) The importance of redox shuttles to pancreatic β-cell energy metabolism and function. Biochem Soc Trans 34:811–814

Bertrand G, Ishiyama N, Nenquin M, Ravier MA, Henquin JC (2002) The elevation of glutamate content and the amplification of insulin secretion in glucose-stimulated pancreatic islets are not causally related. J Biol Chem 277:32883–32891

Bindokas VP, Kuznetsov A, Sreenan S, Polonsky KS, Roe MW, Philipson LH (2003) Visualizing superoxide production in normal and diabetic rat islets of Langerhans. J Biol Chem 278:9796–9801

Brandle M, Lehmann R, Maly FE, Schmid C, Spinas GA (2001) Diminished insulin secretory response to glucose but normal insulin and glucagon secretory responses to arginine in a family with maternally inherited diabetes and deafness caused by mitochondrial tRNA(LEU(UUR)) gene mutation. Diabetes Care 24:1253–1258

Brennan L, Shine A, Hewage C, Malthouse JP, Brindle KM, McClenaghan N, Flatt PR, Newsholme P (2002) A nuclear magnetic resonance-based demonstration of substantial oxidative L-alanine metabolism and L-alanine-enhanced glucose metabolism in a clonal pancreatic β-cell line: metabolism of L-alanine is important to the regulation of insulin secretion. Diabetes 51:1714–1721

Broca C, Brennan L, Petit P, Newsholme P, Maechler P (2003) Mitochondria-derived glutamate at the interplay between branched-chain amino acid and glucose-induced insulin secretion. FEBS Lett 545:167–172

Brun T, Roche E, Assimacopoulos-Jeannet F, Corkey BE, Kim KH, Prentki M (1996) Evidence for an anaplerotic/malonyl-CoA pathway in pancreatic β-cell nutrient signaling. Diabetes 45:190–198

Buchet K, Godinot C (1998) Functional F1-ATPase essential in maintaining growth and membrane potential of human mitochondrial DNA-depleted rho degrees cells. J Biol Chem 273:22983–22989

Carobbio S, Ishihara H, Fernandez-Pascual S, Bartley C, Martin-Del-Rio R, Maechler P (2004) Insulin secretion profiles are modified by overexpression of glutamate dehydrogenase in pancreatic islets. Diabetologia 47:266–276

Carobbio S, Frigerio F, Rubi B, Vetterli L, Bloksgaard M, Gjinovci A, Pournourmohammadi S, Herrera PL, Reith W, Mandrup S, Maechler P (2009) Deletion of glutamate dehydrogenase in β-cells abolishes part of the insulin secretory response not required for glucose homeostasis. J Biol Chem 284:921–929

Casimir M, Lasorsa FM, Rubi B, Caille D, Palmieri F, Meda P, Maechler P (2009a) Mitochondrial glutamate carrier GC1 as a newly identified player in the control of glucose-stimulated insulin secretion. J Biol Chem 284:25004–25014

Casimir M, Rubi B, Frigerio F, Chaffard G, Maechler P (2009b) Silencing of the mitochondrial NADH shuttle component Aspartate-Glutamate Carrier AGC1 (or Aralar1) in INS-1E cells and rat islets. Biochem J 424:459–466

Chance B, Sies H, Boveris A (1979) Hydroperoxide metabolism in mammalian organs. Physiol Rev 59:527–605

Charles MA, Lawecki J, Pictet R, Grodsky GM (1975) Insulin secretion. Interrelationships of glucose, cyclic adenosine 3:5-monophosphate, and calcium. J Biol Chem 250:6134–6140

Chen JJ, Yu BP (1994) Alterations in mitochondrial membrane fluidity by lipid peroxidation products. Free Radic Biol Med 17:411–418

Cline GW, Lepine RL, Papas KK, Kibbey RG, Shulman GI (2004) 13C NMR isotopomer analysis of anaplerotic pathways in INS-1 cells. J Biol Chem 279:44370–44375

Costa NJ, Dahm CC, Hurrell F, Taylor ER, Murphy MP (2003) Interactions of mitochondrial thiols with nitric oxide. Antioxid Redox Signal 5:291–305

Croteau DL, ap Rhys CM, Hudson EK, Dianov GL, Hansford RG, Bohr VA (1997) An oxidative damage-specific endonuclease from rat liver mitochondria. J Biol Chem 272:27338–27344

de Andrade PB, Casimir M, Maechler P (2004) Mitochondrial activation and the pyruvate paradox in a human cell line. FEBS Lett 578:224–228

de Andrade PB, Rubi B, Frigerio F, van den Ouweland JM, Maassen JA, Maechler P (2006) Diabetes-associated mitochondrial DNA mutation A3243G impairs cellular metabolic pathways necessary for β cell function. Diabetologia 49:1816–1826

Deeney JT, Gromada J, Hoy M, Olsen HL, Rhodes CJ, Prentki M, Berggren PO, Corkey BE (2000) Acute stimulation with long chain acyl-CoA enhances exocytosis in insulin-secreting cells (HIT T-15 and NMRI β-cells). J Biol Chem 275:9363–9368

del Arco A, Satrustegui J (1998) Molecular cloning of Aralar, a new member of the mitochondrial carrier superfamily that binds calcium and is present in human muscle and brain. J Biol Chem 273:23327–23334

Detimary P, Van den Berghe G, Henquin JC (1996) Concentration dependence and time course of the effects of glucose on adenine and guanine nucleotides in mouse pancreatic islets. J Biol Chem 271:20559–20565

Drucker DJ (2003) Glucagon-like peptide-1 and the islet β-cell: augmentation of cell proliferation and inhibition of apoptosis. Endocrinology 144:5145–5148

Drucker DJ, Nauck MA (2006) The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet 368:1696–1705

Duchen MR (1999) Contributions of mitochondria to animal physiology: from homeostatic sensor to calcium signalling and cell death. J Physiol 516:1–17

Dyachok O, Idevall-Hagren O, Sagetorp J, Tian G, Wuttke A, Arrieumerlou C, Akusjarvi G, Gylfe E, Tengholm A (2008) Glucose-induced cyclic AMP oscillations regulate pulsatile insulin secretion. Cell Metab 8:26–37

Eliasson L, Abdulkader F, Braun M, Galvanovskis J, Hoppa MB, Rorsman P (2008) Novel aspects of the molecular mechanisms controlling insulin secretion. J Physiol 586:3313–3324

Eto K, Tsubamoto Y, Terauchi Y, Sugiyama T, Kishimoto T, Takahashi N, Yamauchi N, Kubota N, Murayama S, Aizawa T, Akanuma Y, Aizawa S, Kasai H, Yazaki Y, Kadowaki T (1999) Role of NADH shuttle system in glucose-induced activation of mitochondrial metabolism and insulin secretion. Science 283:981–985

Eto K, Yamashita T, Hirose K, Tsubamoto Y, Ainscow EK, Rutter GA, Kimura S, Noda M, Iino M, Kadowaki T (2003) Glucose metabolism and glutamate analog acutely alkalinize pH of insulin secretory vesicles of pancreatic β-cells. Am J Physiol Endocrinol Metab 285: E262–E271

Evans JL, Goldfine ID, Maddux BA, Grodsky GM (2002) Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev 23:599–622

Evans JL, Goldfine ID, Maddux BA, Grodsky GM (2003) Are oxidative stress-activated signaling pathways mediators of insulin resistance and β-cell dysfunction? Diabetes 52:1–8

Fahien LA, MacDonald MJ, Kmiotek EH, Mertz RJ, Fahien CM (1988) Regulation of insulin release by factors that also modify glutamate dehydrogenase. J Biol Chem 263:13610–13614

Farfari S, Schulz V, Corkey B, Prentki M (2000) Glucose-regulated anaplerosis and cataplerosis in pancreatic β-cells: possible implication of a pyruvate/citrate shuttle in insulin secretion. Diabetes 49:718–726

Fransson U, Rosengren AH, Schuit FC, Renstrom E, Mulder H (2006) Anaplerosis via pyruvate carboxylase is required for the fuel-induced rise in the ATP:ADP ratio in rat pancreatic islets. Diabetologia 49:1578–1586

Fridovich I (1995) Superoxide radical and superoxide dismutases. Annu Rev Biochem 64:97–112

Frigerio F, Casimir M, Carobbio S, Maechler P (2008) Tissue specificity of mitochondrial glutamate pathways and the control of metabolic homeostasis. Biochim Biophys Acta 1777:965–972

Gammelsaeter R, Coppola T, Marcaggi P, Storm-Mathisen J, Chaudhry FA, Attwell D, Regazzi R, Gundersen V (2011) A role for glutamate transporters in the regulation of insulin secretion. PLoS One 6:e22960

Gembal M, Gilon P, Henquin JC (1992) Evidence that glucose can control insulin release independently from its action on ATP-sensitive K^+ channels in mouse B cells. J Clin Invest 89:1288–1295

Gilon P, Henquin JC (1992) Influence of membrane potential changes on cytoplasmic Ca^{2+} concentration in an electrically excitable cell, the insulin-secreting pancreatic β-cell. J Biol Chem 267:20713–20720

Guay C, Madiraju SR, Aumais A, Joly E, Prentki M (2007) A role for ATP-citrate lyase, malic enzyme, and pyruvate/citrate cycling in glucose-induced insulin secretion. J Biol Chem 282:35657–35665

Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, Valenzuela DM, Yancopoulos GD, Karow M, Blander G, Wolberger C, Prolla TA, Weindruch R, Alt FW, Guarente L (2006) SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic β cells. Cell 126:941–954

Hellman B, Idahl LA, Sehlin J, Taljedal IB (1975) Influence of anoxia on glucose metabolism in pancreatic islets: lack of correlation between fructose-1,6-diphosphate and apparent glycolytic flux. Diabetologia 11:495–500

Henquin JC (2000) Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 49:1751–1760

Herrero L, Rubi B, Sebastian D, Serra D, Asins G, Maechler P, Prentki M, Hegardt FG (2005) Alteration of the malonyl-CoA/carnitine palmitoyltransferase I interaction in the β-cell impairs glucose-induced insulin secretion. Diabetes 54:462–471

Herzig S, Raemy E, Montessuit S, Veuthey JL, Zamboni N, Westermann B, Kunji ER, Martinou JC (2012) Identification and functional expression of the mitochondrial pyruvate carrier. Science 337:93–96

Hoch FL (1992) Cardiolipins and biomembrane function. Biochim Biophys Acta 1113:71–133

Hoy M, Maechler P, Efanov AM, Wollheim CB, Berggren PO, Gromada J (2002) Increase in cellular glutamate levels stimulates exocytosis in pancreatic β-cells. FEBS Lett 531:199–203

Hudson RC, Daniel RM (1993) L-glutamate dehydrogenases: distribution, properties and mechanism. Comp Biochem Physiol B 106:767–792

Huypens P, Ling Z, Pipeleers D, Schuit F (2000) Glucagon receptors on human islet cells contribute to glucose competence of insulin release. Diabetologia 43:1012–1019

Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, Ogi K, Hosoya M, Tanaka Y, Uejima H, Tanaka H, Maruyama M, Satoh R, Okubo S, Kizawa H, Komatsu H, Matsumura F, Noguchi Y, Shinohara T, Hinuma S, Fujisawa Y, Fujino M (2003) Free fatty acids regulate insulin secretion from pancreatic β cells through GPR40. Nature 422:173–176

Ivarsson R, Quintens R, Dejonghe S, Tsukamoto K, In't Veld P, Renstrom E, Schuit FC (2005) Redox control of exocytosis: regulatory role of NADPH, thioredoxin, and glutaredoxin. Diabetes 54:2132–2142

Iynedjian PB (2009) Molecular physiology of mammalian glucokinase. Cell Mol Life Sci 66:27–42

Jalil MA, Begum L, Contreras L, Pardo B, Iijima M, Li MX, Ramos M, Marmol P, Horiuchi M, Shimotsu K, Nakagawa S, Okubo A, Sameshima M, Isashiki Y, Del Arco A, Kobayashi K, Satrustegui J, Saheki T (2005) Reduced *N*-acetylaspartate levels in mice lacking aralar, a brain- and muscle-type mitochondrial aspartate-glutamate carrier. J Biol Chem 280:31333–31339

Jones PM, Persaud SJ (1998) Protein kinases, protein phosphorylation, and the regulation of insulin secretion from pancreatic β-cells. Endocr Rev 19:429–461

Joseph JW, Odegaard ML, Ronnebaum SM, Burgess SC, Muehlbauer J, Sherry AD, Newgard CB (2007) Normal flux through ATP-citrate lyase or fatty acid synthase is not required for glucose-stimulated insulin secretion. J Biol Chem 282:31592–31600

Kennedy ED, Maechler P, Wollheim CB (1998) Effects of depletion of mitochondrial DNA in metabolism secretion coupling in INS-1 cells. Diabetes 47:374–380

Kibbey RG, Pongratz RL, Romanelli AJ, Wollheim CB, Cline GW, Shulman GI (2007) Mitochondrial GTP regulates glucose-stimulated insulin secretion. Cell Metab 5:253–264

Kowluru A, Chen HQ, Modrick LM, Stefanelli C (2001) Activation of acetyl-CoA carboxylase by a glutamate- and magnesium-sensitive protein phosphatase in the islet β-cell. Diabetes 50:1580–1587

Krus U, Kotova O, Spégel P, Hallgard E, Sharoyko VV, Vedin A, Moritz T, Sugden MC, Koeck T, Mulder H (2010) Pyruvate dehydrogenase kinase 1 controls mitochondrial metabolism and insulin secretion in INS-1 832/13 clonal β-cells. Biochem J 429:205–213

Lang J (1999) Molecular mechanisms and regulation of insulin exocytosis as a paradigm of endocrine secretion. Eur J Biochem 259:3–17

Lehtihet M, Honkanen RE, Sjoholm A (2005) Glutamate inhibits protein phosphatases and promotes insulin exocytosis in pancreatic β-cells. Biochem Biophys Res Commun 328:601–607

Leibowitz G, Khaldi MZ, Shauer A, Parnes M, Oprescu AI, Cerasi E, Jonas JC, Kaiser N (2005) Mitochondrial regulation of insulin production in rat pancreatic islets. Diabetologia 48:1549–1559

Li C, Matter A, Kelly A, Petty TJ, Najafi H, MacMullen C, Daikhin Y, Nissim I, Lazarow A, Kwagh J, Collins HW, Hsu BY, Yudkoff M, Matschinsky FM, Stanley CA (2006) Effects of a GTP-insensitive mutation of glutamate dehydrogenase on insulin secretion in transgenic mice. J Biol Chem 281:15064–15072

Li N, Frigerio F, Maechler P (2008) The sensitivity of pancreatic β-cells to mitochondrial injuries triggered by lipotoxicity and oxidative stress. Biochem Soc Trans 36:930–934

Li N, Brun T, Cnop M, Cunha DA, Eizirik DL, Maechler P (2009) Transient oxidative stress damages mitochondrial machinery inducing persistent β-cell dysfunction. J Biol Chem 284:23602–23612

Liang Y, Matschinsky FM (1991) Content of CoA-esters in perifused rat islets stimulated by glucose and other fuels. Diabetes 40:327–333

Lippe G, Comelli M, Mazzilis D, Sala FD, Mavelli I (1991) The inactivation of mitochondrial F1 ATPase by H_2O_2 is mediated by iron ions not tightly bound in the protein. Biochem Biophys Res Commun 181:764–770

Liu YQ, Moibi JA, Leahy JL (2004) Chronic high glucose lowers pyruvate dehydrogenase activity in islets through enhanced production of long chain acyl-CoA: prevention of impaired glucose oxidation by enhanced pyruvate recycling through the malate-pyruvate shuttle. J Biol Chem 279:7470–7475

Lortz S, Tiedge M (2003) Sequential inactivation of reactive oxygen species by combined overexpression of SOD isoforms and catalase in insulin-producing cells. Free Radic Biol Med 34:683–688

Lowell BB, Shulman GI (2005) Mitochondrial dysfunction and type 2 diabetes. Science 307:384–387

Lu D, Mulder H, Zhao P, Burgess SC, Jensen MV, Kamzolova S, Newgard CB, Sherry AD (2002) 13C NMR isotopomer analysis reveals a connection between pyruvate cycling and glucose-stimulated insulin secretion (GSIS). Proc Natl Acad Sci USA 99:2708–2713

Lynn S, Borthwick GM, Charnley RM, Walker M, Turnbull DM (2003) Heteroplasmic ratio of the A3243G mitochondrial DNA mutation in single pancreatic β cells. Diabetologia 46:296–299

Maassen JA, van Essen E, van den Ouweland JM, Lemkes HH (2001) Molecular and clinical aspects of mitochondrial diabetes mellitus. Exp Clin Endocrinol Diabetes 109:127–134

Maassen JA, Hart LM T, Van Essen E, Heine RJ, Nijpels G, Jahangir Tafrechi RS, Raap AK, Janssen GM, Lemkes HH (2004) Mitochondrial diabetes: molecular mechanisms and clinical presentation. Diabetes 53(Suppl 1):S103–S109

Maassen JA, Janssen GM, Hart LM (2005) Molecular mechanisms of mitochondrial diabetes (MIDD). Ann Med 37:213–221

MacDonald MJ (1982) Evidence for the malate aspartate shuttle in pancreatic islets. Arch Biochem Biophys 213:643–649

MacDonald MJ, Fahien LA (2000) Glutamate is not a messenger in insulin secretion. J Biol Chem 275:34025–34027

Maechler P, Wollheim CB (1999) Mitochondrial glutamate acts as a messenger in glucose-induced insulin exocytosis. Nature 402:685–689

Maechler P, Wollheim CB (2000) Mitochondrial signals in glucose-stimulated insulin secretion in the β cell. J Physiol 529:49–56

Maechler P, Kennedy ED, Pozzan T, Wollheim CB (1997) Mitochondrial activation directly triggers the exocytosis of insulin in permeabilized pancreatic β-cells. EMBO J 16:3833–3841

Maechler P, Kennedy ED, Wang H, Wollheim CB (1998) Desensitization of mitochondrial Ca^{2+} and insulin secretion responses in the β cell. J Biol Chem 273:20770–20778

Maechler P, Jornot L, Wollheim CB (1999) Hydrogen peroxide alters mitochondrial activation and insulin secretion in pancreatic β cells. J Biol Chem 274:27905–27913

Maechler P, Antinozzi PA, Wollheim CB (2000) Modulation of glutamate generation in mitochondria affects hormone secretion in INS-1E β cells. IUBMB Life 50:27–31

Maechler P, Gjinovci A, Wollheim CB (2002) Implication of glutamate in the kinetics of insulin secretion in rat and mouse perfused pancreas. Diabetes 51(S1):S99–S102
Mandrup-Poulsen T (2001) β-cell apoptosis: stimuli and signaling. Diabetes 50(Suppl 1):S58–S63
McCormack JG, Halestrap AP, Denton RM (1990) Role of calcium ions in regulation of mammalian intramitochondrial metabolism. Physiol Rev 70:391–425
Miki T, Nagashima K, Seino S (1999) The structure and function of the ATP-sensitive K^+ channel in insulin-secreting pancreatic β-cells. J Mol Endocrinol 22:113–123
Mukala-Nsengu A, Fernandez-Pascual S, Martin F, Martin-del-Rio R, Tamarit-Rodriguez J (2004) Similar effects of succinic acid dimethyl ester and glucose on islet calcium oscillations and insulin release. Biochem Pharmacol 67:981–988
Newsholme P, Brennan L, Rubi B, Maechler P (2005) New insights into amino acid metabolism, β-cell function and diabetes. Clin Sci (Lond) 108:185–194
Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M (2000) Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 404:787–790
Nolan CJ, Madiraju MS, Delghingaro-Augusto V, Peyot ML, Prentki M (2006) Fatty acid signaling in the β-cell and insulin secretion. Diabetes 55(Suppl 2):S16–S23
Owen OE, Kalhan SC, Hanson RW (2002) The key role of anaplerosis and cataplerosis for citric acid cycle function. J Biol Chem 277:30409–30412
Pagliarini DJ, Wiley SE, Kimple ME, Dixon JR, Kelly P, Worby CA, Casey PJ, Dixon JE (2005) Involvement of a mitochondrial phosphatase in the regulation of ATP production and insulin secretion in pancreatic β cells. Mol Cell 19:197–207
Palmieri L, Pardo B, Lasorsa FM, del Arco A, Kobayashi K, Iijima M, Runswick MJ, Walker JE, Saheki T, Satrustegui J, Palmieri F (2001) Citrin and aralar1 are Ca^{2+}-stimulated aspartate/glutamate transporters in mitochondria. EMBO J 20:5060–5069
Panten U, Zielmann S, Langer J, Zunkler BJ, Lenzen S (1984) Regulation of insulin secretion by energy metabolism in pancreatic β-cell mitochondria. Studies with a non-metabolizable leucine analogue. Biochem J 219:189–196
Panten U, Schwanstecher M, Wallasch A, Lenzen S (1988) Glucose both inhibits and stimulates insulin secretion from isolated pancreatic islets exposed to maximally effective concentrations of sulfonylureas. Naunyn Schmiedebergs Arch Pharmacol 338:459–462
Park KS, Wiederkehr A, Kirkpatrick C, Mattenberger Y, Martinou JC, Marchetti P, Demaurex N, Wollheim CB (2008) Selective actions of mitochondrial fission/fusion genes on metabolism-secretion coupling in insulin-releasing cells. J Biol Chem 283:33347–33356
Patterson GH, Knobel SM, Arkhammar P, Thastrup O, Piston DW (2000) Separation of the glucose-stimulated cytoplasmic and mitochondrial NAD(P)H responses in pancreatic islet β cells. Proc Natl Acad Sci USA 97:5203–5207
Pi J, Bai Y, Zhang Q, Wong V, Floering LM, Daniel K, Reece JM, Deeney JT, Andersen ME, Corkey BE, Collins S (2007) Reactive oxygen species as a signal in glucose-stimulated insulin secretion. Diabetes 56:1783–1791
Pizarro-Delgado J, Hernandez-Fisac I, Martin-Del-Rio R, Tamarit-Rodriguez J (2009) Branched-chain 2-oxoacid transamination increases GABA-shunt metabolism and insulin secretion in isolated islets. Biochem J 419:359–368
Pralong WF, Bartley C, Wollheim CB (1990) Single islet β-cell stimulation by nutrients: relationship between pyridine nucleotides, cytosolic Ca^{2+} and secretion. EMBO J 9:53–60
Prentki M (1996) New insights into pancreatic β-cell metabolic signaling in insulin secretion. Eur J Biochem 134:272–286
Prentki M, Vischer S, Glennon MC, Regazzi R, Deeney JT, Corkey BE (1992) Malonyl-CoA and long chain acyl-CoA esters as metabolic coupling factors in nutrient-induced insulin secretion. J Biol Chem 267:5802–5810
Prentki M, Joly E, El-Assaad W, Roduit R (2002) Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in β-cell adaptation and failure in the etiology of diabetes. Diabetes 51 (Suppl 3):S405–S413

Produit-Zengaffinen N, Davis-Lameloise N, Perreten H, Becard D, Gjinovci A, Keller PA, Wollheim CB, Herrera P, Muzzin P, Assimacopoulos-Jeannet F (2007) Increasing uncoupling protein-2 in pancreatic β cells does not alter glucose-induced insulin secretion but decreases production of reactive oxygen species. Diabetologia 50:84–93

Rabinovitch A (1998) An update on cytokines in the pathogenesis of insulin-dependent diabetes mellitus. Diabetes Metab Rev 14:129–151

Raha S, Robinson BH (2000) Mitochondria, oxygen free radicals, disease and ageing. Trends Biochem Sci 25:502–508

Randle PJ, Priestman DA, Mistry S, Halsall A (1994) Mechanisms modifying glucose oxidation in diabetes mellitus. Diabetologia 37(Suppl 2):S155–S161

Rhee SG (2006) Cell signaling. H_2O_2, a necessary evil for cell signaling. Science 312:1882–1883

Robertson RP (2006) Oxidative stress and impaired insulin secretion in type 2 diabetes. Curr Opin Pharmacol 6:615–619

Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H (2003) Glucose toxicity in β-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. Diabetes 52:581–587

Robertson RP, Harmon J, Tran PO, Poitout V (2004) β-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. Diabetes 53(Suppl 1):S119–S124

Rocheleau JV, Head WS, Piston DW (2004) Quantitative NAD(P)H/flavoprotein autofluorescence imaging reveals metabolic mechanisms of pancreatic islet pyruvate response. J Biol Chem 279:31780–31787

Roduit R, Nolan C, Alarcon C, Moore P, Barbeau A, Delghingaro-Augusto V, Przybykowski E, Morin J, Masse F, Massie B, Ruderman N, Rhodes C, Poitout V, Prentki M (2004) A role for the malonyl-CoA/long-chain acyl-CoA pathway of lipid signaling in the regulation of insulin secretion in response to both fuel and nonfuel stimuli. Diabetes 53:1007–1019

Ronnebaum SM, Jensen MV, Hohmeier HE, Burgess SC, Zhou YP, Qian S, MacNeil D, Howard A, Thornberry N, Ilkayeva O, Lu D, Sherry AD, Newgard CB (2008) Silencing of cytosolic or mitochondrial isoforms of malic enzyme has no effect on glucose-stimulated insulin secretion from rodent islets. J Biol Chem 283:28909–28917

Rorsman P, Eliasson L, Renstrom E, Gromada J, Barg S, Gopel S (2000) The cell physiology of biphasic insulin secretion. News Physiol Sci 15:72–77

Rubi B, Ishihara H, Hegardt FG, Wollheim CB, Maechler P (2001) GAD65-mediated glutamate decarboxylation reduces glucose-stimulated insulin secretion in pancreatic β cells. J Biol Chem 276:36391–36396

Rubi B, Antinozzi PA, Herrero L, Ishihara H, Asins G, Serra D, Wollheim CB, Maechler P, Hegardt FG (2002) Adenovirus-mediated overexpression of liver carnitine palmitoyl-transferase I in INS1E cells: effects on cell metabolism and insulin secretion. Biochem J 364:219–226

Rubi B, del Arco A, Bartley C, Satrustegui J, Maechler P (2004) The malate-aspartate NADH shuttle member Aralar1 determines glucose metabolic fate, mitochondrial activity, and insulin secretion in β cells. J Biol Chem 279:55659–55666

Rutter GA, Burnett P, Rizzuto R, Brini M, Murgia M, Pozzan T, Tavare JM, Denton RM (1996) Subcellular imaging of intramitochondrial Ca^{2+} with recombinant targeted aequorin: significance for the regulation of pyruvate dehydrogenase activity. Proc Natl Acad Sci USA 93:5489–5494

Sato Y, Aizawa T, Komatsu M, Okada N, Yamada T (1992) Dual functional role of membrane depolarization/Ca^{2+} influx in rat pancreatic β-cell. Diabetes 41:438–443

Schuit F, De Vos A, Farfari S, Moens K, Pipeleers D, Brun T, Prentki M (1997) Metabolic fate of glucose in purified islet cells. Glucose-regulated anaplerosis in β cells. J Biol Chem 272:18572–18579

Schuit FC, Huypens P, Heimberg H, Pipeleers DG (2001) Glucose sensing in pancreatic β-cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. Diabetes 50:1–11

Sekine N, Cirulli V, Regazzi R, Brown LJ, Gine E, Tamarit-Rodriguez J, Girotti M, Marie S, MacDonald MJ, Wollheim CB (1994) Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogenase in pancreatic β-cells. Potential role in nutrient sensing. J Biol Chem 269:4895–4902

Sener A, Malaisse WJ (1980) L-leucine and a nonmetabolized analogue activate pancreatic islet glutamate dehydrogenase. Nature 288:187–189

Sener A, Malaisse-Lagae F, Malaisse WJ (1981) Stimulation of pancreatic islet metabolism and insulin release by a nonmetabolizable amino acid. Proc Natl Acad Sci USA 78:5460–5464

Silva JP, Kohler M, Graff C, Oldfors A, Magnuson MA, Berggren PO, Larsson NG (2000) Impaired insulin secretion and β-cell loss in tissue-specific knockout mice with mitochondrial diabetes. Nat Genet 26:336–340

Soejima A, Inoue K, Takai D, Kaneko M, Ishihara H, Oka Y, Hayashi JI (1996) Mitochondrial DNA is required for regulation of glucose-stimulated insulin secretion in a mouse pancreatic β cell line, MIN6. J Biol Chem 271:26194–26199

Stanley CA, Lieu YK, Hsu BY, Burlina AB, Greenberg CR, Hopwood NJ, Perlman K, Rich BH, Zammarchi E, Poncz M (1998) Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. N Engl J Med 338:1352–1357

Storto M, Capobianco L, Battaglia G, Molinaro G, Gradini R, Riozzi B, Di Mambro A, Mitchell KJ, Bruno V, Vairetti MP, Rutter GA, Nicoletti F (2006) Insulin secretion is controlled by mGlu5 metabotropic glutamate receptors. Mol Pharmacol 69:1234–1241

Tiedge M, Lortz S, Drinkgern J, Lenzen S (1997) Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes 46:1733–1742

Tsuruzoe K, Araki E, Furukawa N, Shirotani T, Matsumoto K, Kaneko K, Motoshima H, Yoshizato K, Shirakami A, Kishikawa H, Miyazaki J, Shichiri M (1998) Creation and characterization of a mitochondrial DNA-depleted pancreatic β-cell line: impaired insulin secretion induced by glucose, leucine, and sulfonylureas. Diabetes 47:621–631

Turrens JF, Boveris A (1980) Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochem J 191:421–427

Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, Shirihai OS (2008) Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J 27:433–446

Vallar L, Biden TJ, Wollheim CB (1987) Guanine nucleotides induce Ca^{2+} independent insulin secretion from permeabilized RINm5F cells. J Biol Chem 262:5049–5056

van den Ouweland JM, Lemkes HH, Ruitenbeek W, Sandkuijl LA, de Vijlder MF, Struyvenberg PA, van de Kamp JJ, Maassen JA (1992) Mutation in mitochondrial tRNA(Leu)(UUR) gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. Nat Genet 1:368–371

van den Ouweland JM, Maechler P, Wollheim CB, Attardi G, Maassen JA (1999) Functional and morphological abnormalities of mitochondria harbouring the tRNA(Leu)(UUR) mutation in mitochondrial DNA derived from patients with maternally inherited diabetes and deafness (MIDD) and progressive kidney disease. Diabetologia 42:485–492

Varadi A, Ainscow EK, Allan VJ, Rutter GA (2002) Involvement of conventional kinesin in glucose-stimulated secretory granule movements and exocytosis in clonal pancreatic β-cells. J Cell Sci 115:4177–4189

Velho G, Byrne MM, Clement K, Sturis J, Pueyo ME, Blanche H, Vionnet N, Fiet J, Passa P, Robert JJ, Polonsky KS, Froguel P (1996) Clinical phenotypes, insulin secretion, and insulin sensitivity in kindreds with maternally inherited diabetes and deafness due to mitochondrial tRNALeu(UUR) gene mutation. Diabetes 45:478–487

Verspohl EJ, Handel M, Ammon HP (1979) Pentosephosphate shunt activity of rat pancreatic islets: its dependence on glucose concentration. Endocrinology 105:1269–1274

Vetterli L, Carobbio S, Pournourmohammadi S, Martin-Del-Rio R, Skytt DM, Waagepetersen HS, Tamarit-Rodriguez J, Maechler P (2012) Delineation of glutamate pathways and secretory

responses in pancreatic islets with β-cell specific abrogation of the glutamate dehydrogenase. Mol Biol Cell 123:342–348
Wallace DC (1999) Mitochondrial diseases in man and mouse. Science 283:1482–1488
Watkins DT (1972) Pyridine nucleotide stimulation of insulin release from isolated toadfish insulin secretion granules. Endocrinology 90:272–276
Watkins DT, Moore M (1977) Uptake of NADPH by islet secretion granule membranes. Endocrinology 100:1461–1467
Watkins D, Cooperstein SJ, Dixit PK, Lazarow A (1968) Insulin secretion from toadfish islet tissue stimulated by pyridine nucleotides. Science 162:283–284
Westermann B (2008) Molecular machinery of mitochondrial fusion and fission. J Biol Chem 283:13501–13505
Wiederkehr A, Park KS, Dupont O, Demaurex N, Pozzan T, Cline GW, Wollheim CB (2009) Matrix alkalinization: a novel mitochondrial signal for sustained pancreatic β-cell activation. EMBO J 28:417–428
Yakes FM, Van Houten B (1997) Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc Natl Acad Sci USA 94:514–519
Yan LJ, Sohal RS (1998) Mitochondrial adenine nucleotide translocase is modified oxidatively during aging. Proc Natl Acad Sci USA 95:12896–12901
Yan LJ, Levine RL, Sohal RS (1997) Oxidative damage during aging targets mitochondrial aconitase. Proc Natl Acad Sci USA 94:11168–11172
Yu BP (1994) Cellular defenses against damage from reactive oxygen species. Physiol Rev 74:139–162
Yu W, Niwa T, Fukasawa T, Hidaka H, Senda T, Sasaki Y, Niki I (2000) Synergism of protein kinase A, protein kinase C, and myosin light-chain kinase in the secretory cascade of the pancreatic β-cell. Diabetes 49:945–952
Zawalich WS, Zawalich KC, Cline G, Shulman G, Rasmussen H (1993) Comparative effects of monomethylsuccinate and glucose on insulin secretion from perifused rat islets. Diabetes 42:843–850

IGF-1 and Insulin-Receptor Signalling in Insulin-Secreting Cells: From Function to Survival

23

Susanne Ullrich

Contents

Abstract

Insulin and insulin-like growth factor 1 (IGF-1) receptors are ubiquitously expressed and regulate cell growth, survival, and function. In insulin-secreting cells, they contribute to proper insulin synthesis and secretion, as well as to overall pancreatic β-cell survival. The most convincing proof of the importance of these signalling pathways came from mice deficient in insulin receptors, IGF-1 receptors, or insulin receptor substrate-2 (IRS-2). Knockout of the insulin

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_26,

receptor or IRS-2 leads to life-threatening hyperglycemia and is prevented by β-cell-specific expression of IRS-2. Pancreatic β-cells exist in an insulin-rich environment, and therefore, the regulation and activation of their receptors must differ from cells in peripheral insulin-sensitive tissues. Intriguingly, the downstream signalling of these receptors diverges towards anti- and proapoptotic pathways: receptor activation improves cell function and growth but also induces feedback inhibition and apoptosis. This chapter summarizes current understanding of insulin and IGF-1 receptor regulation; signalling and function in β-cells with special emphasis on the regulation of insulin receptor substrates, IRS-1 and IRS-2; downstream kinases; and feedback mechanisms that impair β-cell function.

Keywords

Insulin-secreting cells • Insulin receptor • IGF-1 receptor • β-cell survival • β-cell differentiation • β-cell mass • Insulin secretion • IRS-1 • IRS-2 • PI3K • AKT • MAPK

Introduction

The role and mode of function of insulin receptors (IR) and insulin-like growth factor 1 receptors (IGF-1R) in pancreatic β-cells have been summarized and discussed in a variety of valuable reviews (Goldfine and Kulkarni 2012; Leibiger et al. 2008; White 2006; Talchai et al. 2009; LeRoith and Accili 2008; Buteau and Accili 2007; van Haeften and Twickler 2004; Rhodes 2005). This chapter sets out to give a historical overview of "insulin action" in insulin-secreting cells and then summarizes and critically discusses important findings about the functional role and molecular mechanisms of IR and IGF-1R signalling in insulin-secreting cells. This includes their effects on insulin secretion, insulin gene regulation, β-cell proliferation, and β-cell death, as well as the underlying molecular mechanisms.

Discovery of Insulin and IGF-1 Receptors: Subtypes, Structures, and Ligand Affinities

In 1971, Roth and coworkers discovered that insulin binds to cell surface receptors in the liver (Freychet et al. 1971). Nine years later, similar binding experiments performed in rat islets using tracer-labeled insulin, enabled Verspohl and Ammon to demonstrate the existence of insulin receptors in the endocrine pancreas (Verspohl and Ammon 1980). Insulin acts in target tissues through activating insulin receptor type A (IR-A) and type B (IR-B), two alternative splice variants of exon 11 from the *INSR* gene (Seino and Bell 1989). Insulin-secreting cells express both IR-A and IR-B (Leibiger et al. 2001). Although structural analysis of IR (and IGF-1R) first started at the beginning of 1980 (Massague et al. 1980; De Meyts

1994; Luo et al. 1999; Hubbard 1997), the molecular structure of the IR including the insulin binding site was only recently resolved (Smith et al. 2010; Menting et al. 2013). IR-A and IR-B belong to the same family of tyrosine kinase receptors as IGF-1R and IGF-2R, sharing 60 % amino acid homology with IGF-1R (Ullrich et al. 1985; Samani et al. 2007). The first evidence of IGF-1R expression in pancreatic endocrine cells was obtained by Pipeleers and coworkers in 1987 (Van Schravendijk et al. 1987).

Insulin binds with a half-maximal concentration (K_d) of 6 pmol/l (1 μIU/ml = 6.945 pmol/l = 40.3 ng/l) to human IR. The K_d measured in isolated rat islets was somewhat higher at 0.46 nmol/l. In addition, insulin also activates IGF-1R but with a more than three orders of magnitude higher concentration (K_d = 20 nmol/l). This cross-activation is thought to play a role in the endocrine pancreas where extracellular insulin concentrations surrounding β-cells reach particularly high levels. IGF-1 binds to the IGF-1R with a K_d of 20 pmol/l (Zhu and Kahn 1997), and, reciprocally, IGF-1 is also a ligand of IR (K_d = 1 nmol/l).

Tightly Controlled Degradation of Ligands and Receptors Terminate Stimulation

Activation of membrane receptors is controlled by both degradation of the ligand and downregulation of the receptor. Insulin, i.e., ligand action, is limited by the relatively short half-life of the hormone. The degradation of insulin is catalyzed by the insulin-degrading enzyme (IDE), a cytoplasmic and peroxisomal zinc metalloprotease. IDE is ubiquitously expressed and secreted into the extracellular space where it degrades extracellular substrates including insulin and amyloid β-protein (Farris et al. 2003). IDE which lacks the signal sequence necessary for the classical secretory pathway is secreted through a newly identified, non-conventional mechanism (Zhao et al. 2009; Glebov et al. 2011). Pharmacological inhibition of IDE increases amylin-induced cytotoxicity in pancreatic β-cells, and mice deficient in IDE develop glucose intolerance due to an impairment of glucose-induced insulin secretion (Bennett et al. 2003; Farris et al. 2003; Steneberg et al. 2013). In humans, genetic variants in the IDE gene, however, do not associate with an increased susceptibility to type 2 diabetes (Florez et al. 2006).

Membrane receptor degradation is a regulated process and involves ligand binding, followed by ubiquitination of the receptor, which is a prerequisite for internalization via endocytosis. Endocytotic vesicles either fuse with lysosomes and enter the proteasomal pathway for degradation or recycle to the plasma membrane. When insulin binds and stimulates the IR, the ligand-receptor complex is internalized via clathrin-coated pit-dependent endocytosis (Carpentier 1994; Vogt et al. 1991). This internalization depends on ubiquitination of IR mediated by an E3 ubiquitin ligase (c-Cbl) and an adaptor protein (APS), but does not automatically result in degradation (Kishi et al. 2007). Interestingly, after internalization of the insulin-IR complex, most of the IR recycles back to the plasma membrane, whereas insulin is rapidly degraded by IDE (Krupp and Lane 1982). This recycling

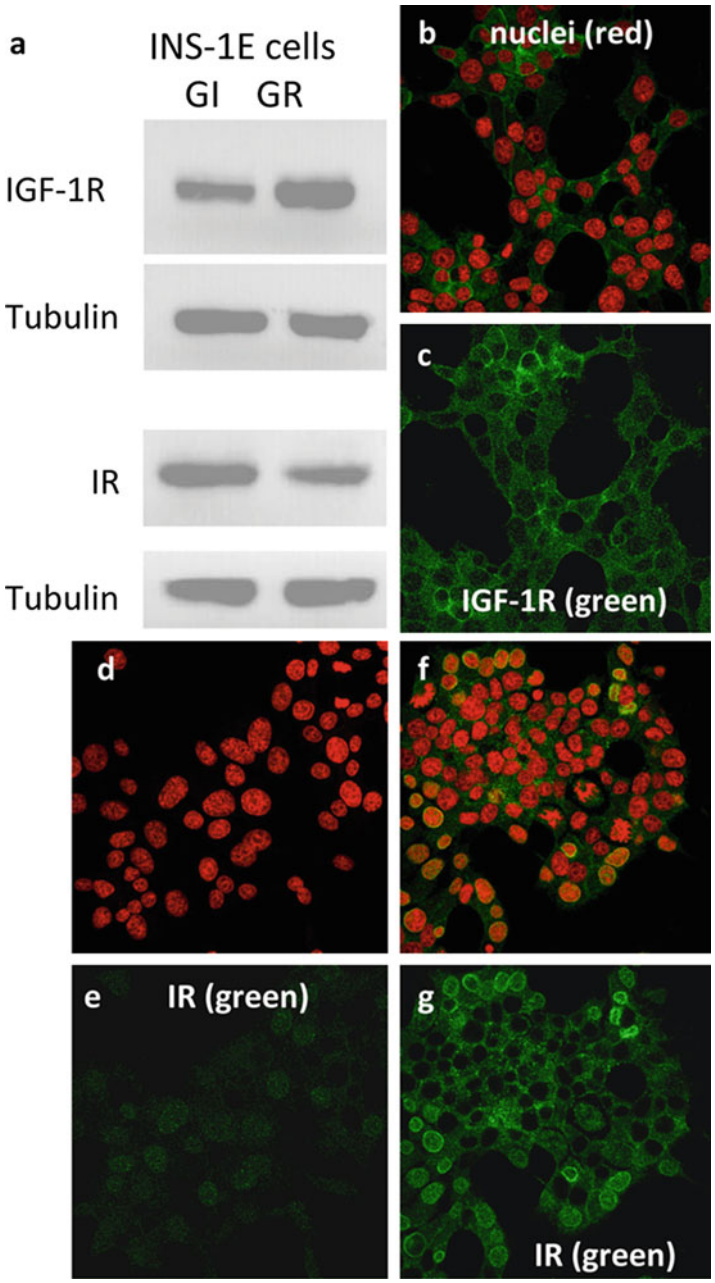

Fig. 1 Expression of IGF-1R and IR in insulin-secreting INS-1E cells. (**a**) INS-1E cells were cultured under standard culture condition and lysed and the proteins of whole-cell homogenates analyzed by Western blotting. Glucose-insensitive, aged cells (*GI*) express less IGF-1R but more IR than glucose-responsive cells (*GR*). Tubulin was used as loading control. (**b**–**g**) Representative

process is important, since the number of IR at the plasma membrane is one of the parameters influencing insulin sensitivity. Development of insulin resistance, i.e., reduced insulin sensitivity, also arises through changes in insulin receptor signalling (Häring 1991). Such mechanisms are discussed below (The Role of IRS Proteins in Insulin-Secreting Cells: Lessons from IRS-1 and IRS-2 Knockout Mice).

Ubiquitination of IGF-1R is catalyzed by the ubiquitin ligase Nedd4 since this receptor contains the signal element of this E3 ubiquitin ligase (Kwak et al. 2012; Higashi et al. 2008; Monami et al. 2008; Vecchione et al. 2003). Nedd4 proteins are expressed in insulin-secreting cells, but the function and regulation of the enzyme are poorly understood (Lopez-Avalos et al. 2006).

Insulin-secreting INS-1E cells under standard culture conditions express both IR and IGF-1R, as visualized by Western blotting (Fig. 1a). Immunocytochemistry suggests that IGF-1R is enriched at the plasma membrane (Fig. 1b, c). Surprisingly, IR is almost completely absent at the plasma membrane (Fig. 1d, e). Only after repeatedly exchanging the extracellular solution to reduce concentrations of insulin released by the cells into the media is IR detected at the plasma membrane and, more pronounced, at intracellular sites (Fig. 1f, g). These observations suggest that the presence of high insulin concentrations in the extracellular medium leads to a selective internalization of insulin-bound IR and consequently to downregulation of plasma membrane IR. This example demonstrates that IR signalling in β-cells could be altered in situations of excessive insulin secretion and hyperinsulinemia.

More than a Negative Feedback Loop: Acute and Prolonged Effects of Insulin on Insulin Secretion

Insulin action on β-cells has been studied since in the mid-1960s, when Frerichs, Reich, and Creutzfeldt published the first in vitro study on the effects of insulin on glucose-induced insulin secretion (Frerichs et al. 1965). They examined insulin secretion in isolated slices of rat pancreata and found that glucose-induced insulin secretion was reduced upon adding high concentrations of insulin (70 nmol/l for 90 min). This prompted the provocative hypothesis that insulin might be a stronger regulator of its own secretion than glucose. Similar observations were made with isolated islets (Sodoyez et al. 1969), perfused canine (Iversen and Miles 1971), and later perfused rat pancreas (Ammon et al. 1991).

The underlying mechanism may involve phosphatidylinositol-3-kinase (PI3K)-dependent activation of K_{ATP} channels by insulin as revealed by patch clamp experiments using isolated islets (Khan et al. 2001). These results confirmed

Fig. 1 (continued) laser scan microscope images show the staining of IGF-1R (**b**) and (**c**) and IR **d**–**g** in *green* and the nuclei stained with TOPRO3 (**b**, **d**, **f**) in *red*. Repeated exchange of medium, followed by 30 min incubation of the cells at 2.8 mmol/l glucose, was performed prior to immunostaining of cells in images (**f**) and (**g**)

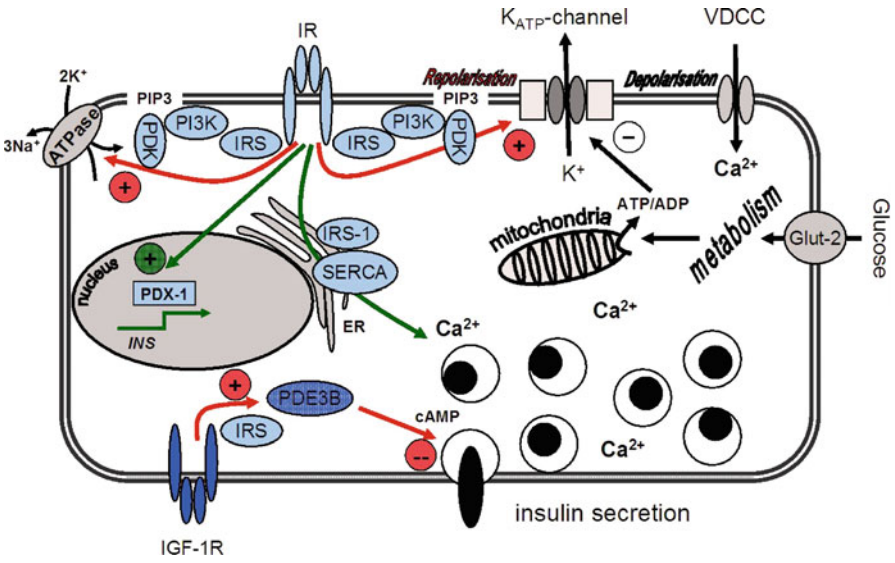

Fig. 2 IR and IGF-1R interfere with glucose-induced insulin secretion. IR and IGF-1R interact with IRS proteins. IR activates K_{ATP} channels and Na^+/K^+ ATPase through PI3K. Stimulation of IR induces hyperpolarization of β-cells, which antagonizes glucose-induced inhibition of K_{ATP} channels, and subsequently stimulates voltage-dependent Ca^{2+} channels (*VDCC*) and insulin release. IGF-1R stimulation reduces cellular cAMP concentrations by activating phosphodiesterase PDE3B. IR and IGF-1R also interfere with Ca^{2+} release from the endoplasmic reticulum (*ER*). Long-term stimulation of IR and IGF-1R increases insulin production by PDX-1-dependent activation of *INS* gene transcription

previous findings showing that insulin stimulates K_{ATP} channels in neuronal and smooth muscle cells (Yasui et al. 2008; Spanswick et al. 2000; O'Malley et al. 2003). In β-cells, acute stimulation of K_{ATP} channels by insulin counteracts the effect of glucose, resulting in hyperpolarization of the cells followed by inhibition of Ca^{2+} channels, reduction of cytosolic Ca^{2+}, $[Ca^{2+}]_i$, and inhibition of insulin secretion (Fig. 2). Interestingly, insulin was also found to hyperpolarize β-cells which do not express functional K_{ATP} channels (Düfer et al. 2009). This study reveals that insulin activates the Na^+/K^+-ATPase. The activation may contribute to the hyperpolarization induced by insulin (see chapter "▶ Electrophysiology of Islet Cells"). Taken together these observations suggest that insulin inhibits its own secretion at high glucose levels by lowering $[Ca^{2+}]_i$.

The inhibitory feedback action of insulin on insulin secretion is not unambiguously accepted (Malaisse et al. 1967). Aspinwall and coworkers published a series of papers providing evidence that insulin augments insulin secretion by increasing cytosolic Ca^{2+} through release of Ca^{2+} from the endoplasmic reticulum (Aspinwall et al. 2000, 1999a). Similar results were obtained with an insulin mimetic (Roper et al. 2002). These experiments were performed using amperometric measurements of secretion involving preloading β-cell vesicles with a charged molecule such as 5-HT (serotonin). It is assumed that the amperometric signal directly reflects insulin secretion (Aspinwall et al. 1999b; Braun et al. 2009).

One important difference between studies suggesting that insulin inhibits its own secretion and those claiming the opposite – that insulin stimulates its release – is the glucose concentration. While insulin inhibits its secretion at high glucose levels, it stimulates secretion at low glucose levels. In a physiological context, the latter assertion seems inappropriate since stimulation of secretion in the presence of basal glucose levels would result in hypoglycemia. In all studies relatively high concentrations of insulin (50–200 nmol/l) were used. At high glucose, insulin concentrations further increase due to endogenous secretion and may reach levels that stimulate IGF-1R (Zhu and Kahn 1997). Indeed, IGF-1R receptor activation inhibits insulin secretion through phosphodiesterase PDE3B-dependent lowering of cAMP (Zhao et al. 1997). This effect seems not to couple to activation of K_{ATP} channels since IGF-1 does not mimic the hyperpolarizing effect of insulin (Khan et al. 2001). Whether a reduction in cAMP levels may affect K_{ATP} channel activity through the guanine-nucleotide exchange factor EPAC-2 (cAMP-GEF-II) remains elusive. This guanine-nucleotide exchange factor, when activated by cAMP, may sensitize the channel to ATP and favor its closure (Shibasaki et al. 2004; Kang et al. 2008).

Further experimental evidence is needed to understand the inhibitory and stimulatory effects of insulin on its secretion.

Stimulation of Insulin Gene Transcription by Insulin: A Positive Feedback Loop

In contrast to the acute, most likely inhibitory effect of insulin on insulin secretion, chronic exposure to high concentrations of glucose and insulin stimulates insulin gene transcription (Melloul et al. 2002; German et al. 1990). This effect increases insulin production, the amount of stored insulin, and hence the capacity of the pancreas to secrete insulin.

Insulin action on insulin (*INS*) gene transcription is transmitted through IR-A, and the signalling pathway involves the activation of PI3K, p70S6K, and Ca^{2+}/calmodulin-dependent kinase (*CaMK*) (Fig. 3; Leibiger et al. 2001, 1998). The IR transmits at least partly the stimulatory effect of glucose on *INS* gene transcription. Accordingly, knockdown of IR in Min6 cells inhibits the accumulation of preproinsulin induced by high glucose (da Silva et al. 2000). Dissecting the effects of glucose and insulin, Leibiger and coworkers showed that during glucose-induced insulin secretion, increased (pro) insulin biosynthesis results equally from insulin-induced activation of transcription and glucose-induced posttranscriptional/post-translational modification (Leibiger et al. 2000). Indeed, the glucose-sensitive factor which binds to the insulin promoter has been identified as the transcription factor pancreatic duodenal homeobox-1 (PDX-1) (Marshak et al. 1996). Glucose modulates the activity of PDX-1 by phosphorylation, which stimulates the nuclear translocation of PDX-1 (MacFarlane et al. 1999, 1994, 1997). The human insulin gene promoter region contains four and the rat three PDX-1 binding sites, and deletion of PDX-1 results in a 40 % reduction in insulin mRNA (Iype et al. 2005). Beside glucose, insulin stimulates its own transcription through PDX-1

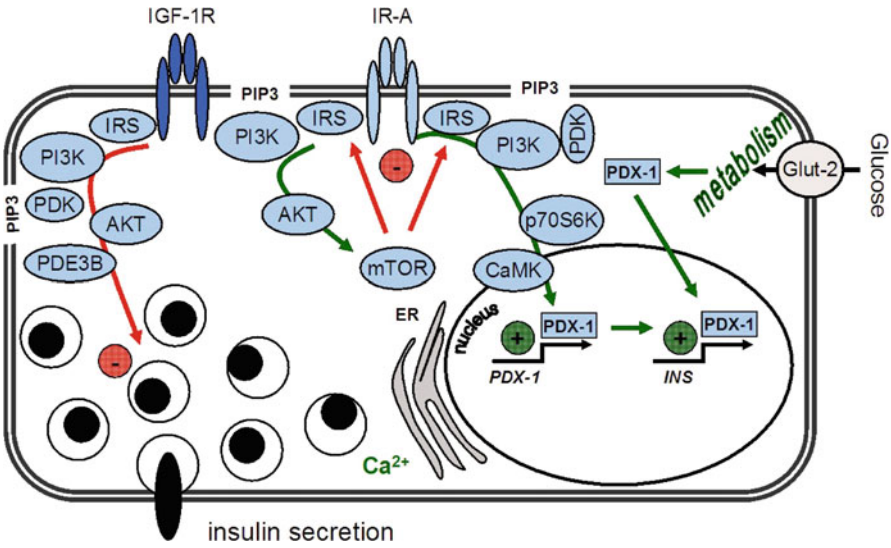

Fig. 3 IR and IGF-1R stimulation of gene transcription. Stimulation of the *INS* gene by insulin is mediated through IR-A, followed by the IRS-dependent activation of PI3K, and involves p70S6K and Ca^{2+} calmodulin-dependent kinase (*CaMK*). This activates PDX-1, a major transcription factor of the *INS* gene. Through the stimulation of mTOR, AKT exerts feedback inhibition of the IRS-dependent pathways

(Wu et al. 1999). Notably, PDX-1 conveys the effects of glucose and insulin to insulin gene transcription (Watada et al. 1996; Melloul 2004). These findings suggest that secreted insulin from the β-cells induces a positive feedback loop resulting in enhanced insulin production.

Since glucose mobilizes granules that contain newly synthesized insulin, the stimulation of insulin gene transcription reflects an important mechanism to satisfy the demand for insulin during hyperglycemia (Hou et al. 2012; Rhodes and Halban 1987). In addition, the regulation of insulin synthesis is essential for adapting to an insulin resistant metabolic state where the demand for insulin is dramatically increased.

Dual and Opposing Effects of Insulin Observed in Humans

In humans in vivo infusion of insulin inhibits insulin secretion (Argoud et al. 1987; Elahi et al. 1982). Infusion of insulin while maintaining constant plasma glucose concentrations using the glucose-clamp technique reduces plasma C peptide levels in normal and obese subjects. This indicates reduced endogenous secretion of insulin, since the C peptide is secreted together with insulin in equal amounts, and confirms the presence of a negative feedback loop in humans as discussed above (Argoud et al. 1987; Elahi et al. 1982). This negative feedback effect of

insulin on endogenous insulin release is persistent in obese subjects, i.e., in the insulin resistant state. It is, therefore, unlikely that this effect is solely mediated by IR. In addition to the direct inhibitory effect transmitted by IR and/or IGF-1R, an indirect neuronal feedback from insulin-sensitive tissues, which have continuously taken up glucose during the hyperinsulinemic-euglycemic clamp, could influence insulin release. However, the maintenance of euglycemia is more efficiently controlled by a direct glucose-insulin feedback loop rather than by insulin-mediated feedback inhibition of its own secretion (Kraegen et al. 1983).

Mirroring the contradictory findings on how insulin affects its own secretion in mice and cell lines discussed above, another in vivo study in healthy humans also claims that insulin augments insulin secretion (Bouche et al. 2010). In this study, insulin was infused for 4 h prior to stimulating endogenous secretion with glucose. In this case, insulin infusion was long enough to activate insulin synthesis. Consequently, higher amounts of insulin were available for subsequent stimulation of secretion.

In conclusion, the apparent contradictory effects of insulin on insulin secretion are explained by (1) an acute stimulatory effect of insulin on K_{ATP} channels and Na^+/K^+-ATPase, which favors hyperpolarization of the cells (Fig. 2). The mechanism most likely involves activation of IR and PI3K and antagonizes the stimulatory effect of glucose on insulin release and (2) prolonged PDX-1-mediated activation of insulin gene transcription through IR-A, PI3K, and CaMK, which increases the insulin content of β-cells and hence augments insulin release (Fig. 3).

Distinct Roles of IR and IGF-1R in β-Cells: Lessons from Mice Deficient in IR and IGF-1R

The ultimate experiments proving that IR and IGF-1R have an important function in β-cells were performed with knockout mice. However, the results were not always anticipated and unequivocal. Whole-body IR knockout (IRKO) mice as well as β-cell-specific KO (β-IRKO) mice lack visible pathophysiological features at birth, which indicates that IR does not contribute to embryonic development in mice (Kulkarni et al. 1999a; Accili et al. 1996). However, this does not extrapolate to humans. Patients with mutations in the IR gene suffer severe intrauterine growth retardation (Taylor 1992; Accili 1995).

IRKO mice represent the most drastic animal model of insulin resistance. The mice develop hyperglycemia and die of ketoacidosis early after birth. The β-IRKO mouse, which is the more appropriate animal model to study IR function in β-cells, only develops a mild phenotype. These mice display impaired insulin secretion, and when they age they progressively develop insulin deficiency. The reduction of glucose-induced insulin release seems to result from the loss of insulin-induced stimulation of insulin synthesis and the development of insulin deficiency during aging from the loss of proliferative activity (Brüning et al. 1997). In β-IRKO mice, diet-induced insulin resistance did not provoke the adaptive increase of β-cell mass, suggesting that proper IR function is important for β-cell hyperplasia in adults.

IGF-1R was unable to compensate for the loss of IR (Okada et al. 2007). Proliferation of adult, differentiated β-cells seemed unverifiable for a long time (Parnaud et al. 2008). Today, it is generally accepted that special stimuli induce proliferation of β-cells not only during development and early life but also in adulthood (Heit et al. 2006).

β-cell-specific knockout of IGF-1R gives a less dramatic phenotype than the deletion of IR (Xuan et al. 2002; Kulkarni et al. 2002). One explanation might be the compensatory upregulation of IR expression in IGF-1RKO mouse islets. This counter-regulation indicates that IR expression is variable and may account for intensive regulation. Surprisingly, the IGF-1RKO mice do not show any change in β-cell mass, but the first phase of glucose-induced insulin secretion is abolished, similar to secretory defects in islets of β-IRKO mice. IGF-1RKO mice acquire impaired glucose tolerance when they age or when developing insulin resistance. Interestingly, hyperinsulinemia is observed under fasting conditions, suggesting that IGF-1R exerts a negative feedback on basal insulin secretion. A 90 % reduction of IR or IGF-1R expression in Min6 cells inhibits glucose-induced insulin secretion, but not the release augmented by KCl-mediated depolarization (da Silva et al. 2004). These observations indicate that IR and IGF-1R signalling and PI3K-dependent phosphatidylinositol-3,4,5-triphosphate (PIP3) synthesis might not affect exocytosis directly.

Concerning the regulation of expression of β-cell specific genes, IR stimulates not only insulin and PDX-1 expression, but also the expression of the glucose sensors, glut-2, and glucokinase, an effect which further improves glucose-induced insulin secretion (Leibiger et al. 2001; da Silva et al. 2000; Kaneto et al. 2008). Whereas the effect on the *INS* gene is transmitted by IR-A (see paragraph 5 of this chapter), stimulation of the glucokinase gene (*GCK*) by insulin is mediated through IR-B and includes activation of AKT (Fig. 4; Leibiger et al. 2001). The differential functions of IR-A and IR-B might be regulated by their distinct spatial plasma membrane localization (Uhles et al. 2003). Besides reduced transcription of the *INS* gene, IR deficiency in β-cells also inhibited glucose-induced stimulation of the glucokinase (*GCK*) gene (da Silva et al. 2004).

In contrast to the inhibition of glucose-induced differentiation markers in the absence of IR, glucose still augments mitogenesis of β-cells through the activation of p42/p44 MAPK and the mammalian target of rapamycin (mTOR)/p70S6K. This activation of proliferation by glucose is mediated by the MAPK pathway, but occurs independently of PI3K (Guillen et al. 2006). That IR but not IGF-1R specifically activates PDX-1 is further supported by the stimulation of PDX-1 and preproinsulin transcription in IGF-1R deficient β-cells, while glucokinase expression is reduced and ATP production inhibited (da Silva et al. 2004). These changes induced by IGF-1R deficiency indicate that IGF-1R and IR-B have redundant roles in β-cells. Furthermore, deletion of IGF-1R may specifically induce compensatory upregulation of IR-A but not IR-B (see also Stimulation of Insulin Gene Transcription by Insulin: A Positive Feedback Loop above in this chapter).

In summary, IR and IGF-1R have redundant, but also complementary, effects on β-cell function. Both IR and IGF-1R transmit signals for differentiation and proliferation and support insulin secretion. The complex regulation of insulin synthesis,

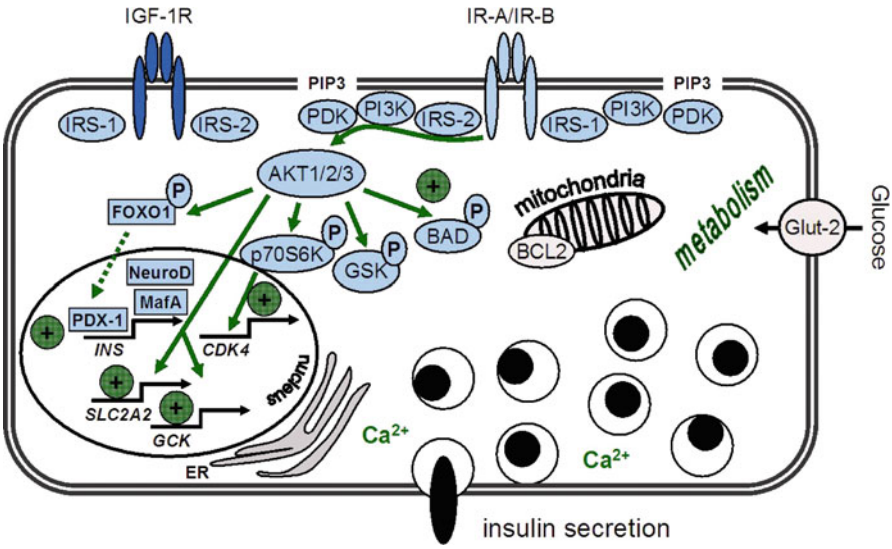

Fig. 4 AKT-dependent stimulation of antiapoptotic pathways. AKT1, AKT2, and AKT3 are expressed in insulin-secreting cells and inhibit mitochondrial-dependent cell death through phosphorylation of BAD. This phosphorylation inhibits the translocation of BAD from the cytosol to the mitochondria and the opening of the mitochondrial transition pore. Nuclear extrusion of FOXO1 by AKT phosphorylation further inhibits the activation of proapoptotic genes. Phosphorylation of S6K induces cell cycle proteins such as cyclin-dependent kinase 4 (*cdk4*) and cyclin D1 and favors proliferation. IR-B transmits the IRS-, PI3K-, and AKT-dependent activation of *GCK* and *SLC2A2* genes, which encode glucokinase and glut-2, respectively

insulin secretion, and β-cell mass makes it difficult to uncover single signalling pathways in knockout animals, particularly due to all the compensatory mechanisms and counter-regulation (Figs. 2 and 3).

The Role of IRS Proteins in Insulin-Secreting Cells: Lessons from IRS-1 and IRS-2 Knockout Mice

IR and IGF-1R signalling is transmitted through IRS proteins. The mechanism involves tyrosine phosphorylation of IRS proteins, first described by Morris White and colleagues (1985). In insulin-secreting cells, both IRS-1 and IRS-2 are expressed and link to phosphatidylinositol-3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways (Velloso et al. 1995; Harbeck et al. 1996). IRS-2 and IRS-1 guarantee proper insulin production and β-cell function, with IRS-2 being more important for adaptive β-cell proliferation than IRS-1.

β-cells lacking IRS-1 produce 50 % less insulin than control cells, and glucose- and arginine-induced insulin secretion is largely impaired (Kulkarni et al. 1999b). Nevertheless, in mice, ablation of IRS-1 causes only mild glucose intolerance

which may be explained at least in part due to the compensatory upregulation of IRS-2 (Hennige et al. 2005). The effect of IRS-1 on insulin secretion involves its binding to the endoplasmic reticulum Ca^{2+} ATPase (SERCA3b), which contributes to the regulation of Ca^{2+} homeostasis (Borge and Wolf 2003). Deletion of IRS-2 in mice results in more dramatic phenotype as the mice develop diabetes and die from hypergylcemia early in life (Withers et al. 1998; Kubota et al. 2000; Cantley et al. 2007). Most convincingly, IRS-2-deficient mice were protected against developing life-threatening diabetes by β-cell-specific rescue of IRS-2 (Hennige et al. 2003). Interestingly, even in type 1 diabetic NOD mice, IRS-2 overexpression retards the progression of β-cell loss, although insulin deficiency is ultimately not prevented (Norquay et al. 2009).

Synthesis and function of IRS proteins are subject to tight regulation (Takamoto et al. 2008). While IRS-1 is more stably expressed in β-cells, IRS-2 is highly regulated (Lingohr et al. 2006). Lingohr and coworkers calculated a protein half-life ($T_{1/2}$) of less than 2 h for IRS-2 protein in rat islets and INS-1E cells, while IRS-1 protein remained stable over the 8-h observation time (Lingohr et al. 2006). IRS-2 expression is stimulated by glucose concentrations slightly elevated over normoglycemia (>6 mmol/l). The stimulatory effect of glucose depends on metabolizable glucose and increased cytosolic Ca^{2+} and involves activation of the calcineurin/NFAT (nuclear factor of activated T cells) pathway (Demozay et al. 2011). In addition, IRS-2 transcription is enhanced by GLP-1 through the cAMP-responsive element-binding protein CREB (Jhala et al. 2003). Chronically elevated glucose, however, can accelerate degradation of IRS-2 via activation of mTOR (Briaud et al. 2005). Degradation of IRS-2 is initiated by ubiquitination, a prerequisite for entering the proteasomal pathway (Rui et al. 2001a).

Besides several tyrosine phosphorylation sites, IRS-1 contains multiple serine and threonine phosphorylation sites (Copps and White 2012). Through differential phosphorylation by a variety of kinases, including several protein kinase Cs (PKCs), mammalian target of rapamycin (mTOR), c-Jun N-terminal kinases (JNK), protein kinase A (PKA), and glycogen synthase kinase 3 (GSK-3), the activity of IRS proteins is highly regulated (Schmitz-Peiffer and Whitehead 2003; Pirola et al. 2004; Gual et al. 2005; Liu et al. 2001). These posttranslational modifications change insulin signalling and are mostly studied for IRS-1 in muscle cells and adipocytes, but not in β-cells (Liu et al. 2004; Gual et al. 2003; Weigert et al. 2005).

In muscle cells mutations of IRS-1 at Ser302, Ser307, and Ser612 converted to alanine result in protection against impaired glucose tolerance after high fat feeding (Morino et al. 2008). Additional phosphorylation of Ser318, Ser357, and Ser307 also correlates with attenuation of insulin signalling and insulin resistance in skeletal muscle cells and adipocytes (Gual et al. 2003; Moeschel et al. 2004; Hennige et al. 2006; Werner et al. 2004). Phosphorylation of IRS-1 Ser307 is linked to degradation by mTOR-dependent PP2A activation (Gual et al. 2003; Hartley and Cooper 2002; Jiang et al. 2003; Shah et al. 2004). This phosphorylation site is also a target of cytokine TNF-α, which plays a decisive role in β-cell failure (Kanety et al. 1995; Paz et al. 1997; Rui et al. 2001b; Cantley et al. 2011; Kharroubi et al. 2004). The Ser357

phosphorylation by PKC-delta reduces insulin-dependent tyrosine phosphorylation and attenuates activation of AKT and GSK-3 in muscle cells (Waraich et al. 2008). In insulin-secreting cells, fatty acid-induced β-cell death depends on activation of PKC-delta and is accompanied by a transient reduction in AKT activation (Hennige et al. 2010). Whether PKC-delta-dependent serine/threonine phosphorylations of IRS-1 (and/or IRS-2) contribute to fatty acid-induced reduction of AKT phosphorylation in β-cells remains speculative.

Besides the negative effects of serine/threonine phosphorylations on IRS-1 protein function, insulin signalling is improved by insulin-, glucose- or amino acid-stimulated phosphorylation of IRS-1 at Ser302 and Ser789, mediated by mTOR and AMPK, respectively (Giraud et al. 2004; Jakobsen et al. 2001).

In analogy to IRS-1, IRS-2 serine and threonine phosphorylations modify its stability and function. From 24 serine and threonine phosphorylation sites in IRS-2, Ser573 is identified as a binding site of 14-3-3 proteins, which are cellular binding proteins that regulate intracellular signalling pathways. Phosphorylation of Ser573 in IRS-2 triggers IRS-2 binding to 14-3-3 and this inhibits insulin signalling (Neukamm et al. 2012). IRS-2 function is also compromised by phosphorylation of Ser907, which prevents insulin-stimulated tyrosine phosphorylation of adjacent Tyr911 of IRS-2 (Fritsche et al. 2011). In addition, phosphorylation at Ser675 by mTOR accelerates degradation of IRS-2 (Fritsche et al. 2011).

Both IR and IGF-1R stimulation induce tyrosine phosphorylation of IRS-1 and IRS-2 in insulin-secreting cells. In β-cells the regulation of IRS-1 and IRS-2 by serine and threonine phosphorylations and the role this plays are largely unknown. Future studies are required to understand the mechanisms underlying differential activation of these signalling pathways.

The Downstream Pathways PI3K-PDK1-AKT Regulate Secretion, Proliferation, and Differentiation

Insulin and IGF-1 stimulate the PI3K-PDK1-AKT pathways. Insulin-secreting cells express class I and II isoforms of PI3K (Pigeau et al. 2009; Dominguez et al. 2011). Inhibition of PI3K by isoform-nonselective PI3K inhibitors, wortmannin and LY294002, results in the potentiation of glucose-induced insulin secretion (Eto et al. 2002; Zawalich and Zawalich 2000; Hagiwara et al. 1995). This effect is mimicked by acute inhibition of the class IA PI3K-PDK1-AKT signalling pathway and involves recruitment of new granules to the plasma membrane (Aoyagi et al. 2012). This observation is in agreement with the inhibitory effect of insulin on glucose-induced insulin release and, furthermore, implies that AKT inhibits the mobility of secretory granules towards the plasma membrane. In contrast to this acute effect of PI3K inhibition, chronic inhibition or ablation of PI3K reduces insulin secretion; this effect is mediated by the class IB and II PI3K isoforms (MacDonald et al. 2004; Leibiger et al. 2010). Impaired insulin synthesis could be one explanation for this reduction in insulin secretion after chronic inhibition of PI3K.

Stimulation of AKT, the downstream target of PI3K, is decisive for β-cell survival and proliferation (Elghazi and Bernal-Mizrachi 2009; Elghazi et al. 2007). Insulin-secreting cells express all three AKT (AKT1, AKT2, AKT3, also known as protein kinase B) isoforms, and these isoforms are functionally interchangeable (Kaiser et al. 2013). Constitutively active AKT protects against cell death, while a kinase-dead AKT promotes apoptosis of Min6 cells (Srinivasan et al. 2002). One prominent substrate of AKT is the proapoptotic Bcl-2 protein BAD. Phosphorylated BAD is bound to 14-3-3 protein and resides in the cytosol. Inhibition of AKT results in dephosphorylation and translocation of BAD to mitochondria where BAD contributes to mitochondrial-dependent apoptosis (Datta et al. 1997). A mouse model expressing a kinase-dead mutant of AKT in β-cells displays impaired insulin secretion, but unexpectedly no significant changes in β-cell mass are observed (Bernal-Mizrachi et al. 2004). The reduced AKT activity is mirrored by reduced phosphorylation of GSK3, p70S6K, and FOXO1, which, however, is not sufficient to induce apoptosis, but does impair Ca^{2+} signalling (Fig. 4). In accordance, transgenic mice expressing constitutively active AKT secrete more insulin, which results in improved glucose tolerance.

The excess AKT activity in these mice was tumorigenic, an effect that is blunted by S6K1 deletion (Alliouachene et al. 2008). The underlying mechanism involves cell cycle progression due to activation of *cyclin-dependent kinase 4* (*CDK*4), cyclin D1, and cyclin D2 (Fatrai et al. 2006). Proliferation depends not only on S6K1, since overexpression of S6K does not induce uncontrolled cell growth and β-cell mass remains normal due to a concomitant increase in proliferation and apoptosis. Increased S6K activity is paralleled by downregulation of IRS-2. Nevertheless, insulin secretion and glucose tolerance are improved in these mice (Elghazi et al. 2010).

AKT regulates master-transcription factors of β-cells: PDX-1 and the forkhead box protein O1 (FOXO1). FOXO1 regulates genes involved in survival and cell death (Buteau and Accili 2007; Kobayashi et al. 2012). Phosphorylation of FOXO1 by AKT is accompanied by cytosolic accumulation of the transcription factor, while PDX-1 is preferentially localized to nuclei in β-cells (Kitamura et al. 2002, 2005; Kitamura and Ido 2007; Martinez et al. 2006). Under stress situations and reduced AKT activity, FOXO1 translocates to nuclei, which in parallel triggers nuclear extrusion of PDX-1 (Kawamori et al. 2006). Additional transcription factors regulating β-cell differentiation, such as MafA and NeuroD/β2, transmit the favorable effects of FOXO1 on β-cell function (Kitamura et al. 2005). In particular, transcription of insulin and the glucose sensors, glut-2 and glucokinase, are regulated by these factors (Wang et al. 2007).

Activation of FOXO1 and its nuclear localization is related to the antiproliferative and proapoptotic action of this transcription factor (Hennige et al. 2010; Okamoto et al. 2006). Although nuclear accumulation of FOXO1 correlates with an increase in IRS-2 mRNA, in rodent islets and INS-1E cells, the counter-regulatory effect of an increase in IRS-2 expression upon AKT inhibition is mediated by FOXO3a and involves JNK3, whereas FOXO1 has only a minor effect (Tsunekawa et al. 2011). The switch of FOXO1 from an anti- to

proapoptotic function in β-cells may involve deacetylation by SIRT-1 and phosphorylation by JNK (Kawamori et al. 2006; Hughes et al. 2011). One proapoptotic gene induced by FOXO1 after inhibition of AKT is the Bcl-2 protein BIM (Kaiser et al. 2013). Cell death induced by chronic exposure to fatty acids depends on the stimulation of JNK, but inhibition of this stress kinase does not antagonize nuclear accumulation of FOXO1 (Hennige et al. 2010). Rather, the nuclear accumulation of FOXO1 depends on PKC-delta activity (Hennige et al. 2010). These examples, among numerous other effects of PI3K-PDK1-AKT signalling, demonstrate the importance of these pathways on β-cell function.

Stimulation of MAPK by Insulin and IGF-1 Receptor Signalling

Mitogen-activated protein kinases (MAPK) comprise three groups: the extracellular signal-related kinases (ERK1 and ERK2) and the p38-MAPK and c-Jun-N-terminal kinases (JNK). The ERK1/ERK2 kinases are regulated by a variety of growth receptors, including IGF-1R, as well as by metabolites, such as glucose and fatty acids, and are the ones discussed here.

The ERK1/ERK2 pathway primarily regulates cell proliferation and cell cycle progression (Heit et al. 2006). The effect of glucose, which is in part transmitted by insulin, includes the activation of serine/threonine protein kinase RAF-1 and depends on increased cytosolic Ca^{2+} and activation of protein kinase A (PKA) (Briaud et al. 2003; Alejandro et al. 2010). It remains unclear whether both IR and IGF-1R transmit ERK1/ERK2 activation and which of the IRS proteins is involved (Rhodes 2000). Retrograde regulation of IR signalling was recently deciphered where ERK1/ERK2 phosphorylated IRS-2 at Ser675, and this phosphorylation accelerated degradation of IRS-2 (Fritsche et al. 2011 and The Downstream Pathways PI3K-PDK1-AKT Regulate Secretion, Proliferation, and Differentiation above in this chapter). Whether ERK1/ERK2 interferes with IR signalling in insulin-secreting cells through such a mechanism remains to be determined.

The function of ERK1/ERK2 remains rather controversial. Under some circumstances, ERK1/ERK2 activation associates with apoptosis, whereas other observations suggest a proliferative and antiapoptotic effect. The apparently opposing conclusions probably reflect the fact that proliferating rather than quiescent cells enter apoptotic pathways (Alenzi 2004). In rat islets ERK1/ERK2 seems to exert proapoptotic effects since the MEK1/MEK2-inhibitor PD98059 prevents apoptotic cell death (Fei et al. 2008; Pavlovic et al. 2000). In proliferating insulin-secreting cell lines, inhibiting ERK1/ERK2 by PD98059 does not prevent apoptosis but reduces proliferation of RIN cells (Hennige et al. 2002) and, more intriguingly, counteracts the antiapoptotic effect of IGF-1 in INS-1E cells (Avram et al. 2008). ERK1/ERK2 regulates cell cycle progression through activating transcription factors c-fos and myc, which induce cyclin D1 (Fig. 5; Chambard et al. 2007). Thus, AKT and ERK1/ERK2 stimulation have redundant effects on cell cycle progression.

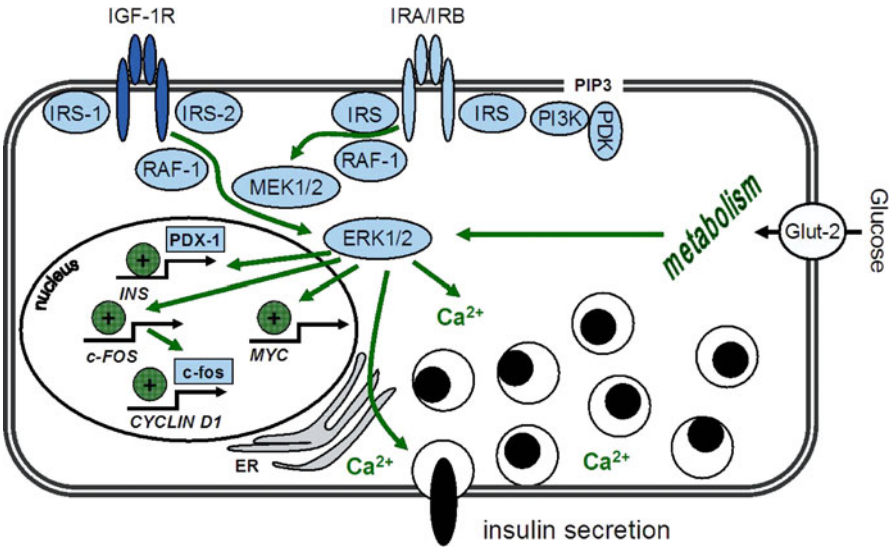

Fig. 5 Stimulation of early genes by ERK1/ERK2. IR and IGF-1R activate the protein kinase RAF-1 which further leads to the stimulation of the mitogen-activated kinase (*MAPK*) pathway. ERK1/ERK2 stimulation induces early genes such as c-fos and myc and activates cell cycle proteins, cyclin D1. ERK1/ERK2 also interferes with the function of the endoplasmic reticulum (*ER*) and is especially involved in ER stress

ERK1/ERK2 relates to insulin secretion through regulating insulin gene activity (Benes et al. 1999). In this context, ERK1/ERK2 supports rather than disturbs insulin secretion. In Min6 cells and rat islets, ERK1/ERK2 interacts with synapsin1, and inhibition of ERK1/ERK2 activity reduces glucose-induced insulin secretion (Longuet et al. 2005). In agreement, reduced ERK1/ERK2 activity associates with lowered insulin secretion (Watson et al. 2011). In contrast, in INS-1 cells, inhibition of ERK1/ERK2 activity has no acute effect on glucose-induced insulin secretion (Khoo and Cobb 1997). It remains unexplained why the effect of ERK1/ERK2 inhibition on insulin secretion does not always become apparent. It is likely that activation of ERK1/ERK2 positively affect insulin secretion by improving insulin gene transcription, which increases the amount of stored insulin and by this the secretory capacity of β-cells.

Alterations in Insulin Signalling During β-Cell Failure and the Development of Type 2 Diabetes Mellitus

Increased β-cell apoptosis and reduced β-cells mass have been observed in patients with type 2 diabetes (Folli et al. 2011; Kloppel et al. 1985; Butler et al. 2003; Yoon et al. 2003). In the study by Folli and coworkers, residual β-cells as well as α-cells

expressed the proliferating cell nuclear antigen (PCNA), a proliferation marker indicative for adaptive β-cell growth in response to peripheral insulin resistance (Folli et al. 2011). It should be noted that the rate of proliferation and apoptosis of adult human β-cells is much less pronounced than that of mouse and rat islet cells and cell lines, which were used for the majority of studies discussed so far. The capacity of human β-cells to proliferate is largely age dependent and, concomitantly, apoptosis is more pronounced in proliferating than nonproliferating β-cells (Kohler et al. 2011). Nonetheless, defective insulin signalling may induce β-cell death. Thus, human islets from donors with the common Arg972 polymorphism in insulin receptor substrate-1 display increased apoptotic cell death when compared to human islets from carrier of the wild-type allele (Federici et al. 2001).

Quite likely, defective IR and IGF-1R signalling contribute to β-cell failure in humans. Insulin receptor signalling is indeed impaired in islets of patients with type 2 diabetes (Folli et al. 2011; Hribal et al. 2003; Gunton et al. 2005). Interestingly, hyperglycemia changes the splicing of IR, which reduces expression of IR-A, but increases expression of IR-B (Hribal et al. 2003). In fact, insulin stimulates insulin gene expression through IR-A. By this mechanism hyperglycemia could contribute to insulin deficiency, a major cause for the development of diabetes. Multiple observations are consistent with the conclusion that impaired regulation of insulin gene transcription in respond to altered insulin demand may be decisive for the development of type 2 diabetes mellitus.

Most of the studies above examined changes in expression levels of IR and IGF-1R signalling proteins. Multiple mechanisms are under consideration for affecting proper β-cell function, including oxidative stress induced by chronic hyperglycemia, ER stress provoked by elevated saturated fatty acids, and inflammation responses mediated by cytokines. Numerous actions of these and other stress factors and pathways on IR and IGF-1R signalling have been described and are discussed in other chapters (see also chapters "► Inflammatory Pathways Linked to β Cell Demise in Diabetes", "► Mechanisms of Pancreatic β-Cell Apoptosis in Diabetes and Its Therapies", "► β-Cell Function in Obese-Hyperglycemic Mice [*ob*/*ob* Mice]", "► The β-Cell in Human Type 2 Diabetes", and "► Pancreatic β Cells in Metabolic Syndrome").

Summary

Without doubt, IR and IGF-1R signalling pathways are fundamental for proper β-cell function. Not only cell replication and survival but also differentiation and secretion depend on these pathways. The most prominent function is attributed to IR, IRS-2, AKT1-3, PDX-1, and *INS* gene. IR and IRS-2 are highly regulated and IRS-2 is almost absent in aged β-cells. The knowledge of molecular mechanisms regulating IR and IGF-1R signalling in β-cells will help us to better understand and treat β-cell failure during the development of type 2 diabetes mellitus.

Cross-References

- ▶ Electrophysiology of Islet Cells
- ▶ Inflammatory Pathways Linked to β Cell Demise in Diabetes
- ▶ Mechanisms of Pancreatic ß-Cell Apoptosis in Diabetes and Its Therapies
- ▶ Pancreatic β Cells in Metabolic Syndrome
- ▶ The β-Cell in Human Type 2 Diabetes

References

Accili D (1995) Molecular defects of the insulin receptor gene. Diabetes Metab Rev 11:47–62

Accili D, Drago J, Lee EJ, Johnson MD, Cool MH, Salvatore P, Asico LD, Jose PA, Taylor SI, Westphal H (1996) Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. Nat Genet 12:106–109

Alejandro EU, Kalynyak TB, Taghizadeh F, Gwiazda KS, Rawstron EK, Jacob KJ, Johnson JD (2010) Acute insulin signaling in pancreatic β-cells is mediated by multiple Raf-1 dependent pathways. Endocrinology 151:502–512

Alenzi FQ (2004) Links between apoptosis, proliferation and the cell cycle. Br J Biomed Sci 61:99–102

Alliouachene S, Tuttle RL, Boumard S, Lapointe T, Berissi S, Germain S, Jaubert F, Tosh D, Birnbaum MJ, Pende M (2008) Constitutively active Akt1 expression in mouse pancreas requires S6 kinase 1 for insulinoma formation. J Clin Invest 118:3629–3638

Ammon HP, Reiber C, Verspohl EJ (1991) Indirect evidence for short-loop negative feedback of insulin secretion in the rat. J Endocrinol 128:27–34

Aoyagi K, Ohara-Imaizumi M, Nishiwaki C, Nakamichi Y, Ueki K, Kadowaki T, Nagamatsu S (2012) Acute inhibition of PI3K-PDK1-Akt pathway potentiates insulin secretion through upregulation of newcomer granule fusions in pancreatic β-cells. PLoS One 7(10):e47381

Argoud GM, Schade DS, Eaton RP (1987) Insulin suppresses its own secretion in vivo. Diabetes 36:959–962

Aspinwall CA, Lakey JR, Kennedy RT (1999a) Insulin-stimulated insulin secretion in single pancreatic β cells. J Biol Chem 274:6360–6365

Aspinwall CA, Huang L, Lakey JR, Kennedy RT (1999b) Comparison of amperometric methods for detection of exocytosis from single pancreatic β-cells of different species. Anal Chem 71:5551–5556

Aspinwall CA, Qian WJ, Roper MG, Kulkarni RN, Kahn CR, Kennedy RT (2000) Roles of insulin receptor substrate-1, phosphatidylinositol 3-kinase, and release of intracellular Ca^{2+} stores in insulin-stimulated insulin secretion in β -cells. J Biol Chem 275:22331–22338

Avram D, Ranta F, Hennige AM, Berchtold S, Hopp S, Häring HU, Lang F, Ullrich S (2008) IGF-1 protects against dexamethasone-induced cell death in insulin secreting INS-1 cells independent of AKT/PKB phosphorylation. Cell Physiol Biochem 21:455–462

Benes C, Poitout V, Marie JC, Martin-Perez J, Roisin MP, Fagard R (1999) Mode of regulation of the extracellular signal-regulated kinases in the pancreatic β-cell line MIN6 and their implication in the regulation of insulin gene transcription. Biochem J 340:219–225

Bennett RG, Hamel FG, Duckworth WC (2003) An insulin-degrading enzyme inhibitor decreases amylin degradation, increases amylin-induced cytotoxicity, and increases amyloid formation in insulinoma cell cultures. Diabetes 52:2315–2320

Bernal-Mizrachi E, Fatrai S, Johnson JD, Ohsugi M, Otani K, Han Z, Polonsky KS, Permutt MA (2004) Defective insulin secretion and increased susceptibility to experimental diabetes are induced by reduced Akt activity in pancreatic islet β cells. J Clin Invest 114:928–936

Borge PD Jr, Wolf BA (2003) Insulin receptor substrate 1 regulation of sarco-endoplasmic reticulum calcium ATPase 3 in insulin-secreting β-cells. J Biol Chem 278:11359–11368

Bouche C, Lopez X, Fleischman A, Cypess AM, O'Shea S, Stefanovski D, Bergman RN, Rogatsky E, Stein DT, Kahn CR, Kulkarni RN, Goldfine AB (2010) Insulin enhances glucose-stimulated insulin secretion in healthy humans. Proc Natl Acad Sci USA 107:4770–4775

Braun M, Ramracheya R, Johnson PR, Rorsman P (2009) Exocytotic properties of human pancreatic β-cells. Ann N Y Acad Sci 1152:187–193

Briaud I, Lingohr MK, Dickson LM, Wrede CE, Rhodes CJ (2003) Differential activation mechanisms of Erk-1/2 and p70(S6K) by glucose in pancreatic β-cells. Diabetes 52: 974–983

Briaud I, Dickson LM, Lingohr MK, McCuaig JF, Lawrence JC, Rhodes CJ (2005) Insulin receptor substrate-2 proteasomal degradation mediated by a mammalian target of rapamycin (mTOR)-induced negative feedback down-regulates protein kinase B-mediated signaling pathway in β-cells. J Biol Chem 280:2282–2293

Brüning JC, Winnay J, Bonner-Weir S, Taylor SI, Accili D, Kahn CR (1997) Development of a novel polygenic model of NIDDM in mice heterozygous for IR and IRS-1 null alleles. Cell 88:561–572

Buteau J, Accili D (2007) Regulation of pancreatic β-cell function by the forkhead protein FoxO1. Diabetes Obes Metab 9(Suppl 2):140–146

Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC (2003) β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 52:102–110

Cantley J, Choudhury AI, Asare-Anane H, Selman C, Lingard S, Heffron H, Herrera P, Persaud SJ, Withers DJ (2007) Pancreatic deletion of insulin receptor substrate 2 reduces β and α cell mass and impairs glucose homeostasis in mice. Diabetologia 50:1248–1256

Cantley J, Boslem E, Laybutt DR, Cordery DV, Pearson G, Carpenter L, Leitges M, Biden TJ (2011) Deletion of protein kinase Cdelta in mice modulates stability of inflammatory genes and protects against cytokine-stimulated β cell death in vitro and in vivo. Diabetologia 54:380–389

Carpentier JL (1994) Insulin receptor internalization: molecular mechanisms and physiopathological implications. Diabetologia 37(Suppl 2):S117–S124

Chambard JC, Lefloch R, Pouyssegur J, Lenormand P (2007) ERK implication in cell cycle regulation. Biochim Biophys Acta 1773:1299–1310

Copps KD, White MF (2012) Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. Diabetologia 55:2565–2582

da Silva X, Varadi A, Ainscow EK, Rutter GA (2000) Regulation of gene expression by glucose in pancreatic β-cells (MIN6) via insulin secretion and activation of phosphatidylinositol 3′-kinase. J Biol Chem 275:36269–36277

da Silva X, Qian Q, Cullen PJ, Rutter GA (2004) Distinct roles for insulin and insulin-like growth factor-1 receptors in pancreatic β-cell glucose sensing revealed by RNA silencing. Biochem J 377:149–158

Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME (1997) Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91:231–241

De Meyts P (1994) The structural basis of insulin and insulin-like growth factor-I receptor binding and negative co-operativity, and its relevance to mitogenic versus metabolic signalling. Diabetologia 37(Suppl 2):S135–S148

Demozay D, Tsunekawa S, Briaud I, Shah R, Rhodes CJ (2011) Specific glucose-induced control of insulin receptor substrate-2 expression is mediated via Ca^{2+}-dependent calcineurin/NFAT signaling in primary pancreatic islet β-cells. Diabetes 60:2892–2902

Dominguez V, Raimondi C, Somanath S, Bugliani M, Loder MK, Edling CE, Divecha N, da Silva-Xavier G, Marselli L, Persaud SJ, Turner MD, Rutter GA, Rutter GA, Falasca M, Maffucci T (2011) Class II phosphoinositide 3-kinase regulates exocytosis of insulin granules in pancreatic β cells. J Biol Chem 286:4216–4225

Düfer M, Haspel D, Krippeit-Drews P, Aguilar-Bryan L, Bryan J, Drews G (2009) Activation of the Na^+/K^+-ATPase by insulin and glucose as a putative negative feedback mechanism in pancreatic β-cells. Pflugers Arch 457:1351–1360

Elahi D, Nagulesparan M, Hershcopf RJ, Müller DC, Tobin JD, Blix PM, Rubenstein AH, Unger RH, Andres R (1982) Feedback inhibition of insulin secretion by insulin: relation to the hyperinsulinemia of obesity. N Engl J Med 306:1196–1202

Elghazi L, Rachdi L, Weiss AJ, Cras-Meneur C, Bernal-Mizrachi E (2007) Regulation of β-cell mass and function by the Akt/protein kinase B signalling pathway. Diabetes Obes Metab 9(Suppl 2):147–157

Elghazi L, Bernal-Mizrachi E (2009) Akt and PTEN: β-cell mass and pancreas plasticity. Trends Endocrinol Metab 20:243–251

Elghazi L, Balcazar N, Blandino-Rosano M, Cras-Meneur C, Fatrai S, Gould AP, Chi MM, Moley KH, Bernal-Mizrachi E (2010) Decreased IRS signaling impairs β-cell cycle progression and survival in transgenic mice overexpressing S6K in β-cells. Diabetes 59:2390–2399

Eto K, Yamashita T, Tsubamoto Y, Terauchi Y, Hirose K, Kubota N, Yamashita S, Taka J, Satoh S, Sekihara H, Tobe K, Iino M, Noda M, Kimura S, Kadowaki T (2002) Phosphatidylinositol 3-kinase suppresses glucose-stimulated insulin secretion by affecting post-cytosolic $[Ca^{2+}]$ elevation signals. Diabetes 51:87–97

Farris W, Mansourian S, Chang Y, Lindsley L, Eckman EA, Frosch MP, Eckman CB, Tanzi RE, Selkoe DJ, Guenette S (2003) Insulin-degrading enzyme regulates the levels of insulin, amyloid β-protein, and the β-amyloid precursor protein intracellular domain in vivo. Proc Natl Acad Sci USA 100:4162–4167

Fatrai S, Elghazi L, Balcazar N, Cras-Meneur C, Krits I, Kiyokawa H, Bernal-Mizrachi E (2006) Akt induces β-cell proliferation by regulating cyclin D1, cyclin D2, and p21 levels and cyclin-dependent kinase-4 activity. Diabetes 55:318–325

Federici M, Hribal ML, Ranalli M, Marselli L, Porzio O, Lauro D, Borboni P, Lauro R, Marchetti P, Melino G, Sesti G (2001) The common Arg972 polymorphism in insulin receptor substrate-1 causes apoptosis of human pancreatic islets. FASEB J 15:22–24

Fei H, Zhao B, Zhao S, Wang Q (2008) Requirements of calcium fluxes and ERK kinase activation for glucose- and interleukin-1β-induced β-cell apoptosis. Mol Cell Biochem 315:75–84

Florez JC, Wiltshire S, Agapakis CM, Burtt NP, de Bakker PI, Almgren P, Bengtsson BK, Tuomi T, Gaudet D, Daly MJ, Hirschhorn JN, McCarthy MI, Altshuler D, Groop L (2006) High-density haplotype structure and association testing of the insulin-degrading enzyme (IDE) gene with type 2 diabetes in 4,206 people. Diabetes 55:128–135

Folli F, Okada T, Perego C, Gunton J, Liew CW, Akiyama M, D'Amico A, La Rosa S, Placidi C, Lupi R, Marchetti P, Sesti G, Hellerstein M, Perego L, Kulkarni RN (2011) Altered insulin receptor signalling and β-cell cycle dynamics in type 2 diabetes mellitus. PLoS ONE 6:e28050

Frerichs H, Reich U, Creutzfeldt W (1965) Insulin secretion in vitro. I. Inhibition of glucose-induced insulin release by insulin. Klin Wochenschr 43:136–140

Freychet P, Roth J, Neville DM Jr (1971) Insulin receptors in the liver: specific binding of (125 I) insulin to the plasma membrane and its relation to insulin bioactivity. Proc Natl Acad Sci USA 68:1833–1837

Fritsche L, Neukamm SS, Lehmann R, Kremmer E, Hennige AM, Hunder-Gugel A, Schenk M, Häring HU, Schleicher ED, Weigert C (2011) Insulin-induced serine phosphorylation of IRS-2 via ERK1/2 and mTOR: studies on the function of Ser675 and Ser907. Am J Physiol Endocrinol Metab 300:E824–E836

German MS, Moss LG, Rutter WJ (1990) Regulation of insulin gene expression by glucose and calcium in transfected primary islet cultures. J Biol Chem 265:22063–22066

Giraud J, Leshan R, Lee YH, White MF (2004) Nutrient-dependent and insulin-stimulated phosphorylation of insulin receptor substrate-1 on serine 302 correlates with increased insulin signaling. J Biol Chem 279:3447–3454

Glebov K, Schutze S, Walter J (2011) Functional relevance of a novel SlyX motif in non-conventional secretion of insulin-degrading enzyme. J Biol Chem 286:22711–22715

Goldfine AB, Kulkarni RN (2012) Modulation of β-cell function: a translational journey from the bench to the bedside. Diabetes Obes Metab 14(Suppl 3):152–160

Gual P, Gremeaux T, Gonzalez T, Marchand-Brustel Y, Tanti JF (2003) MAP kinases and mTOR mediate insulin-induced phosphorylation of insulin receptor substrate-1 on serine residues 307, 612 and 632. Diabetologia 46:1532–1542

Gual P, Marchand-Brustel Y, Tanti JF (2005) Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. Biochimie 87:99–109

Guillen C, Navarro P, Robledo M, Valverde AM, Benito M (2006) Differential mitogenic signaling in insulin receptor-deficient fetal pancreatic β-cells. Endocrinol 147:1959–1968

Gunton JE, Kulkarni RN, Yim S, Okada T, Hawthorne WJ, Tseng YH, Roberson RS, Ricordi C, O'Connell PJ, Gonzalez FJ, Kahn CR (2005) Loss of ARNT/HIF1β mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. Cell 122: 337–349

Hagiwara S, Sakurai T, Tashiro F, Hashimoto Y, Matsuda Y, Nonomura Y, Miyazaki J (1995) An inhibitory role for phosphatidylinositol 3-kinase in insulin secretion from pancreatic β cell line MIN6. Biochem Biophys Res Commun 214:51–59

Harbeck MC, Louie DC, Howland J, Wolf BA, Rothenberg PL (1996) Expression of insulin receptor mRNA and insulin receptor substrate 1 in pancreatic islet β-cells. Diabetes 45:711–717

Häring HU (1991) The insulin receptor: signalling mechanism and contribution to the pathogenesis of insulin resistance. Diabetologia 34:848–861

Hartley D, Cooper GM (2002) Role of mTOR in the degradation of IRS-1: regulation of PP2A activity. J Cell Biochem 85:304–314

Heit JJ, Karnik SK, Kim SK (2006) Intrinsic regulators of pancreatic β-cell proliferation. Annu Rev Cell Dev Biol 22:311–338

Hennige AM, Fritsche A, Strack V, Weigert C, Mischak H, Borboni P, Renn W, Häring HU, Kellerer M (2002) PKC ζ enhances insulin-like growth factor 1-dependent mitogenic activity in the rat clonal β cell line RIN 1046-38. Biochem Biophys Res Commun 290:85–90

Hennige AM, Burks DJ, Ozcan U, Kulkarni RN, Ye J, Park S, Schubert M, Fisher TL, Dow MA, Leshan R, Zakaria M, Mossa-Basha M, White MF (2003) Upregulation of insulin receptor substrate-2 in pancreatic β cells prevents diabetes. J Clin Invest 112:1521–1532

Hennige AM, Ozcan U, Okada T, Jhala US, Schubert M, White MF, Kulkarni RN (2005) Alterations in growth and apoptosis of IRS-1 deficient β-cells. Am J Physiol Endocrinol Metab 289:E337–E346

Hennige AM, Stefan N, Kapp K, Lehmann R, Weigert C, Beck A, Moeschel K, Mushack J, Schleicher E, Häring HU (2006) Leptin down-regulates insulin action through phosphorylation of serine-318 in insulin receptor substrate 1. FASEB J 20:1206–1208

Hennige AM, Ranta F, Heinzelmann I, Düfer M, Michael D, Braumüller H, Lutz SZ, Lammers R, Drews G, Bosch F, Häring HU, Ullrich S (2010) Overexpression of kinase-negative protein kinase Cdelta in pancreatic β-cells protects mice from diet-induced glucose intolerance and β-cell dysfunction. Diabetes 59:119–127

Higashi Y, Sukhanov S, Parthasarathy S, Delafontaine P (2008) The ubiquitin ligase Nedd4 mediates oxidized low-density lipoprotein-induced downregulation of insulin-like growth factor-1 receptor. Am J Physiol Heart Circ Physiol 295:H1684–H1689

Hou N, Mogami H, Kubota-Murata C, Sun M, Takeuchi T, Torii S (2012) Preferential release of newly synthesized insulin assessed by a multi-label reporter system using pancreatic β-cell line MIN6. PLoS ONE 7:e47921

Hribal ML, Perego L, Lovari S, Andreozzi F, Menghini R, Perego C, Finzi G, Usellini L, Placidi C, Capella C, Guzzi V, Lauro D, Bertuzzi F, Davalli A, Pozza G, Pontiroli A, Federici M, Lauro R, Brunetti A, Folli F, Sesti G (2003) Chronic hyperglycemia impairs insulin secretion by affecting insulin receptor expression, splicing, and signaling in RIN β cell line and human islets of Langerhans. FASEB J 17:1340–1342

Hubbard SR (1997) Crystal structure of the activated insulin receptor tyrosine kinase in complex with peptide substrate and ATP analog. EMBO J 16:5572–5581

Hughes KJ, Meares GP, Hansen PA, Corbett JA (2011) FoxO1 and SIRT1 regulate β-cell responses to nitric oxide. J Biol Chem 286:8338–8348

Iversen J, Miles DW (1971) Evidence for a feedback inhibition of insulin on insulin secretion in the isolated, perfused canine pancreas. Diabetes 20:1–9

Iype T, Francis J, Garmey JC, Schisler JC, Nesher R, Weir GC, Becker TC, Newgard CB, Griffen SC, Mirmira RG (2005) Mechanism of insulin gene regulation by the pancreatic transcription factor Pdx-1: application of pre-mRNA analysis and chromatin immunoprecipitation to assess formation of functional transcriptional complexes. J Biol Chem 280:16798–16807

Jakobsen SN, Hardie DG, Morrice N, Tornqvist HE (2001) 5′-AMP-activated protein kinase phosphorylates IRS-1 on Ser-789 in mouse C2C12 myotubes in response to 5-aminoimidazole-4-carboxamide riboside. J Biol Chem 276:46912–46916

Jhala US, Canettieri G, Screaton RA, Kulkarni RN, Krajewski S, Reed J, Walker J, Lin X, White M, Montminy M (2003) cAMP promotes pancreatic β-cell survival via CREB-mediated induction of IRS2. Genes Dev 17:1575–1580

Jiang G, Dallas-Yang Q, Liu F, Moller DE, Zhang BB (2003) Salicylic acid reverses phorbol 12-myristate-13-acetate (PMA)- and tumor necrosis factor α (TNFα)-induced insulin receptor substrate 1 (IRS1) serine 307 phosphorylation and insulin resistance in human embryonic kidney 293 (HEK293) cells. J Biol Chem 278:180–186

Kaiser G, Gerst F, Michael D, Berchtold S, Friedrich B, Strutz-Seebohm N, Lang F, Häring HU, Ullrich S (2013) Regulation of forkhead box O1 (FOXO1) by protein kinase B and glucocorticoids: different mechanisms of induction of β cell death in vitro. Diabetologia 56:1587–1595

Kaneto H, Miyatsuka T, Kawamori D, Yamamoto K, Kato K, Shiraiwa T, Katakami N, Yamasaki Y, Matsuhisa M, Matsuoka TA (2008) PDX-1 and MafA play a crucial role in pancreatic β-cell differentiation and maintenance of mature β-cell function. Endocr J 55:235–252

Kanety H, Feinstein R, Papa MZ, Hemi R, Karasik A (1995) Tumor necrosis factor α-induced phosphorylation of insulin receptor substrate-1 (IRS-1). Possible mechanism for suppression of insulin-stimulated tyrosine phosphorylation of IRS-1. J Biol Chem 270:23780–23784

Kang G, Leech CA, Chepurny OG, Coetzee WA, Holz GG (2008) Role of the cAMP sensor Epac as a determinant of K_{ATP} channel ATP sensitivity in human pancreatic β-cells and rat INS-1 cells. J Physiol 586:1307–1319

Kawamori D, Kaneto H, Nakatani Y, Matsuoka TA, Matsuhisa M, Hori M, Yamasaki Y (2006) The forkhead transcription factor Foxo1 bridges the JNK pathway and the transcription factor PDX-1 through its intracellular translocation. J Biol Chem 281:1091–1098

Khan FA, Goforth PB, Zhang M, Satin LS (2001) Insulin activates ATP-sensitive K^+ channels in pancreatic β-cells through a phosphatidylinositol 3-kinase-dependent pathway. Diabetes 50:2192–2198

Kharroubi I, Ladriere L, Cardozo AK, Dogusan Z, Cnop M, Eizirik DL (2004) Free fatty acids and cytokines induce pancreatic β-cell apoptosis by different mechanisms: role of nuclear factor-kappaB and endoplasmic reticulum stress. Endocrinology 145:5087–5096

Khoo S, Cobb MH (1997) Activation of mitogen-activating protein kinase by glucose is not required for insulin secretion. Proc Natl Acad Sci USA 94:5599–5604

Kishi K, Mawatari K, Sakai-Wakamatsu K, Yuasa T, Wang M, Ogura-Sawa M, Nakaya Y, Hatakeyama S, Ebina Y (2007) APS-mediated ubiquitination of the insulin receptor enhances its internalization, but does not induce its degradation. Endocr J 54:77–88

Kitamura T, Nakae J, Kitamura Y, Kido Y, Biggs WH III, Wright CV, White MF, Arden KC, Accili D (2002) The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic β cell growth. J Clin Invest 110:1839–1847

Kitamura YI, Kitamura T, Kruse JP, Raum JC, Stein R, Gu W, Accili D (2005) FoxO1 protects against pancreatic β cell failure through NeuroD and MafA induction. Cell Metab 2:153–163

Kitamura T, Ido KY (2007) Role of FoxO Proteins in Pancreatic β Cells. Endocr J 54:507–515

Kloppel G, Lohr M, Habich K, Oberholzer M, Heitz PU (1985) Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. Surv Synth Pathol Res 4:110–125

Kobayashi M, Kikuchi O, Sasaki T, Kim HJ, Yokota-Hashimoto H, Lee YS, Amano K, Kitazumi T, Susanti VY, Kitamura YI, Kitamura T (2012) FoxO1 as a double-edged sword in the pancreas: analysis of pancreas- and β-cell-specific FoxO1 knockout mice. Am J Physiol Endocrinol Metab 302:E603–E613

Kohler CU, Olewinski M, Tannapfel A, Schmidt WE, Fritsch H, Meier JJ (2011) Cell cycle control of β-cell replication in the prenatal and postnatal human pancreas. Am J Physiol Endocrinol Metab 300:E221–E230

Kraegen EW, Lazarus L, Campbell LV (1983) Failure of insulin infusion during euglycemia to influence endogenous basal insulin secretion. Metabolism 32:622–627

Krupp MN, Lane MD (1982) Evidence for different pathways for the degradation of insulin and insulin receptor in the chick liver cell. J Biol Chem 257:1372–1377

Kubota N, Tobe K, Terauchi Y, Eto K, Yamauchi T, Suzuki R, Tsubamoto Y, Komeda K, Nakano R, Miki H, Satoh S, Sekihara H, Sciacchitano S, Lesniak M, Aizawa S, Nagai R, Kimura S, Akanuma Y, Taylor SI, Kadowaki T (2000) Disruption of insulin receptor substrate 2 causes type 2 diabetes because of liver insulin resistance and lack of compensatory β-cell hyperplasia. Diabetes 49:1880–1889

Kulkarni RN, Brüning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR (1999a) Tissue-specific knockout of the insulin receptor in pancreatic β cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell 96:329–339

Kulkarni RN, Winnay JN, Daniels M, Brüning JC, Flier SN, Hanahan D, Kahn CR (1999b) Altered function of insulin receptor substrate-1-deficient mouse islets and cultured β-cell lines. J Clin Invest 104:R69–R75

Kulkarni RN, Holzenberger M, Shih DQ, Ozcan U, Stoffel M, Magnuson MA, Kahn CR (2002) β-cell-specific deletion of the IGF1 receptor leads to hyperinsulinemia and glucose intolerance but does not alter β-cell mass. Nat Genet 31:111–115

Kwak YD, Wang B, Li JJ, Wang R, Deng Q, Diao S, Chen Y, Xu R, Masliah E, Xu H, Sung JJ, Liao FF (2012) Upregulation of the E3 ligase NEDD4-1 by oxidative stress degrades IGF-1 receptor protein in neurodegeneration. J Neurosci 32:10971–10981

Leibiger IB, Leibiger B, Moede T, Berggren PO (1998) Exocytosis of insulin promotes insulin gene transcription via the insulin receptor/PI-3 kinase/p70 s6 kinase and CaM kinase pathways. Mol Cell 1:933–938

Leibiger B, Wahlander K, Berggren PO, Leibiger IB (2000) Glucose-stimulated insulin biosynthesis depends on insulin-stimulated insulin gene transcription. J Biol Chem 275:30153–30156

Leibiger B, Leibiger IB, Moede T, Kemper S, Kulkarni RN, Kahn CR, de Vargas LM, Berggren PO (2001) Selective insulin signaling through A and B insulin receptors regulates transcription of insulin and glucokinase genes in pancreatic β cells. Mol Cell 7:559–570

Leibiger IB, Leibiger B, Berggren PO (2008) Insulin signaling in the pancreatic β-cell. Annu Rev Nutr 28:233–251

Leibiger B, Moede T, Uhles S, Barker CJ, Creveaux M, Domin J, Berggren PO, Leibiger IB (2010) Insulin-feedback via PI3K-C2α activated PKBα/Akt1 is required for glucose-stimulated insulin secretion. FASEB J 24:1824–1837

LeRoith D, Accili D (2008) Mechanisms of disease: using genetically altered mice to study concepts of type 2 diabetes. Nat Clin Pract Endocrinol Metab 4:164–172

Lingohr MK, Briaud I, Dickson LM, McCuaig JF, Alarcon C, Wicksteed BL, Rhodes CJ (2006) Specific regulation of IRS-2 expression by glucose in rat primary pancreatic islet β -cells. J Biol Chem 281:15884–15892

Liu YF, Paz K, Herschkovitz A, Alt A, Tennenbaum T, Sampson SR, Ohba M, Kuroki T, LeRoith D, Zick Y (2001) Insulin stimulates PKCζ -mediated phosphorylation of insulin receptor substrate-1 (IRS-1). A self-attenuated mechanism to negatively regulate the function of IRS proteins. J Biol Chem 276:14459–14465

Liu YF, Herschkovitz A, Boura-Halfon S, Ronen D, Paz K, LeRoith D, Zick Y (2004) Serine phosphorylation proximal to its phosphotyrosine binding domain inhibits insulin receptor substrate 1 function and promotes insulin resistance. Mol Cell Biol 24:9668–9681

Longuet C, Broca C, Costes S, Hani EH, Bataille D, Dalle S (2005) Extracellularly regulated kinases 1/2 (p44/42 mitogen-activated protein kinases) phosphorylate synapsin I and regulate insulin secretion in the MIN6 β-cell line and islets of Langerhans. Endocrinol 146:643–654

Lopez-Avalos MD, Duvivier-Kali VF, Xu G, Bonner-Weir S, Sharma A, Weir GC (2006) Evidence for a role of the ubiquitin-proteasome pathway in pancreatic islets. Diabetes 55:1223–1231

Luo RZ, Beniac DR, Fernandes A, Yip CC, Ottensmeyer FP (1999) Quaternary structure of the insulin-insulin receptor complex. Science 285:1077–1080

MacDonald PE, Joseph JW, Yau D, Diao J, Asghar Z, Dai F, Oudit GY, Patel MM, Backx PH, Wheeler MB (2004) Impaired glucose-stimulated insulin secretion, enhanced IP insulin tolerance and increased β-cell mass in mice lacking the p110gamma isoform of PI3-kinase. Endocrinol 145:4078–4083

MacFarlane WM, Read ML, Gilligan M, Bujalska I, Docherty K (1994) Glucose modulates the binding activity of the β-cell transcription factor IUF1 in a phosphorylation-dependent manner. Biochem J 303:625–631

MacFarlane WM, Smith SB, James RF, Clifton AD, Doza YN, Cohen P, Docherty K (1997) The p38/reactivating kinase mitogen-activated protein kinase cascade mediates the activation of the transcription factor insulin upstream factor 1 and insulin gene transcription by high glucose in pancreatic β-cells. J Biol Chem 272:20936–20944

MacFarlane WM, McKinnon CM, Felton-Edkins ZA, Cragg H, James RF, Docherty K (1999) Glucose stimulates translocation of the homeodomain transcription factor PDX1 from the cytoplasm to the nucleus in pancreatic β-cells. J Biol Chem 274:1011–1016

Malaisse WJ, Malaisse-Lagae F, Lacy PE, Wright PH (1967) Insulin secretion by isolated islets in presence of glucose, insulin and anti-insulin serum. Proc Soc Exp Biol Med 124:497–500

Marshak S, Totary H, Cerasi E, Melloul D (1996) Purification of the β-cell glucose-sensitive factor that transactivates the insulin gene differentially in normal and transformed islet cells. Proc Natl Acad Sci USA 93:15057–15062

Martinez SC, Cras-Meneur C, Bernal-Mizrachi E, Permutt MA (2006) Glucose regulates Foxo1 through insulin receptor signaling in the pancreatic islet β-cell. Diabetes 55:1581–1591

Massague J, Pilch PF, Czech MP (1980) Electrophoretic resolution of three major insulin receptor structures with unique subunit stoichiometries. Proc Natl Acad Sci USA 77:7137–7141

Melloul D, Marshak S, Cerasi E (2002) Regulation of insulin gene transcription. Diabetologia 45:309–326

Melloul D (2004) Transcription factors in islet development and physiology: role of PDX-1 in β-cell function. Ann N Y Acad Sci 1014:28–37

Menting JG, Whittaker J, Margetts MB, Whittaker LJ, Kong GK, Smith BJ, Watson CJ, Zakova L, Kletvikova E, Jiracek J, Chan SJ, Steiner DF, Dodson GG, Brzozowski AM, Weiss MA, Ward CW, Lawrence MC (2013) How insulin engages its primary binding site on the insulin receptor. Nature 493:241–245

Moeschel K, Beck A, Weigert C, Lammers R, Kalbacher H, Voelter W, Schleicher ED, Häring HU, Lehmann R (2004) Protein kinase C-ζ-induced phosphorylation of Ser318 in insulin receptor substrate-1 (IRS-1) attenuates the interaction with the insulin receptor and the tyrosine phosphorylation of IRS-1. J Biol Chem 279:25157–25163

Monami G, Emiliozzi V, Morrione A (2008) Grb10/Nedd4-mediated multiubiquitination of the insulin-like growth factor receptor regulates receptor internalization. J Cell Physiol 216:426–437

Morino K, Neschen S, Bilz S, Sono S, Tsirigotis D, Reznick RM, Moore I, Nagai Y, Samuel V, Sebastian D, White M, Philbrick W, Shulman GI (2008) Muscle-specific IRS-1 Ser- > Ala transgenic mice are protected from fat-induced insulin resistance in skeletal muscle. Diabetes 57:2644–2651

Neukamm SS, Toth R, Morrice N, Campbell DG, Mackintosh C, Lehmann R, Häring HU, Schleicher ED, Weigert C (2012) Identification of the amino acids 300–600 of IRS-2 as 14-3-3 binding region with the importance of IGF-1/insulin-regulated phosphorylation of Ser-573. PLoS ONE 7:e43296

Norquay LD, D'Aquino KE, Opare-Addo LM, Kuznetsova A, Haas M, Bluestone JA, White MF (2009) Insulin receptor substrate-2 in β-cells decreases diabetes in nonobese diabetic mice. Endocrinology 150:4531–4540

Okada T, Liew CW, Hu J, Hinault C, Michael MD, Kürtzfeldt J, Yin C, Holzenberger M, Stoffel M, Kulkarni RN (2007) Insulin receptors in β-cells are critical for islet compensatory growth response to insulin resistance. Proc Natl Acad Sci USA 104:8977–8982

Okamoto H, Hribal ML, Lin HV, Bennett WR, Ward A, Accili D (2006) Role of the forkhead protein FoxO1 in β cell compensation to insulin resistance. J Clin Invest 116: 775–782

O'Malley D, Shanley LJ, Harvey J (2003) Insulin inhibits rat hippocampal neurones via activation of ATP-sensitive K^+ and large conductance Ca^{2+}-activated K^+ channels. Neuropharmacology 44:855–863

Parnaud G, Bosco D, Berney T, Pattou F, Kerr-Conte J, Donath MY, Bruun C, Mandrup-Poulsen T, Billestrup N, Halban PA (2008) Proliferation of sorted human and rat β cells. Diabetologia 51:91–100

Pavlovic D, Andersen NA, Mandrup-Poulsen T, Eizirik DL (2000) Activation of extracellular signal-regulated kinase (ERK)1/2 contributes to cytokine-induced apoptosis in purified rat pancreatic β-cells. Eur Cytokine Netw 11:267–274

Paz K, Hemi R, LeRoith D, Karasik A, Elhanany E, Kanety H, Zick Y (1997) A molecular basis for insulin resistance. Elevated serine/threonine phosphorylation of IRS-1 and IRS-2 inhibits their binding to the juxtamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation. J Biol Chem 272:29911–29918

Pigeau GM, Kolic J, Ball BJ, Hoppa MB, Wang YW, Rückle T, Woo M, Manning Fox JE, MacDonald PE (2009) Insulin granule recruitment and exocytosis is dependent on p110gamma in insulinoma and human β-cells. Diabetes 58:2084–2092

Pirola L, Johnston AM, Van Obberghen E (2004) Modulation of insulin action. Diabetologia 47:170–184

Rhodes CJ, Halban PA (1987) Newly synthesized proinsulin/insulin and stored insulin are released from pancreatic B cells predominantly via a regulated, rather than a constitutive, pathway. J Cell Biol 105:145–153

Rhodes CJ (2000) IGF-I and GH post-receptor signaling mechanisms for pancreatic β-cell replication. J Mol Endocrinol 24:303–311

Rhodes CJ (2005) Type 2 diabetes-a matter of β-cell life and death? Science 307:380–384

Roper MG, Qian WJ, Zhang BB, Kulkarni RN, Kahn CR, Kennedy RT (2002) Effect of the insulin mimetic L-783,281 on intracellular Ca^{2+} and insulin secretion from pancreatic β-cells. Diabetes 51(Suppl 1):S43–S49

Rui L, Fisher TL, Thomas J, White MF (2001a) Regulation of insulin/insulin-like growth factor-1 signaling by proteasome-mediated degradation of insulin receptor substrate-2. J Biol Chem 276:40362–40367

Rui L, Aguirre V, Kim JK, Shulman GI, Lee A, Corbould A, Dunaif A, White MF (2001b) Insulin/IGF-1 and TNF-α stimulate phosphorylation of IRS-1 at inhibitory Ser307 via distinct pathways. J Clin Invest 107:181–189

Samani AA, Yakar S, LeRoith D, Brodt P (2007) The role of the IGF system in cancer growth and metastasis: overview and recent insights. Endocr Rev 28:20–47

Schmitz-Peiffer C, Whitehead JP (2003) IRS-1 regulation in health and disease. IUBMB Life 55:367–374

Seino S, Bell GI (1989) Alternative splicing of human insulin receptor messenger RNA. Biochem Biophys Res Commun 159:312–316

Shah OJ, Wang Z, Hunter T (2004) Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. Curr Biol 14:1650–1656

Shibasaki T, Sunaga Y, Fujimoto K, Kashima Y, Seino S (2004) Interaction of ATP sensor, cAMP sensor, Ca^{2+} sensor, and voltage-dependent Ca^{2+} channel in insulin granule exocytosis. J Biol Chem 279:7956–7961

Smith BJ, Huang K, Kong G, Chan SJ, Nakagawa S, Menting JG, Hu SQ, Whittaker J, Steiner DF, Katsoyannis PG, Ward CW, Weiss MA, Lawrence MC (2010) Structural resolution of a tandem hormone-binding element in the insulin receptor and its implications for design of peptide agonists. Proc Natl Acad Sci USA 107:6771–6776

Sodoyez JC, Sodoyez-Goffaux F, Foa PP (1969) Evidence for an insulin-induced inhibition of insulin release by isolated islets of Langerhans. Proc Soc Exp Biol Med 130:568–571

Spanswick D, Smith MA, Mirshamsi S, Routh VH, Ashford ML (2000) Insulin activates ATP-sensitive K^+ channels in hypothalamic neurons of lean, but not obese rats. Nat Neurosci 3:757–758

Srinivasan S, Bernal-Mizrachi E, Ohsugi M, Permutt MA (2002) Glucose promotes pancreatic islet β-cell survival through a PI 3-kinase/Akt-signaling pathway. Am J Physiol Endocrinol Metab 283:E784–E793

Steneberg P, Bernardo L, Edfalk S, Backlund F, Lundberg L, Stenson CG, Edlund H (2013) The type 2 diabetes associated gene Ide is required for insulin secretion and suppression of α-synuclein levels in β-cells. Diabetes 62:2004–2014

Takamoto I, Terauchi Y, Kubota N, Ohsugi M, Ueki K, Kadowaki T (2008) Crucial role of insulin receptor substrate-2 in compensatory β-cell hyperplasia in response to high fat diet-induced insulin resistance. Diabetes Obes Metab 10(Suppl 4):147–156

Talchai C, Lin HV, Kitamura T, Accili D (2009) Genetic and biochemical pathways of β-cell failure in type 2 diabetes. Diabetes Obes Metab 11(Suppl 4):38–45

Taylor SI (1992) Lilly lecture: molecular mechanisms of insulin resistance. Lessons from patients with mutations in the insulin-receptor gene. Diabetes 41:1473–1490

Tsunekawa S, Demozay D, Briaud I, McCuaig J, Accili D, Stein R, Rhodes CJ (2011) FoxO feedback control of basal IRS-2 expression in pancreatic β-cells is distinct from that in hepatocytes. Diabetes 60:2883–2891

Uhles S, Moede T, Leibiger B, Berggren PO, Leibiger IB (2003) Isoform-specific insulin receptor signaling involves different plasma membrane domains. J Cell Biol 163:1327–1337

Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli LM, Dull TJ, Gray A, Coussens L, Liao YC, Tsubokawa M (1985) Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. Nature 313:756–761

van Haeften TW, Twickler TB (2004) Insulin-like growth factors and pancreas β cells. Eur J Clin Invest 34:249–255

Van Schravendijk CF, Foriers A, Van den Brande JL, Pipeleers DG (1987) Evidence for the presence of type I insulin-like growth factor receptors on rat pancreatic A and B cells. Endocrinol 121:1784–1788

Vecchione A, Marchese A, Henry P, Rotin D, Morrione A (2003) The Grb10/Nedd4 complex regulates ligand-induced ubiquitination and stability of the insulin-like growth factor I receptor. Mol Cell Biol 23:3363–3372

Velloso LA, Carneiro EM, Crepaldi SC, Boschero AC, Saad MJ (1995) Glucose- and insulin-induced phosphorylation of the insulin receptor and its primary substrates IRS-1 and IRS-2 in rat pancreatic islets. FEBS Lett 377:353–357

Verspohl EJ, Ammon HP (1980) Evidence for presence of insulin receptors in rat islets of Langerhans. J Clin Invest 65:1230–1237

Vogt B, Carrascosa JM, Ermel B, Ullrich A, Häring HU (1991) The two isotypes of the human insulin receptor (HIR-A and HIR-B) follow different internalization kinetics. Biochem Biophys Res Commun 177:1013–1018

Wang H, Brun T, Kataoka K, Sharma AJ, Wollheim CB (2007) MafA controls genes implicated in insulin biosynthesis and secretion. Diabetologia 50:348–358

Waraich RS, Weigert C, Kalbacher H, Hennige AM, Lutz S, Häring HU, Schleicher ED, Voelter W, Lehmann R (2008) Phosphorylation of Ser357of rat insulin receptor substrate-1 mediates adverse effects of protein kinase C-delta on insulin action in skeletal muscle cells. J Biol Chem 283:11226–11233

Watada H, Kajimoto Y, Miyagawa J, Hanafusa T, Hamaguchi K, Matsuoka T, Yamamoto K, Matsuzawa Y, Kawamori R, Yamasaki Y (1996) PDX-1 induces insulin and glucokinase gene expressions in αTC1 clone 6 cells in the presence of betacellulin. Diabetes 45:1826–1831

Watson ML, Macrae K, Marley AE, Hundal HS (2011) Chronic effects of palmitate overload on nutrient-induced insulin secretion and autocrine signalling in pancreatic MIN6 β cells. PLoS ONE 6:e25975

Weigert C, Hennige AM, Brischmann T, Beck A, Moeschel K, Schauble M, Brodbeck K, Häring HU, Schleicher ED, Lehmann R (2005) The phosphorylation of Ser318 of insulin receptor substrate 1 is not per se inhibitory in skeletal muscle cells but is necessary to trigger the attenuation of the insulin-stimulated signal. J Biol Chem 280:37393–37399

Werner ED, Lee J, Hansen L, Yuan M, Shoelson SE (2004) Insulin resistance due to phosphorylation of insulin receptor substrate-1 at serine 302. J Biol Chem 279:35298–35305

White MF, Maron R, Kahn CR (1985) Insulin rapidly stimulates tyrosine phosphorylation of a Mr-185,000 protein in intact cells. Nature 318:183–186

White MF (2006) Regulating insulin signaling and β-cell function through IRS proteins. Can J Physiol Pharmacol 84:725–737

Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, White MF (1998) Disruption of IRS-2 causes type 2 diabetes in mice. Nature 391:900–904

Wu H, MacFarlane WM, Tadayyon M, Arch JR, James RF, Docherty K (1999) Insulin stimulates pancreatic-duodenal homoeobox factor-1 (PDX1) DNA-binding activity and insulin promoter activity in pancreatic β cells. Biochem J 344:813–818

Xuan S, Kitamura T, Nakae J, Politi K, Kido Y, Fisher PE, Morroni M, Cinti S, White MF, Herrera PL, Accili D, Efstratiadis A (2002) Defective insulin secretion in pancreatic β cells lacking type 1 IGF receptor. J Clin Invest 110:1011–1019

Yasui S, Mawatari K, Kawano T, Morizumi R, Hamamoto A, Furukawa H, Koyama K, Nakamura A, Hattori A, Nakano M, Harada N, Hosaka T, Takahashi A, Oshita S, Nakaya Y (2008) Insulin activates ATP-sensitive potassium channels via phosphatidylinositol 3-kinase in cultured vascular smooth muscle cells. J Vasc Res 45:233–243

Yoon KH, Ko SH, Cho JH, Lee JM, Ahn YB, Song KH, Yoo SJ, Kang MI, Cha BY, Lee KW, Son HY, Kang SK, Kim HS, Lee IK, Bonner-Weir S (2003) Selective β-cell loss and α-cell expansion in patients with type 2 diabetes mellitus in Korea. J Clin Endocrinol Metab 88:2300–2308

Zawalich WS, Zawalich KC (2000) A link between insulin resistance and hyperinsulinemia: inhibitors of phosphatidylinositol 3-kinase augment glucose-induced insulin secretion from islets of lean, but not obese, rats. Endocrinology 141:3287–3295

Zhao AZ, Zhao H, Teague J, Fujimoto W, Beavo JA (1997) Attenuation of insulin secretion by insulin-like growth factor 1 is mediated through activation of phosphodiesterase 3B. Proc Natl Acad Sci USA 94:3223–3228

Zhao J, Li L, Leissring MA (2009) Insulin-degrading enzyme is exported via an unconventional protein secretion pathway. Mol Neurodegener 4:4

Zhu J, Kahn CR (1997) Analysis of a peptide hormone-receptor interaction in the yeast two-hybrid system. Proc Natl Acad Sci USA 94:13063–13068

Circadian Control of Islet Function

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Jeongkyung Lee, Mousumi Moulik, and Vijay K. Yechoor

Contents

Abstract

Circadian clocks are evolutionarily conserved from single-celled organisms all the way to humans. These oscillators generate rhythms in gene expression and in physiological processes in cells and organisms that maintain a ~24 h periodicity to coincide with the light and dark cycles generated by the earth's rotation around its own axis. These clocks are self-sustaining and entrainable by external cues. They are generated by transcriptional and translational auto-feedback loops present in every cell. The suprachiasmatic nucleus (SCN) of the

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_43,

hypothalamus forms the central clock and is the pacemaker in mammalian systems. This synchronizes all the peripheral clocks present in other tissues and cells via neurohumoral pathways.

Disruption of this circadian rhythm, most notably with shift work, has been associated with many pathophysiological processes and disease states in humans, including diabetes. Disruption of the circadian clock, either by environmental or by genetic disruption, has been shown in rodent models to result in significant β-cell dysfunction. Tissue-specific deletion models of the core clock genes have demonstrated convincingly the critical regulatory role and cell-autonomous function of the molecular clock in β-cells function. Understanding these regulatory pathways and applying them to prevent human disease remain the objective of circadian biology.

Keywords

Circadian • Rhythms • Clock • Bmal1 • Rev-erb • GSIS • Oxidative stress • ROS • Nrf2 • Circadian disruption • Uncoupling • Ucp2 • Mitochondria

Introduction

Circadian (from Latin: circa – around and diem – day) rhythm is the daily oscillations, with a ~24 h periodicity, in the physiology and behavior of organisms (Hastings et al. 2003; Maywood et al. 2006). Both eukaryotes and prokaryotes demonstrate this synchronized daily variation in virtually all biologic and physiologic functions, mediated by an internal circadian timing system commonly known as the "circadian clock". This phasic and predictive diurnal variation in function is integral to the physiologic functioning of and demonstrated by, the whole organism, specific organ systems as well individual tissues and cells. The circadian clock is synchronized to the ~24 h day-night cycles resulting from the earth's rotation. This rhythmic oscillation is self-sustained and is an innate characteristic of cells and organisms and can function even in the absence of external environmental cues. However, the oscillator generating this rhythm remains entrainable, i.e., rest by appropriate external cues. The sleep-wake cycle and diurnal feeding behavior patterns are the two most overt examples of synchronized circadian variation in the functioning of the whole organism. A wide range of not-so-obvious and occult internal rhythms, with well-defined phase and amplitude, occur with a ~24 h circadian periodicity within the body. Some examples of these internal rhythms include the diurnal variation of adrenal corticosteroid and pituitary hormone release, body temperature regulation, neurotransmitter and neuropeptide levels, levels of sympathetic activation, as well as diurnal regulation of multiple aspects of energy metabolism including lipolysis, gluconeogenesis, insulin sensitivity, and basal metabolic rate (Laposky et al. 2008).

The circadian clock and the rhythms it generates have been evolutionarily selected for the inherent advantage accorded to organisms in adapting their metabolism and behavior to anticipate predictable changes in their environment. Hence, disruption of these rhythms has been shown to be associated with many diseases states including metabolic disorders (Karlsson et al. 2001, 2003; Muller et al. 1987), cardiovascular disorders (Boggild and Knutsson 1999; Knutsson and Boggild 2000; Hermansson et al. 2007), sleep disorders (Hastings et al. 2008), and cancer (Halberg et al. 2006). Interestingly, recent genome-wide association studies have implicated the circadian rhythm-related gene, *MTNR1b*, as being associated with increased glucose levels, diabetes, and impaired β-cell function (Prokopenko et al. 2009; Lyssenko et al. 2009; Ronn et al. 2009). Circadian misalignment, as occurs in shift workers, has been associated with obesity, metabolic syndrome, and increased cardiovascular mortality (Karlsson et al. 2001, 2003; Hermansson et al. 2007; Kroenke et al. 2007; Scheer et al. 2009a).

The last two decade has seen significant breakthroughs in understanding the molecular basis of circadian rhythmicity (Green et al. 2008). Many animal models with targeted disruption of circadian clock genes have been shown to have significant metabolic abnormalities (Rudic et al. 2004; Turek et al. 2005; Green et al. 2008; Prasai et al. 2008; Marcheva et al. 2009; Ramsey and Bass 2009; Staels 2006; Duez and Staels 2008; Le Martelot et al. 2009). Many of the clock-controlled genes have direct effect on many metabolic processes reflected by a circadian rhythm in many plasma metabolites (Minami et al. 2009). There is a rhythmic oscillation in the plasma glucose and insulin secretion with insulin secretion being maximal in the morning (La Fleur et al. 1999; La Fleur 2003).

Molecular Basis of the Circadian Clock

Circadian rhythms are driven by cell-autonomous oscillating circadian clocks built upon molecular feedback loops (Young and Kay 2001; Borgs et al. 2009). The core circadian clock which drives these rhythmic oscillations is composed of two proteins Bmal1 (Brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein-1 and also called as Mop3 or Arntl – Aryl hydrocarbon receptor nuclear translocator-like) and Clock (Circadian Locomotor Output Cycles Kaput). Bmal1 and Clock (or in certain tissues, its orthologue, Npas2 (Reick et al. 2001) are both basic helix-loop-helix PAS domain (bHLH-PAS) transcription factors that form a heterodimer and bind to E-boxes on cis-promoter regions to activate the transcription of downstream clock-controlled genes (CCG). The core clock therefore consists of Bmal1 and Clock binding to the promoters of two other sets of core clock genes – period (Per) 1, 2, 3 and cryptochrome (Cry) 1, 2. Per and Cry proteins form a complex along with casein kinase Iε and after phosphorylation translocate to the nucleus. Once their concentration reaches a threshold, this complex binds to and inhibits the transactivation activity of Bmal1/Clock heterodimeric complex, thus inhibiting their own transcription (Antoch et al. 1997; King et al. 1997;

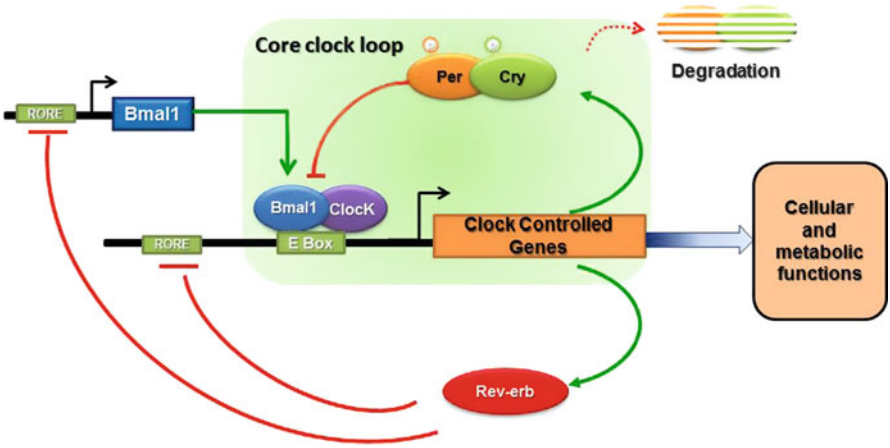

Fig. 1 Schematic model of the molecular clock depicting the core clock genes Bmal1 and Clock binding to E-box elements of clock-controlled genes. Bmal1 and Clock activate the transcription of Per and Cry genes. Per and Cry proteins heterodimerize and after phosphorylation return to the nucleus to inhibit the transactivation by Bmal1/Clock, thus completing an auto-feedback inhibitory loop. Bmal1/Clock also activates Rev-erb α/β and ROR that feedback to regulate Bmal1 transcription

Bunger et al. 2000; Vitaterna et al. 1994, 1999; van der Horst et al. 1999; Okamura et al. 1999; Teboul et al. 2008; Green et al. 2008; Albrecht et al. 1997; Zheng et al. 2001; Tei et al. 1997; Hogenesch et al. 1998) and also that of other clock-controlled genes (Fig. 1). This leads to a decrease in their expression and consequent derepression of the Bmal1/Clock complex, and this whole cycle takes ~24 h and repeats all over again. This oscillating mechanism composed of the transcriptional positive limb and the translational inhibitory negative limb form an autoregulatory loop that is the basis of the molecular clock and the ~24 h rhythmicity. Light serves as an entraining signal by activating Per gene transcription. Other interlocking loops involving Rev-erb α and Rev-erb β (Preitner et al. 2002; Yin et al. 2007; Duez and Staels 2008; Liu et al. 2008), RORs (Jetten 2009; Yang et al. 2006a; Duez and Staels 2008; Liu et al. 2008) with Rev-erb inhibiting and ROR activating the transcription of Bmal1, stabilize and add robustness to the core clock machinery (La Fleur et al. 1999; La Fleur 2003). The heterodimer Bmal1/Clock binds to promoter regions containing E-boxes on clock-controlled genes and regulate many homeostatic processes including cell cycle control, DNA damage response genes, and nuclear hormone receptors such as PPARα (Canaple et al. 2006; Oishi et al. 2005; Inoue et al. 2005; Teboul et al. 2008) directly or indirectly by regulating other transcription factors such as DBP, TEF, and E4BP4 (Cowell 2002). On the other hand, the clock proteins are themselves regulated by certain metabolic sensors, such as the NAD-dependent histone deacetylase, Sirt1 (Hirayama et al. 2007; Nakahata et al. 2008; Asher et al. 2008) and Sirt3 (Peek et al. 2013), and PGC-1α (Liu et al. 2007; Grimaldi and Sassone-Corsi 2007).

Central Versus Peripheral Clocks

The master pacemaker neurons within the hypothalamic suprachiasmatic nucleus (SCN) compose the central clock, the topmost hierarchy of the circadian clock, and influence the activity of a network of extra-SCN and peripheral clocks through neural outputs. The SCN is primarily entrained by the environmental light-dark cycle, but interestingly not by feeding, unlike the peripheral clocks which may be reset by a variety of factors ("zeitgebers," so-called time givers), such as food availability, glucocorticoid level, and temperature.

The core clock machinery is present in all tissues and can be maintained even in isolated cells in culture (Balsalobre et al. 1998). To achieve synchronization between all these clocks, the master central clock, residing in the hypothalamic suprachiasmatic nucleus (SCN) (Saini et al. 2011; Dibner et al. 2010; Schibler 2009; Cuninkova and Brown 2008a; Damiola et al. 2000a), coordinates all the peripheral clocks residing in other tissues (Fig. 2). The central clock receives light input from the retinohypothalamic tract (light entrainment) and functions as the central pacemaker for the organism (Stephan and Zucker 1972). Thus, light is the primary entrainment signal (zeitgeber – German for "time giver") to the central

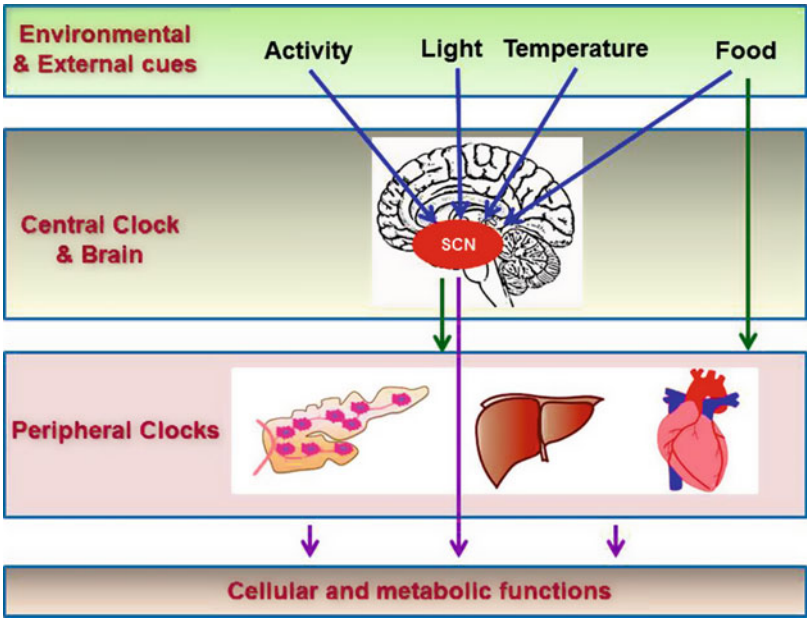

Fig. 2 Schematic representation of the hierarchy of the central and peripheral clocks. The central clock is entrained by many external cues of which light via the retinohypothalamic tract is the primary entraining signal. The central clock in turn regulates the peripheral clocks in every tissue via neurohumoral outputs. The central clock and peripheral clocks in turn regulate all the cellular and metabolic functions

clock. In addition, other entrainment signals include activity, temperature, and food – though food impacts peripheral clocks to a much larger degree. The SCN has neural outputs into other parts of the brain and via the autonomic nervous system to all other tissues in the body. Thus, the pace (period) in all the peripheral clocks is set by this central clock (Cuninkova and Brown 2008b). Food has been shown to be an important synchronizing signal for metabolically active tissues (Damiola et al. 2000b; Le Minh et al. 2001; Preitner et al. 2003), such as the liver, heart, and pancreas. These peripheral clocks receive two important inputs, one from the central clock and the other from food-derived nutrient signals. The importance of the peripheral clocks has been demonstrated by elegant experiments via restricted feeding and more recently by tissue-specific disruption or activation of the circadian clock genes (Lamia et al. 2008; Kornmann et al. 2007; McDearmon et al. 2006).

Circadian Rhythm in Energy Balance and Metabolism

In humans, many aspects of metabolism display circadian cycles, including 24-h variation of glucose, insulin, and leptin levels. Recent reports delineate some of the mechanisms underlying the circadian control of metabolic processes, many of which play active roles in the regulation of β-cell function. PPARα, a major regulator of fatty acid oxidation, is a direct target of Bmal1/Clock (Inoue et al. 2005). PGC1α, a transcriptional coactivator and a key player in energy metabolism, regulates the circadian clock via Bmal1 Rev-erbα and ROR and integrates circadian clock and energy metabolism (Liu et al. 2007). Furthermore, PGC1α itself exhibits circadian expression (Liu et al. 2007). Furthermore, Rev-erbα, a key clock gene and a nuclear receptor, regulates bile acid homeostasis and Srebp1c, the key transcription factor that regulates lipid and cholesterol metabolism (Le Martelot et al. 2009). The NAD^+-dependent protein deacetylase Sirt1, which has been shown to regulate metabolism in many tissues including the β-cell (Bordone et al. 2006), is regulated by the circadian clock. Bmal1/Clock has been shown to regulate Nampt, the rate-limiting enzyme in the generation of NAD^+ (Nakahata et al. 2009; Ramsey et al. 2009). Thus, Bmal1/Clock by regulating the level of NAD^+ in a circadian pattern regulates Sirt1, which is NAD^+ dependent for its protein deacetylation activity. To complete this intriguing loop, Sirt1 has been shown to regulate the clock genes by opposing the histone acetylation activity of Clock protein (Nakahata et al. 2008) and promoting the deacetylation and subsequent degradation of Per2 (Asher et al. 2008). Thus, NAD^+/NADH, key indicators of the redox state of the cell and its metabolic status, directly regulate and are regulated by core clock genes Bmal1 and Clock. More recently, the circadian clock has also been shown to regulate the activity of mitochondrial Sirt3 and its deacetylase activity by regulation of NAD^+ levels in the mitochondria (Peek et al. 2013). This is especially relevant in tissues that display a high metabolic activity.

Another piece of evidence that illustrates the close interaction between metabolic processes and the circadian clock is the transcriptome analysis performed in

various tissues that revealed that about 10 % of all the transcripts in any given tissue have a circadian rhythm (Panda et al. 2002). Interestingly, this circadian control appears to be tissue-specific as these 10 % transcripts that are changed mostly differ between various tissues (Panda et al. 2002; Storch et al. 2002). In addition, a third of all nuclear receptors have a circadian rhythm, and these nuclear receptors play a central role in energy and metabolic homeostasis (Teboul et al. 2008; Yang et al. 2006b) including β-cell function. Furthermore, the circadian control of various metabolic pathways appears to be most apparent on rate-limiting steps (Panda et al. 2002), compelling evidence that circadian control is required for normal homeostasis and that disruption of this control will likely result in adverse consequences.

Evidence for Circadian Function of Pancreatic Islets

Mammals adapt their activity pattern alternating between high activity and rest periods to the diurnal variations in light intensity. Plasma glucose concentrations display a daily rhythm with peak values at the beginning of the active period (Bolli et al. 1984; Jolin and Montes 1973; Lesault et al. 1991; Van Cauter 1990). This is shown in Fig. 3, wherein the normal circadian rhythm of blood glucose in mice is in stark contrast to the significant loss of rhythm in mice with a genetic disruption of the circadian clock due to a deletion of Bmal1 globally.

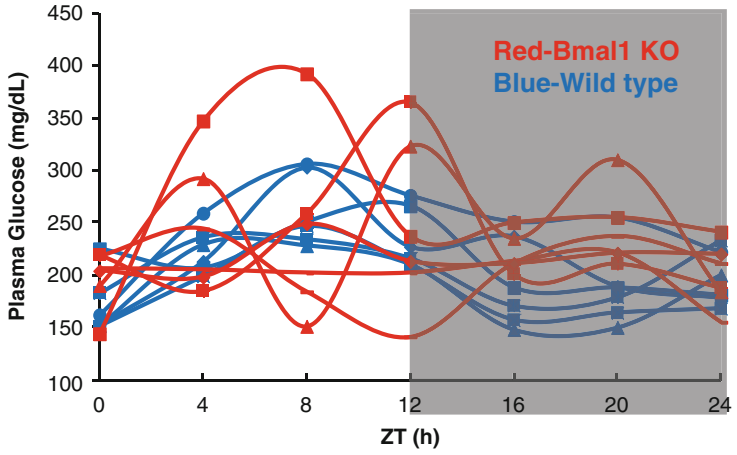

Fig. 3 Circadian rhythm of plasma glucose in wild-type C57/Bl6 mice is shown. Each *blue line* represents an individual mouse sampled every 4 h during a normal 12:12 h light-dark cycle. Zt 0 h (zeitgeber time) represents lights on (7 AM). The *red lines* represent individual mice in which Bmal1 is deleted globally in all tissues. The loss of circadian rhythm in plasma glucose is striking in Bmal1 null mice

In addition circadian rhythm in gene expression in pancreatic islets has been demonstrated almost a decade ago (Allaman-Pillet et al. 2004; Muhlbauer et al. 2004). Of the five endocrine cell subsets of the pancreatic islets, circadian rhythms of β-cell function is the most recognized with recent key advances in understanding the underlying molecular mechanism. The metabolic oscillations in islets are also reviewed in the chapter "▶ Electrical, Calcium, and Metabolic Oscillations in Pancreatic Islets."

β-cell Clock

Evidence that the β-cells have a functional peripheral clock has been accumulating over the last two decades. It has been shown more than a decade ago that many β-cell genes exhibit a circadian rhythm and more recently functional clocks in islets ex vivo has been demonstrated using Per2 luciferase mice wherein the islets retain their rhythmic expression of luciferase under the Per2 promoter even when in culture. The β-cells express all the components of the molecular clock, and the critical importance of the cell-autonomous function of the β-cell molecular clock has been shown in mice by deleting Bmal1 and disrupting the molecular clock only in the β-cells. As with other more peripheral tissues, the expression of the core clock genes has been demonstrated in isolated human islets, rat pancreas, and cultured β-cell lines (Allaman-Pillet et al. 2004; Muhlbauer et al. 2004).

Peripheral Clocks in Other Islet Cells

There is very little data as to the role of and the regulation by the circadian clock on α cell function. One recent study looking at α-cell lines found that Rev-erb α (a negative regulator of clock function) activation stimulated calcium currents in α cells and promoted glucagon secretion, and it was also shown that Rev-erb α is required for the normal low glucose-induced glucagon secretion (Vieira et al. 2013). Though this physiological function in vivo has not been explored, this coordinate regulation of insulin from β-cells and glucagon secretion from α-cells by the circadian clock fits with the broad physiological picture of close coordinate function between the various islet cell types.

Disruptions of the Circadian Clock and Metabolic Disorders

Disruption of the circadian rhythm has been shown to result in metabolic abnormalities in both humans and animals. A higher incidence of diabetes, obesity, and cardiovascular events has been noted in shift workers, who have a work necessitated disruption of the awake-sleep cycle (Bass and Takahashi 2010; Spiegel et al. 2009). In the general population, short sleep, sleep deprivation, and poor sleep quality are associated with diabetes, metabolic syndrome, hypoleptinemia,

increased appetite, and obesity (Van Cauter et al. 2007; Spiegel et al. 2004; Taheri et al. 2004; Laposky et al. 2008). The mechanism underlying this association is unclear. Forced circadian misalignment in humans results in hypoleptinemia, insulin resistance, inverted cortisol rhythms, and increased blood pressure (Scheer et al. 2009a). Narcolepsy, a sleep disorder resulting in excessive day time sleepiness, is associated with elevated BMI and increased incidence of obesity (Kotagal et al. 2004; Schuld et al. 2000). Patients with "nighttime eating syndrome" (NES) have a higher incidence of obesity (even though total food intake is similar to control subjects) and show abnormal metabolic rhythms including decreased nocturnal rise in leptin, phase shift in insulin, cortisol and ghrelin, and inverted 24-h rhythms of blood glucose (Birketvedt et al. 1999; Goel et al. 2009).

Normal alignment of feeding and activity with the environmental light-dark cycle has been shown to be critical for energy homeostasis in rodents. Rats exposed to daily 8-h activity schedule during their normal resting phase (light cycle) have an increased food intake during their resting stage along with associated obesity and diminished rhythmicity of glucose and locomotor activity. Shifting food intake back to the active phase (dark cycle) restored their metabolic rhythms and prevented obesity in the same animals (Salgado-Delgado et al. 2010).

The central role of the circadian clock in metabolic homeostasis is exemplified by the metabolic disturbances seen in the many mouse models with disrupted clock genes. A mutation of the Clock gene (Clock mutant mice) led to obesity and metabolic syndrome (Turek et al. 2005), similar to Bmal1 knockout and Rev-erbα knockout mice that have significant metabolic alterations (Rudic et al. 2004; Lamia et al. 2008; Yin et al. 2007; Le Martelot et al. 2009).

Disruptions of the β-cell Clock Cause β-cell Dysfunction and Diabetes

Global deletion of Bmal1, the nonredundant core clock gene, in mice leads to loss of behavioral rhythm (Bunger et al. 2000) and profound metabolic disruptions, premature aging, and early death (Kondratov et al. 2006; Rudic et al. 2004). These mice display significant impairments in glucose homeostasis and significant hypoglycemia on fasting secondary to impairments in gluconeogenesis in the liver. However, in the fed state they display glucose intolerance despite no significant insulin resistance. This is due to impairment in glucose-stimulated insulin secretion in vivo. Isolated islet studies from these mice display impairment in ex vivo glucose-stimulated insulin secretion. Since many of these mice become very sick and die by 7–8 months of age, it was necessary to specifically address whether this impairment in β-cell function in the global Bmal1 knockout mice was secondary to the critical function of the molecular clock and Bmal1 in β-cells.

A striking confirmation of the importance of the cell-autonomous function of the β-cell clock came from three independent labs (Lee et al. 2011, 2013; Marcheva et al. 2010; Sadacca et al. 2011) reporting the phenotype of mice in which Bmal1

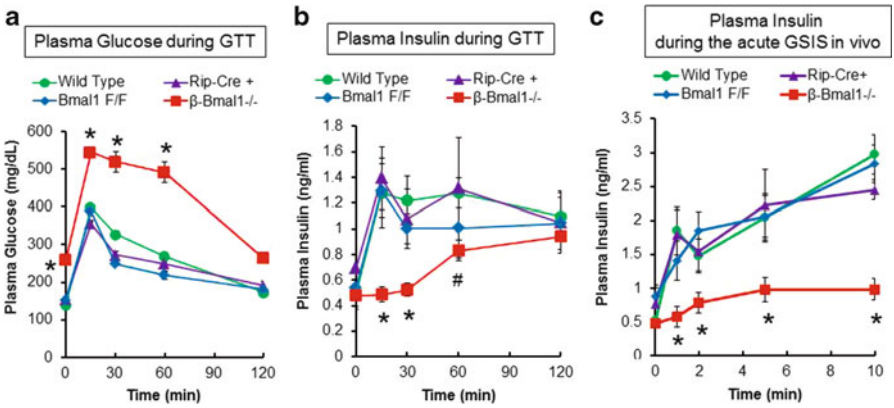

Fig. 4 Glucose intolerance in β-cell-specific Bmal1$^{-/-}$ mice. **a**–**b** Glucose tolerance test (*GTT*) was performed in overnight fasted mice after a 1.5 g/kg dextrose intraperitoneal injection. Plasma glucose (**a**) and plasma insulin (**b**) are shown in β-cell-specific Bmal1$^{-/-}$ mice and their controls. (**c**) Plasma insulin during the first phase insulin response in response to an acute glucose challenge (3 g/kg IP Dextrose) is shown in β-cell-specific Bmal1$^{-/-}$ mice and their controls

was deleted in a tissue-specific manner, using Bmal1 floxed mice which had the DNA binding domain flanked by LoxP sites, in the whole pancreas (Bmal1 floxed mice crossed with Pdx1-Cre transgenic mice), or only in the β-cells of the islet (Bmal1 floxed mice crossed with Rip-Cre transgenic mice). Though both of these transgenes under Pdx1 and Rip promoters are expressed widely in the brain, they were shown not to disrupt Bmal1 in the SCN excluding confounding results from central clock disruption. Both of these models displayed diabetes and significant impairment in glucose-stimulated insulin secretion (GSIS) due to β-cell dysfunction (Figs. 4 and 5). Further in vivo experiments in these mice with a disrupted clock, in β-cells and in vitro using genetic knockdown in insulinoma cells, revealed that deletion of Bmal1 was sufficient to impair GSIS in β-cells (Lee et al. 2011, 2013). Furthermore, this was shown to be a result of impairment in mitochondrial OXPHOS, as shown by a decrease in glucose-induced hyperpolarization of the inner mitochondrial membrane (assessed by the JC-1 assay). In this assay, addition of the dye JC-1 to β-cells leads to a green cytoplasmic fluorescence from monomers of the dye in the cytosol. On glucose stimulation, there is increased metabolism of glucose through the TCA cycle and subsequent increase in potential gradient across the inner mitochondrial membrane. This hyperpolarization leads to the import of the JC-1 dye into the mitochondria, wherein it polymerizes and emits red fluorescence. This ratio of red/green fluorescence is a measure of the glucose-induced changes in potential gradient across the inner mitochondrial membrane. As shown in Fig. 6, disruption of the β-cell clock in β-cell-specific Bmal1 knockout mouse islets leads to impairment in hyperpolarization of the mitochondria on glucose stimulation. This results in a reduction in the glucose-induced ATP/ADP ratio, the critical signal to the ATP-responsive K_{ATP} channels and subsequent insulin granule exocytosis. Other experiments have also implicated changes in vesicular

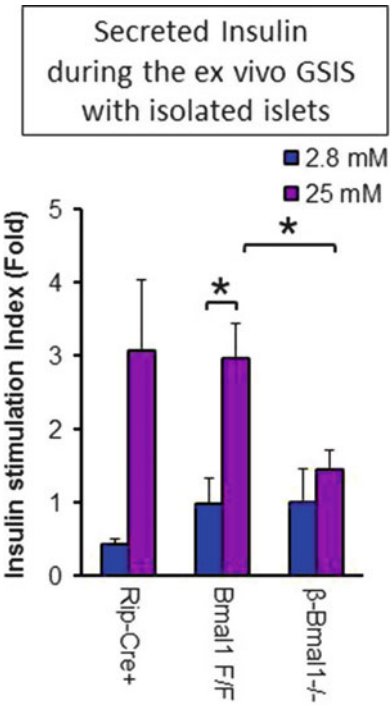

Fig. 5 Ex vivo glucose-stimulated insulin secretion from isolated islets. β-cell-specific Bmal1$^{-/-}$islets display a blunted GSIS in response to 25 mM glucose stimulation for 30 min

trafficking and exocytosis-related genes in β cells in clock-disrupted islets (Marcheva et al. 2010). β-cell mitochondrial dysfunction is also reviewed in the chapter "► Role of Mitochondria in β-cell Function and Dysfunction."

Interestingly, Bmal1 and the β-cell clock also appear to control other aspects of the β-cell function including regulating the oxidative stress response by direct transcriptional regulation of antioxidant response genes. Bmal1 directly binds to an E-box element in the promoter of the master antioxidant transcription factor Nfe2l2 (nuclear factor erythroid-derived 2 like 2 or also called Nrf2), shown in Fig. 7, and this results in a circadian rhythm to the gene expression of Nrf2 in β-cells. In Bmal1 null islets this circadian regulation is lost along with a decrease in expression of critical antioxidant genes (Lee et al. 2013). Since β-cells have a very low antioxidant reserve as compared to other metabolically active tissues (Acharya and Ghaskadbi 2010; Robertson 2006; Kaneto et al. 2005; Lenzen et al. 1996), this decrease in antioxidant response leads to accumulation of reactive oxygen species (ROS), as shown in Fig. 7, and a consequent upregulation of the uncoupling protein Ucp2. This uncoupling of the mitochondria contributes to the impairment in glucose-induced changes in ATP/ADP ratio in Bmal1-null islets. This regulation of the antioxidant response and oxidative stress has been reported in other tissues, including the kidney, heart, and spleen (Kondratov et al. 2006, 2009). Indeed the circadian regulation of antioxidant gene expression of antioxidant genes has also been reported in other metabolically active tissues such as the liver

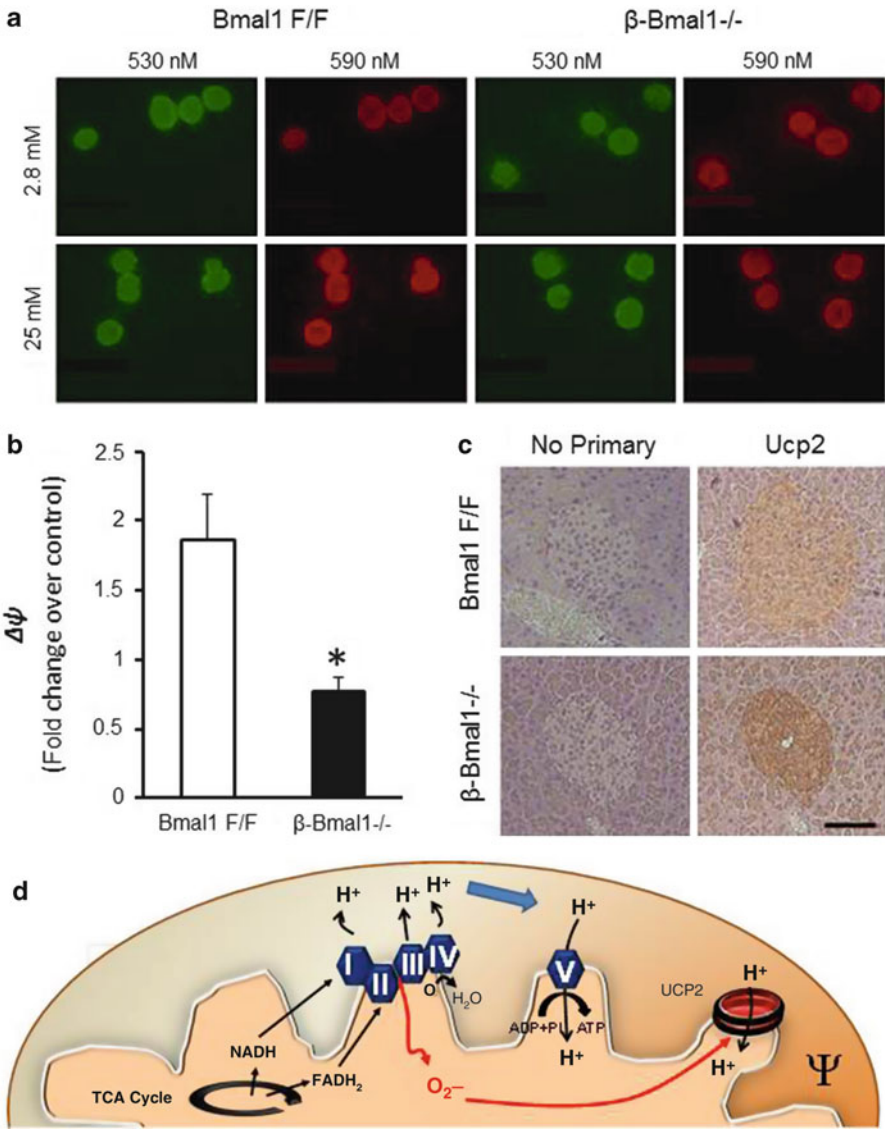

Fig. 6 Bmal1 is required for normal β-cell mitochondrial OXPHOS coupling. **a**–**b** JC-1 assay showing a reduced hyperpolarization of the inner mitochondrial membrane (ψ) in isolated β-cell-specific Bmal1$^{-/-}$islets, exposed to 30 min of 25 mM glucose. The reduced *red*/*green fluorescence* ratio is quantitated in (**b**). This is in part due to increased expression of the uncoupling protein Ucp2 in islets from β-cell-specific Bmal1$^{-/-}$(**c**). Model of the mitochondria with representation of the electron transport system and the uncoupling protein Ucp2 (*in red*) is shown schematically. With an increased expression of Ucp2, the oxidative phosphorylation is uncoupled and ATP production from glucose oxidation in β-cells in Bmal1 null islets (**d**). The negative controls are the sections on the left that have been stained with no primary antibody

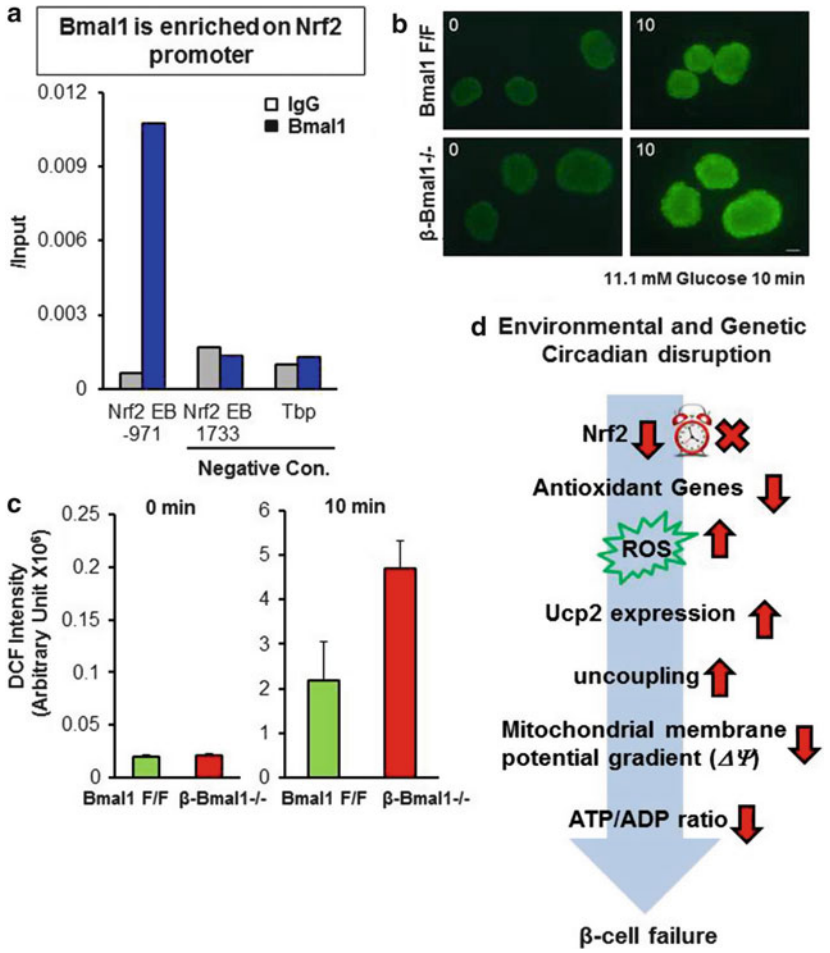

Fig. 7 Bmal1 regulates the antioxidant response in β-cells. (**a**) Chromatin immunoprecipitation (*ChIP*) assay reveals Bmal1 binding to cis-E-box element in the Nrf2 promoter. A different E-box in Nrf2 promoter and TBP promoter E-box element are also shown as negative controls. (**b**) H2-DCF-DA (2′,7′-dichloro dihydrofluorescein acetate) a non-fluorescent dye is oxidized by ROS, after intracellular cleavage of the acetate group, in β-cells exposed to 11.1 mM glucose for 10 min and changes to a *fluorescent green*. This is quantitated and shown in the (**c**) and is significantly increased in Bmal1 null islets. (**d**) Schematic model of circadian clock regulation of the antioxidant response in β-cells and the changes with circadian disruption are indicated by the *red arrows*

(Panda et al. 2002; Xu et al. 2012). It has also been reported that there is a significant increase in ROS in other tissues in Bmal1 null mice that can be rescued in part by the antioxidant compound, N-acetyl cysteine (NAC), and this has led to an improvement in the lifespan of global Bmal1 null mice that otherwise have premature aging and death (Kondratov et al. 2009). This phenomenon is also been shown to be true in β-cells, as NAC has been shown to rescue the impairment in GSIS in ex vivo experiments on Bmal1-null islets (Lee et al. 2013).

Other studies have been done using environmental circadian disruption to test β-cell function, and these have also revealed that circadian disruption by altering the light-dark cycles leads to impairment in glucose homeostasis in mice and this is secondary to β-cell dysfunction and an impairment in GSIS (Lee et al. 2013; Qian et al. 2013). Furthermore, using a genetic model prone to β-cell apoptosis, it has been shown that imposition of circadian disruption by altering the light-dark cycles alone was sufficient to induce diabetes resulting from an increase in β-cell apoptosis (Gale et al. 2011). Human studies have also confirmed the effects of circadian disruption on β-cell function. Acute circadian disruption imposed by light phase advancement resulted in an increase in postprandial hyperglycemia with an insufficient β-cell compensation in healthy control subjects (Scheer et al. 2009b) that was significantly worsened with superimposed sleep deprivation (Buxton et al. 2012).

Clinical Implications

Circadian oscillations have been shown in human islets (Pulimeno et al. 2013; Stamenkovic et al. 2012), and human studies in healthy volunteers have shown the effects of experimental circadian disruption by environmental manipulation to affect glucose homeostasis and β-cell function (Scheer et al. 2009b; Buxton et al. 2012). Epidemiological studies abound that demonstrate the correlation of circadian disruption and shift work with adverse metabolic consequences, including diabetes (Karlsson et al. 2001, 2003; Muller et al. 1987; Hermansson et al. 2007; Kroenke et al. 2007; Scheer et al. 2009b; Pan et al. 2011; Kivimaki et al. 2011). There have been some recent studies that raise the possibility that with a better understanding of the molecular pathways of circadian regulation, it may be possible pharmacologically to prevent the deleterious consequences of circadian disruption. Sik1, the salt-inducible kinase 1, a member of the AMPK family, when inhibited has been shown to promote a rapid re-entrainment after jetlag in mice. Similarly, deletion of vasopressin receptors V1a and V1b (Yamaguchi et al. 2013) also have a similar benefit in mouse models. Meanwhile, a better understanding of the time course of the metabolic disruptions with circadian disruption is needed to see if workplace interventions such as those to avoid rapid shifts in light-dark cycles may be of some benefit along with possible benefit of antioxidants to prevent circadian disruption-induced oxidative stress. Further studies of the metabolic benefits of these approaches need to be conducted both in animal models and humans to better address the urgent need to prevent and treat the consequences of circadian disruption. Circadian disruption is an unavoidable consequence of modern day lifestyle and is an occupational consequence of shift work. As detailed above, there is strong emerging data that these disruptions are sufficient to impair islet function raising the possibility that circadian disruption could be contributing to the increasing incidence of diabetes. However, avoiding circadian disruption is often not practical. Hence, there is an urgent need for further studies to come up with measures that prevent or mitigate the consequences of circadian disruption.

Conclusion

Many pressing questions still remain to be explored. A more comprehensive understanding of the pathways that regulate the metabolic, stress adaptive and survival functions of the β-cells and other cell types in the islet need to be understood. In addition, measures to prevent, treat and reverse the adverse metabolic consequences need to be tested and instituted in high-risk populations, based on sound understanding of underlying the molecular regulatory pathways. Though, circadian control of islet function is still in its infancy, the interest and complementary work being done in many labs offers optimism to deal with this problem.

References

Acharya JD, Ghaskadbi SS (2010) Islets and their antioxidant defense. Islets 2:225–235

Albrecht U, Sun ZS, Eichele G, Lee CC (1997) A differential response of two putative mammalian circadian regulators, mper1 and mper2, to light. Cell 91:1055–1064

Allaman-Pillet N, Roduit R, Oberson A, Abdelli S, Ruiz J, Beckmann JS, Schorderet DF, Bonny C (2004) Circadian regulation of islet genes involved in insulin production and secretion. Mol Cell Endocrinol 226:59–66

Antoch MP, Song EJ, Chang AM, Vitaterna MH, Zhao Y, Wilsbacher LD, Sangoram AM, King DP, Pinto LH, Takahashi JS (1997) Functional identification of the mouse circadian clock gene by transgenic BAC rescue. Cell 89:655–667

Asher G, Gatfield D, Stratmann M, Reinke H, Dibner C, Kreppel F, Mostoslavsky R, Alt FW, Schibler U (2008) SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell 134:317–328

Balsalobre A, Damiola F, Schibler U (1998) A serum shock induces circadian gene expression in mammalian tissue culture cells. Cell 93:929–937

Bass J, Takahashi JS (2010) Circadian integration of metabolism and energetics. Science 330:1349–1354

Birketvedt GS, Florholmen J, Sundsfjord J, Osterud B, Dinges D, Bilker W, Stunkard A (1999) Behavioral and neuroendocrine characteristics of the night-eating syndrome. JAMA 282:657–663

Boggild H, Knutsson A (1999) Shift work, risk factors and cardiovascular disease. Scand J Work Environ Health 25:85–99

Bolli GB, De Feo P, De Cosmo S, Perriello G, Ventura MM, Calcinaro F, Lolli C, Campbell P, Brunetti P, Gerich JE (1984) Demonstration of a dawn phenomenon in normal human volunteers. Diabetes 33:1150–1153

Bordone L, Motta MC, Picard F, Robinson A, Jhala US, Apfeld J, McDonagh T, Lemieux M, McBurney M, Szilvasi A, Easlon EJ, Lin SJ, Guarente L (2006) Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic β cells. PLoS Biol 4:e31

Borgs L, Beukelaers P, Vandenbosch R, Belachew S, Nguyen L, Malgrange B (2009) Cell "circadian" cycle: new role for mammalian core clock genes. Cell Cycle 8:832–837

Bunger MK, Wilsbacher LD, Moran SM, Clendenin C, Radcliffe LA, Hogenesch JB, Simon MC, Takahashi JS, Bradfield CA (2000) Mop3 is an essential component of the master circadian pacemaker in mammals. Cell 103:1009–1017

Buxton OM, Cain SW, O'Connor SP, Porter JH, Duffy JF, Wang W, Czeisler CA, Shea SA (2012) Adverse metabolic consequences in humans of prolonged sleep restriction combined with circadian disruption. Sci Transl Med 4:129ra43

Canaple L, Rambaud J, Dkhissi-Benyahya O, Rayet B, Tan NS, Michalik L, Delaunay F, Wahli W, Laudet V (2006) Reciprocal regulation of brain and muscle Arnt-like protein 1 and peroxisome proliferator-activated receptor α defines a novel positive feedback loop in the rodent liver circadian clock. Mol Endocrinol 20:1715–1727

Cowell IG (2002) E4BP4/NFIL3, a PAR-related bZIP factor with many roles. Bioessays 24:1023–1029

Cuninkova L, Brown SA (2008) Peripheral circadian oscillators: interesting mechanisms and powerful tools. Ann N Y Acad Sci 1129:358–370

Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U (2000) Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes Dev 14:2950–2961

Dibner C, Schibler U, Albrecht U (2010) The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu Rev Physiol 72:517–549

Duez H, Staels B (2008) The nuclear receptors Rev-erbs and RORs integrate circadian rhythms and metabolism. Diab Vasc Dis Res 5:82–88

Gale JE, Cox HI, Qian J, Block GD, Colwell CS, Matveyenko AV (2011) Disruption of circadian rhythms accelerates development of diabetes through pancreatic β-cell loss and dysfunction. J Biol Rhythms 26:423–433

Goel N, Stunkard AJ, Rogers NL, Van Dongen HP, Allison KC, O'Reardon JP, Ahima RS, Cummings DE, Heo M, Dinges DF (2009) Circadian rhythm profiles in women with night eating syndrome. J Biol Rhythms 24:85–94

Green CB, Takahashi JS, Bass J (2008) The meter of metabolism. Cell 134:728–742

Grimaldi B, Sassone-Corsi P (2007) Circadian rhythms: metabolic clockwork. Nature 447:386–387

Halberg F, Cornelissen G, Ulmer W, Blank M, Hrushesky W, Wood P, Singh RK, Wang Z (2006) Cancer chronomics III. Chronomics for cancer, aging, melatonin and experimental therapeutics researchers. J Exp Ther Oncol 6:73–84

Hastings MH, Reddy AB, Maywood ES (2003) A clockwork web: circadian timing in brain and periphery, in health and disease. Nat Rev Neurosci 4:649–661

Hastings MH, Maywood ES, Reddy AB (2008) Two decades of circadian time. J Neuroendocrinol 20:812–819

Hermansson J, Gillander GK, Karlsson B, Lindahl B, Stegmayr B, Knutsson A (2007) Ischemic stroke and shift work. Scand J Work Environ Health 33:435–439

Hirayama J, Sahar S, Grimaldi B, Tamaru T, Takamatsu K, Nakahata Y, Sassone-Corsi P (2007) CLOCK-mediated acetylation of BMAL1 controls circadian function. Nature 450:1086–1090

Hogenesch JB, Gu YZ, Jain S, Bradfield CA (1998) The basic-helix-loop-helix-PAS orphan MOP3 forms transcriptionally active complexes with circadian and hypoxia factors. Proc Natl Acad Sci U S A 95:5474–5479

Inoue I, Shinoda Y, Ikeda M, Hayashi K, Kanazawa K, Nomura M, Matsunaga T, Xu H, Kawai S, Awata T, Komoda T, Katayama S (2005) CLOCK/BMAL1 is involved in lipid metabolism via transactivation of the peroxisome proliferator-activated receptor (PPAR) response element. J Atheroscler Thromb 12:169–174

Jetten AM (2009) Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism. Nucl Recept Signal 7:e003

Jolin T, Montes A (1973) Daily rhythm of plasma glucose and insulin levels in rats. Horm Res 4:153–156

Kaneto H, Kawamori D, Matsuoka TA, Kajimoto Y, Yamasaki Y (2005) Oxidative stress and pancreatic β-cell dysfunction. Am J Ther 12:529–533

Karlsson B, Knutsson A, Lindahl B (2001) Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. Occup Environ Med 58:747–752

Karlsson BH, Knutsson AK, Lindahl BO, Alfredsson LS (2003) Metabolic disturbances in male workers with rotating three-shift work. Results of the WOLF study. Int Arch Occup Environ Health 76:424–430

King DP, Zhao Y, Sangoram AM, Wilsbacher LD, Tanaka M, Antoch MP, Steeves TD, Vitaterna MH, Kornhauser JM, Lowrey PL, Turek FW, Takahashi JS (1997) Positional cloning of the mouse circadian clock gene. Cell 89:641–653

Kivimaki M, Batty GD, Hublin C (2011) Shift work as a risk factor for future type 2 diabetes: evidence, mechanisms, implications, and future research directions. PLoS Med 8:e1001138

Knutsson A, Boggild H (2000) Shiftwork and cardiovascular disease: review of disease mechanisms. Rev Environ Health 15:359–372

Kondratov RV, Kondratova AA, Gorbacheva VY, Vykhovanets OV, Antoch MP (2006) Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock. Genes Dev 20:1868–1873

Kondratov RV, Vykhovanets O, Kondratova AA, Antoch MP (2009) Antioxidant N-acetyl-L-cysteine ameliorates symptoms of premature aging associated with the deficiency of the circadian protein BMAL1. Aging (Albany NY) 1:979–987

Kornmann B, Schaad O, Bujard H, Takahashi JS, Schibler U (2007) System-driven and oscillator-dependent circadian transcription in mice with a conditionally active liver clock. PLoS Biol 5:e34

Kotagal S, Krahn LE, Slocumb N (2004) A putative link between childhood narcolepsy and obesity. Sleep Med 5:147–150

Kroenke CH, Spiegelman D, Manson J, Schernhammer ES, Colditz GA, Kawachi I (2007) Work characteristics and incidence of type 2 diabetes in women. Am J Epidemiol 165:175–183

La Fleur SE (2003) Daily rhythms in glucose metabolism: suprachiasmatic nucleus output to peripheral tissue. J Neuroendocrinol 15:315–322

La Fleur SE, Kalsbeek A, Wortel J, Buijs RM (1999) A suprachiasmatic nucleus generated rhythm in basal glucose concentrations. J Neuroendocrinol 11:643–652

Lamia KA, Storch KF, Weitz CJ (2008) Physiological significance of a peripheral tissue circadian clock. Proc Natl Acad Sci USA 105:15172–15177

Laposky AD, Bass J, Kohsaka A, Turek FW (2008) Sleep and circadian rhythms: key components in the regulation of energy metabolism. FEBS Lett 582:142–151

Le Martelot G, Claudel T, Gatfield D, Schaad O, Kornmann B, Sasso GL, Moschetta A, Schibler U (2009) REV-ERB α participates in circadian SREBP signaling and bile acid homeostasis. PLoS Biol 7:e1000181

Le Minh N, Damiola F, Tronche F, Schutz G, Schibler U (2001) Glucocorticoid hormones inhibit food-induced phase-shifting of peripheral circadian oscillators. EMBO J 20:7128–7136

Lee J, Kim MS, Li R, Liu VY, Fu L, Moore DD, Ma K, Yechoor VK (2011) Loss of Bmal1 leads to uncoupling and impaired glucose-stimulated insulin secretion in β-cells. Islets 3:381–388

Lee J, Moulik M, Fang Z, Saha P, Zou F, Xu Y, Nelson DL, Ma K, Moore DD, Yechoor VK (2013) Bmal1 and β-cell clock are required for adaptation to circadian disruption, and their loss of function leads to oxidative stress-induced β-cell failure in mice. Mol Cell Biol 33:2327–2338

Lenzen S, Drinkgern J, Tiedge M (1996) Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. Free Radic Biol Med 20:463–466

Lesault A, Elchinger B, Desbals B (1991) Circadian rhythms of food intake, plasma glucose and insulin levels in fed and fasted rabbits. Horm Metab Res 23:515–516

Liu C, Li S, Liu T, Borjigin J, Lin JD (2007) Transcriptional coactivator PGC-1α integrates the mammalian clock and energy metabolism. Nature 447:477–481

Liu AC, Tran HG, Zhang EE, Priest AA, Welsh DK, Kay SA (2008) Redundant function of REV-ERB α and β and non-essential role for Bmal1 cycling in transcriptional regulation of intracellular circadian rhythms. PLoS Genet 4:e1000023

Lyssenko V, Nagorny CL, Erdos MR, Wierup N, Jonsson A, Spegel P, Bugliani M, Saxena R, Fex M, Pulizzi N, Isomaa B, Tuomi T, Nilsson P, Kuusisto J, Tuomilehto J, Boehnke M, Altshuler D, Sundler F, Eriksson JG, Jackson AU, Laakso M, Marchetti P, Watanabe RM, Mulder H, Groop L (2009) Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. Nat Genet 41:82–88

Marcheva B, Ramsey KM, Affinati A, Bass J (2009) Clock genes and metabolic disease. J Appl Physiol 107:1638

Marcheva B, Ramsey KM, Buhr ED, Kobayashi Y, Su H, Ko CH, Ivanova G, Omura C, Mo S, Vitaterna MH, Lopez JP, Philipson LH, Bradfield CA, Crosby SD, JeBailey L, Wang X, Takahashi JS, Bass J (2010) Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature 466:627–631

Maywood ES, O'Neill J, Wong GK, Reddy AB, Hastings MH (2006) Circadian timing in health and disease. Prog Brain Res 153:253–269

McDearmon EL, Patel KN, Ko CH, Walisser JA, Schook AC, Chong JL, Wilsbacher LD, Song EJ, Hong HK, Bradfield CA, Takahashi JS (2006) Dissecting the functions of the mammalian clock protein BMAL1 by tissue-specific rescue in mice. Science 314:1304–1308

Minami Y, Kasukawa T, Kakazu Y, Iigo M, Sugimoto M, Ikeda S, Yasui A, van der Horst GT, Soga T, Ueda HR (2009) Measurement of internal body time by blood metabolomics. Proc Natl Acad Sci U S A 106:9890–9895

Muhlbauer E, Wolgast S, Finckh U, Peschke D, Peschke E (2004) Indication of circadian oscillations in the rat pancreas. FEBS Lett 564:91–96

Muller JE, Ludmer PL, Willich SN, Tofler GH, Aylmer G, Klangos I, Stone PH (1987) Circadian variation in the frequency of sudden cardiac death. Circulation 75:131–138

Nakahata Y, Kaluzova M, Grimaldi B, Sahar S, Hirayama J, Chen D, Guarente LP, Sassone-Corsi P (2008) The NAD^+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell 134:329–340

Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P (2009) Circadian control of the NAD^+ salvage pathway by CLOCK-SIRT1. Science 324:654–657

Oishi K, Shirai H, Ishida N (2005) CLOCK is involved in the circadian transactivation of peroxisome-proliferator-activated receptor α (PPAR α) in mice. Biochem J 386:575–581

Okamura H, Miyake S, Sumi Y, Yamaguchi S, Yasui A, Muijtjens M, Hoeijmakers JH, van der Horst GT (1999) Photic induction of mPer1 and mPer2 in cry-deficient mice lacking a biological clock. Science 286:2531–2534

Pan A, Schernhammer ES, Sun Q, Hu FB (2011) Rotating night shift work and risk of type 2 diabetes: two prospective cohort studies in women. PLoS Med 8:e1001141

Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi JS, Hogenesch JB (2002) Coordinated transcription of key pathways in the mouse by the circadian clock. Cell 109:307–320

Peek CB, Affinati AH, Ramsey KM, Kuo HY, Yu W, Sena LA, Ilkayeva O, Marcheva B, Kobayashi Y, Omura C, Levine DC, Bacsik DJ, Gius D, Newgard CB, Goetzman E, Chandel NS, Denu JM, Mrksich M, Bass J (2013) Circadian clock NAD^+ cycle drives mitochondrial oxidative metabolism in mice. Science 342:1243417

Prasai MJ, George JT, Scott EM (2008) Molecular clocks, type 2 diabetes and cardiovascular disease. Diab Vasc Dis Res 5:89–95

Preitner N, Damiola F, Lopez-Molina L, Zakany J, Duboule D, Albrecht U, Schibler U (2002) The orphan nuclear receptor REV-ERB α controls circadian transcription within the positive limb of the mammalian circadian oscillator. Cell 110:251–260

Preitner N, Brown S, Ripperger J, Le Minh N, Damiola F, Schibler U (2003) Orphan nuclear receptors, molecular clockwork, and the entrainment of peripheral oscillators. Novartis Found Symp 253:89–99

Prokopenko I, Langenberg C, Florez JC, Saxena R, Soranzo N, Thorleifsson G, Loos RJ, Manning AK et al (2009) Variants in MTNR1B influence fasting glucose levels. Nat Genet 41:77–81

Pulimeno P, Mannic T, Sage D, Giovannoni L, Salmon P, Lemeille S, Giry-Laterriere M, Unser M, Bosco D, Bauer C, Morf J, Halban P, Philippe J, Dibner C (2013) Autonomous and self-sustained circadian oscillators displayed in human islet cells. Diabetologia 56:497–507

Qian J, Block GD, Colwell CS, Matveyenko AV (2013) Consequences of exposure to light at night on the pancreatic islet circadian clock and function in rats. Diabetes 62:3469–3478

Ramsey KM, Bass J (2009) Obeying the clock yields benefits for metabolism. Proc Natl Acad Sci USA 106:4069–4070

Ramsey KM, Yoshino J, Brace CS, Abrassart D, Kobayashi Y, Marcheva B, Hong HK, Chong JL, Buhr ED, Lee C, Takahashi JS, Imai S, Bass J (2009) Circadian clock feedback cycle through NAMPT-mediated NAD^+ biosynthesis. Science 324:651–654

Reick M, Garcia JA, Dudley C, McKnight SL (2001) NPAS2: an analog of clock operative in the mammalian forebrain. Science 293:506–509

Robertson RP (2006) Oxidative stress and impaired insulin secretion in type 2 diabetes. Curr Opin Pharmacol 6:615–619

Ronn T, Wen J, Yang Z, Lu B, Du Y, Groop L, Hu R, Ling C (2009) A common variant in MTNR1B, encoding melatonin receptor 1B, is associated with type 2 diabetes and fasting plasma glucose in Han Chinese individuals. Diabetologia 52:830

Rudic RD, McNamara P, Curtis AM, Boston RC, Panda S, Hogenesch JB, FitzGerald GA (2004) BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. PLoS Biol 2:e377

Sadacca LA, Lamia KA, Delemos AS, Blum B, Weitz CJ (2011) An intrinsic circadian clock of the pancreas is required for normal insulin release and glucose homeostasis in mice. Diabetologia 54:120

Saini C, Suter DM, Liani A, Gos P, Schibler U (2011) The mammalian circadian timing system: synchronization of peripheral clocks. Cold Spring Harb Symp Quant Biol 76:39

Salgado-Delgado R, Angeles-Castellanos M, Saderi N, Buijs RM, Escobar C (2010) Food intake during the normal activity phase prevents obesity and circadian desynchrony in a rat model of night work. Endocrinology 151:1019–1029

Scheer FAJL, Hilton MF, Mantzoros CS, Shea SA (2009) Adverse metabolic and cardiovascular consequences of circadian misalignment. Proc Natl Acad Sci 106:4453

Schibler U (2009) The 2008 Pittendrigh/Aschoff lecture: peripheral phase coordination in the mammalian circadian timing system. J Biol Rhythms 24:3–15

Schuld A, Hebebrand J, Geller F, Pollmacher T (2000) Increased body-mass index in patients with narcolepsy. Lancet 355:1274–1275

Spiegel K, Tasali E, Penev P, Van Cauter E (2004) Brief communication: sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. Ann Intern Med 141:846–850

Spiegel K, Tasali E, Leproult R, Van Cauter E (2009) Effects of poor and short sleep on glucose metabolism and obesity risk. Nat Rev Endocrinol 5:253–261

Staels B (2006) When the clock stops ticking, metabolic syndrome explodes. Nat Med 12:54–55

Stamenkovic JA, Olsson AH, Nagorny CL, Malmgren S, Dekker-Nitert M, Ling C, Mulder H (2012) Regulation of core clock genes in human islets. Metabolism 61:978–985

Stephan FK, Zucker I (1972) Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc Natl Acad Sci USA 69:1583–1586

Storch KF, Lipan O, Leykin I, Viswanathan N, Davis FC, Wong WH, Weitz CJ (2002) Extensive and divergent circadian gene expression in liver and heart. Nature 417:78–83

Taheri S, Lin L, Austin D, Young T, Mignot E (2004) Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. PLoS Med 1:e62

Teboul M, Guillaumond F, Grechez-Cassiau A, Delaunay F (2008) The nuclear hormone receptor family round the clock. Mol Endocrinol 22:2573–2582

Tei H, Okamura H, Shigeyoshi Y, Fukuhara C, Ozawa R, Hirose M, Sakaki Y (1997) Circadian oscillation of a mammalian homologue of the Drosophila period gene. Nature 389:512–516

Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen DR, Eckel RH, Takahashi JS, Bass J (2005) Obesity and metabolic syndrome in circadian clock mutant mice. Science 308:1043–1045

Van Cauter E (1990) Diurnal and ultradian rhythms in human endocrine function: a minireview. Horm Res 34:45–53

Van Cauter E, Holmback U, Knutson K, Leproult R, Miller A, Nedeltcheva A, Pannain S, Penev P, Tasali E, Spiegel K (2007) Impact of sleep and sleep loss on neuroendocrine and metabolic function. Horm Res 67(Suppl 1):2–9

van der Horst GT, Muijtjens M, Kobayashi K, Takano R, Kanno S, Takao M, de Wit J, Verkerk A, Eker AP, van Leenen D, Buijs R, Bootsma D, Hoeijmakers JH, Yasui A (1999) Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. Nature 398:627–630

Vieira E, Marroqui L, Figueroa AL, Merino B, Fernandez-Ruiz R, Nadal A, Burris TP, Gomis R, Quesada I (2013) Involvement of the clock gene Rev-erb α in the regulation of glucagon secretion in pancreatic α-cells. PLoS One 8:e69939

Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, McDonald JD, Dove WF, Pinto LH, Turek FW, Takahashi JS (1994) Mutagenesis and mapping of a mouse gene, clock, essential for circadian behavior. Science 264:719–725

Vitaterna MH, Selby CP, Todo T, Niwa H, Thompson C, Fruechte EM, Hitomi K, Thresher RJ, Ishikawa T, Miyazaki J, Takahashi JS, Sancar A (1999) Differential regulation of mammalian period genes and circadian rhythmicity by cryptochromes 1 and 2. Proc Natl Acad Sci USA 96:12114–12119

Xu YQ, Zhang D, Jin T, Cai DJ, Wu Q, Lu Y, Liu J, Klaassen CD (2012) Diurnal variation of hepatic antioxidant gene expression in mice. PLoS One 7:e44237

Yamaguchi Y, Suzuki T, Mizoro Y, Kori H, Okada K, Chen Y, Fustin JM, Yamazaki F, Mizuguchi N, Zhang J, Dong X, Tsujimoto G, Okuno Y, Doi M, Okamura H (2013) Mice genetically deficient in vasopressin V1a and V1b receptors are resistant to jet lag. Science 342:85–90

Yang X, Downes M, Yu RT, Bookout AL, He W, Straume M, Mangelsdorf DJ, Evans RM (2006) Nuclear receptor expression links the circadian clock to metabolism. Cell 126:801–810

Yin L, Wu N, Curtin JC, Qatanani M, Szwergold NR, Reid RA, Waitt GM, Parks DJ, Pearce KH, Wisely GB, Lazar MA (2007) Rev-erb α, a heme sensor that coordinates metabolic and circadian pathways. Science 318:1786–1789

Young MW, Kay SA (2001) Time zones: a comparative genetics of circadian clocks. Nat Rev Genet 2:702–715

Zheng B, Albrecht U, Kaasik K, Sage M, Lu W, Vaishnav S, Li Q, Sun ZS, Eichele G, Bradley A, Lee CC (2001) Nonredundant roles of the mPer1 and mPer2 genes in the mammalian circadian clock. Cell 105:683–694

Wnt Signaling in Pancreatic Islets

25

Joel F. Habener and Zhengyu Liu

Contents

Abstract

The Wnt signaling pathway is critically important not only for stem cell amplification, but also for the differentiation and migration and for organogenesis and the development of the body plan. β-catenin/TCF7L2-dependent

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_13,

Wnt signaling (the canonical pathway) is involved in pancreas development, islet function, and insulin production and secretion. The glucoincretin hormone glucagon-like peptide-1 and the chemokine stromal cell-derived factor-1 modulate canonical Wnt signaling in β cells which is obligatory for their mitogenic and cytoprotective actions.

Genome-wide association studies have uncovered approximately 90 gene loci that confer susceptibility for the development of type 2 diabetes (Marchetti P, Syed F, Suleiman M, Bugliani M, Marselli L, Islets 4:323–332, 2012). The majority of these diabetes risk alleles encode proteins that are implicated in islet growth and functioning (Marchetti P, Syed F, Suleiman M, Bugliani M, Marselli L, Islets 4:323–332, 2012, Ahlqvist E, Ahluwalia TS, Groop L, Clin Chem 57:241–254, 2011). At least 20 of the type 2 diabetes genes that affect islet functions are either components of or known target genes for Wnt signaling. The transcription factor TCF7L2 is particularly strongly associated with risk for diabetes and appears to be fundamentally important in both canonical Wnt signaling and β cell functioning. Experimental loss of TCF7L2 function in islets and polymorphisms in TCF7L2 alleles in humans impair glucose-stimulated insulin secretion suggesting that perturbations in the Wnt signaling pathway may contribute substantially to the susceptibility for, and pathogenesis of, type 2 diabetes. This review focuses on considerations of the hormonal regulation of Wnt signaling in islets and implications for mutations in components of the Wnt signaling pathway as a source for risk alleles for type 2 diabetes.

Keywords

β cell regeneration • Cell proliferation • Cytoprotection • Glucagon-like peptide-1 • Stromal cell-derived factor-1 • Tcf7L2

The Diabetes Problem

The prevalence of diabetes mellitus and its accompanying complications is increasing in populations throughout the world (American Diabetes Association, http://www.diabetes.org/about-diabetes.jsp). Diabetes results from a deficiency of the β cells of the islets of Langerhans to produce insulin in amounts sufficient to meet the body's needs, either absolute deficiency (type 1 diabetes) or relative deficiency (type 2 diabetes). In both forms of diabetes, the remaining β cells are placed under stress by (1) being forced to overproduce insulin to compensate for the lost β cells, (2) insulin resistance, and (3) by the glucotoxic effects of prolonged, sustained hyperglycemia. In the USA, 20 million individuals are currently afflicted with some form of diabetes, while an estimated 12 million additional people in the USA have diabetes but do not know it yet (Juvenile Diabetes Research Foundation, http://www.jdrf.org/index.cfm?fuseaction=home.viewPage&page_id=71927021-99EA-4D04-92E8463E607C84E1). Worldwide, an estimated 347 million people have the disease, and this global figure is expected to continue to increase in epidemic

proportions (World Health Organization fact sheet #312, March 2013; http://who.int/topics/diabetes_mellitus/en/). Type 2 diabetes is the most prevalent form of diabetes comprising >90 % of all diabetes. Most individuals who develop type 2 diabetes do so in association with obesity (Jin and Patti 2009). Because a common feature of both type 1 and type 2 diabetes is a reduction in β cell mass, understanding the factors and the cellular mechanisms that govern β cell growth and survival may lead to new effective treatments for diabetes.

In adult rats and mice the entire mass of the β cells in the pancreas turns over approximately every 50 days (2–3 % per day) by processes of apoptosis counterbalanced by replication from existing β cells and neogenesis from progenitor cells believed to be located in the pancreatic ducts and possibly within the islets (Bonner-Weir and Sharma 2006). The adult pancreas of rodents, including the endocrine islets, has a substantial capacity for regeneration (Jensen et al. 2005). Rodent models of pancreatic injuries are followed by partial to nearly complete regeneration of the exocrine and endocrine pancreas. Such models of pancreas regeneration include partial pancreatectomy (Pauls and Bancroft 1950), streptozotocin-mediated ablation of the β cells (Cheta 1998; Rees and Alcolado 2005), duct ligation, and caerulein treatments (Sakaguchi et al. 2006).

Whether or not endocrine stem/progenitor cells exist in the adult pancreas and can give rise to new β cells has been a topic of debate (Bonner-Weir and Sharma 2006; Dor et al. 2004). Lineage tracing studies in mice during pancreas regeneration following partial pancreatectomy found that the majority of new β cells derive from preexisting insulin-expressing cells without evidence of regeneration from stem/progenitor cells (Dor et al. 2004; Cano et al. 2008). Compelling new evidence, however, indicates that stem-like progenitor cells reside in the duct linings of adult mouse pancreas (Xu et al. 2008; Collombat et al. 2009; Al-Hasani et al. 2013; Courtney et al. in press). Pancreas injury induced by partial duct ligation promotes the appearance and the expansion of a population of cells within the duct lining that express the transcription factor neurogenin-3 (Ngn3), a modulator of Wnt signaling (Gradwohl et al. 2000) and a major determinant of endocrine lineage commitment during pancreas development (Serafimidis et al. 2008). In adult mouse models with manipulations of the functions of the transcription factors Pax4 (paired homeobox-4) and Arx (Aristalis homeobox), the Ngn3-expressing cells differentiate into α cells and then transdifferentiate into β cells in response to metabolic stress and/or injuries of β cells (Collombat et al. 2009; Al-Hasani et al. 2013; Courtney et al. in press). Moreover, the β cells of mice undergoing metabolic stresses, such as glucotoxicity, dedifferentiate into Ngn3-expressing cells, many of which differentiate into α cells (Talchai et al. 2012).

Genome-wide scans of several large populations of diabetic cohorts have uncovered 70 or more of the genes associated with type 2 diabetes (Lyssenko et al. 2008; Florez 2008a; Van Hoek et al. 2008; Florez 2008b; Ahlqvist et al. 2011; Marchetti et al. 2012). Of note, the majority of the diabetes genes identified thus far appear to be involved in islet development and functions and, most notably, the functions of

the insulin-producing β cells in the islets (Ahlqvist et al. 2011; Marchetti et al. 2012). Furthermore, as discussed later in this chapter, several of these genes appear to be involved in the Wnt signaling pathway, either components of the Wnt signaling system itself or target genes for downstream Wnt signaling by β-catenin and TCF7L2. The Wnt signaling pathway may be involved in the dysfunction of β cells in type 2 diabetes (Lee et al. 2008). Attention is directed to recent reviews on the role of Wnt signaling in pancreas development and function (Welters and Kulkarni 2008; Murtaugh 2008; Jin 2008) and the importance of the transcription factor TCF7L2 in pancreatic islet function and diabetes (Marchetti et al. 2012; Lyssenko 2008; Jin and Liu 2008; Perry and Frayling 2008; Cauchi and Froguel 2008; Bordonaro 2009; Hattersley 2007; Weedon 2007; Chiang et al. 2012; Florez 2007; Xiong et al. 2012; Owen and McCarthy 2007a; Smith 2007). In this review evidence is considered for the regulation of islet β cell functions by β-catenin/TCF7L2 induced by glucagon-like peptide-1 and stromal cell-derived factor-1, and speculations are presented on the potential involvement of the Wnt signaling pathway in the genetic predisposition to type 2 diabetes.

Wnt Signaling Pathways

The Wnt signaling cascade controls several cellular functions, including differentiation, proliferation, and migration (Kikuchi et al. 2006; Willert and Jones 2006; Moon et al. 2004; Nelson and Nusse 2004; Logan and Nusse 2004; Gordon and Nusse 2006; Nusse 2008). Useful brief summaries of the Wnt signaling pathways are provided in references MacDonald et al. 2007 and Semenov et al. 2007. The Wnt proteins form a large family of cell-secreted factors that control diverse aspects of development and organogenesis. Wnt proteins exert their effect by binding to cell-surface G-protein-coupled Frizzled (Fz) receptors and the lipoprotein receptor-like proteins, LRP5/6 co-receptors, and modulate the expression of various target genes through a series of intracellular processes ultimately leading to the regulation of transcription. There are several recognized Wnt signaling pathways: the β-catenin dependent, so-called canonical Wnt pathway that is dependent on the activation of the transcriptional complex of proteins consisting of β-catenin and TCF/LEF (Fig. 1), and several (at least nine) distinct and complex β-catenin, TCF/LEF-independent, noncanonical pathways, two of which are illustrated (Logan and Nusse 2004 and Fig. 2). In addition to Wnt signaling pathways activated by Wnt interactions with frizzed receptors, certain hormones, such as glucagon-like peptide-1 (GLP-1) and stromal cell-derived factor-1 (CXCL12) that activate G-protein-coupled receptors, can activate downstream Wnt signaling pathways via unique mechanisms culminating in the formation of transactivating complexes consisting of β-catenin and the DNA-binding proteins of the TCF/LEF family (see below) (Xiong et al. 2012; Liu and Habener 2008, 2009).

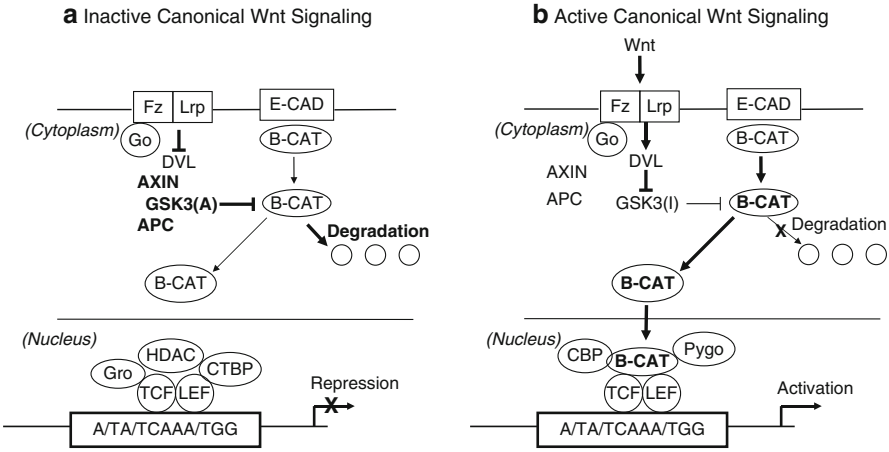

Fig. 1 Models depicting the canonical, β-catenin/TCF/LEF-dependent Wnt signaling pathway in inactive and active states. (**a**). Inactive Wnt signaling. In the absence of Wnt ligand-mediated activation of its receptor Frizzled (Fz), β-catenin in the cytoplasm is phosphorylated by the protein kinases glycogen synthase kinase-3β (GSK3β) and casein kinase 1 α (CK1α) leading to its degradation by proteosome complexes. GSK3β and CK1α are constitutively activated by the cofactors adenomatous polyposis coli (APC) and axin, which along with GSK3β and CK1α are known as the destruction complex. In the absence of sufficient levels of cytosolic β-catenin, nuclear levels are depleted and the DNA-binding transcription factors TCF and LEF act as repressors of gene transcription by the recruitment of corepressors such as Groucho and CtBP. (**b**). Active Wnt signaling. In the presence of Wnt ligands, Fz is activated via G-protein Gαi/o and small GTPases leading to the activation of disheveled (DVL) that disrupts the destruction complex composed of GSK3, CK1, APC, and axin, thereby inhibiting the activities of GSK3 and CK1. In the absence of phosphorylation, unphosphorylated β-catenin is stabilized and translocated to the nucleus where it non-covalently associates with TCF/LEF DNA-binding proteins and recruits co-activators such as CBP and Pygo resulting in the activation of gene transcription

The Canonical Wnt Signaling Pathway

The downstream canonical Wnt signaling pathway is defined as the pathway that ends in the formation of active, productive transcriptional, transactivation complexes composed of β-catenin and the DNA-binding proteins TCF (T-cell factor) and LEF (lymphocyte enhancer factor) (Fig. 1). It involves β-catenin that when stabilized translocates to the nucleus where it associates with the TCF/LEF family of transcription factors to regulate the expression of canonical Wnt target genes. In the absence of a Wnt signal, β-catenin is efficiently captured by the scaffold protein axin, which is present within a protein complex (referred to as the destruction complex) that also harbors adenomatous polyposis coli (APC), glycogen synthase kinase (GSK)-3, and casein kinase 1 (CSNK1) (Fig. 1a). The resident CSNK1 and GSK3 protein kinases sequentially phosphorylate conserved serine and threonine residues in the N-terminus of β-catenin subsequently targeting it for ubiquitination and degradation. The efficient suppression of β-catenin levels

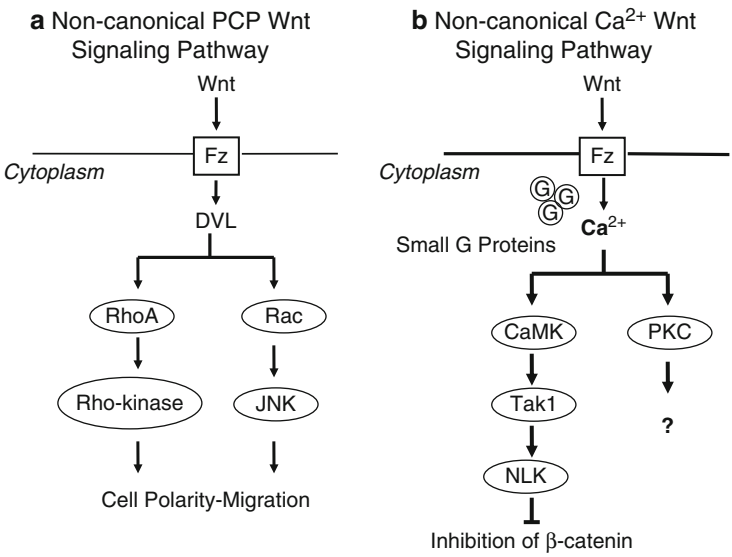

Fig. 2 Two models depicting noncanonical β-catenin-independent Wnt signaling pathways. (**a**). The planar cell polarity (PCP) pathway. The activation of Fz by Wnts leads to the activation of DVL and small G-proteins such as RhoA and Rac and the kinases Rho-kinase and Jun kinase (JNK). Through as yet undefined pathways, Rho-kinase and JNK modulate changes in the cytoskeleton involved in cell migration and polarity. (**b**). The Ca^{2+} pathway. Wnt ligands such as Wnt 5a activate Ca^{2+}-activated calmodulin kinases. CaMK and downstream kinases TAK1 and NLK. This pathway inhibits the canonical β-catenin-dependent Wnt signaling pathway and is active during gastrulation. The Ca^{2+} pathway also activates protein kinase C (PKC)

ensures that Groucho (TLE) proteins are free to bind members of the lymphocyte enhancer factor (LEF)/T-cell factor (TCF) family of transcription factors occupying the promoters and enhancers of Wnt target genes in the nucleus. These transcriptionally repressive complexes actively suppress the Wnt target genes such as c-Myc and cyclin D1, thereby silencing an array of biological responses, including cell proliferation. Rapid activation of the canonical pathway occurs when Wnt proteins interact with specific receptor complexes comprising members of the Frizzled family of proteins and the low-density lipid co-receptor LRP5 or LRP6 (Fig. 1b). The ligand-receptor binding activates the intracellular protein, disheveled (Dvl), which inhibits APC-GSK3β-axin activity and subsequently blocks degradation of β-catenin. This stabilization of β-catenin allows it to accumulate and translocate to the nucleus where it forms a transcriptionally active complex with the DNA-binding TCF transcription factors to activate the expression of Wnt signaling target genes.

In pancreatic β cells TCF7L2 is a major form of TCF involved in downstream Wnt signaling responsible for the activation of growth-promoting and survival (anti-apoptosis) genes in response to glucagon-like peptide-1 (GLP-1) agonists (Liu and Habener 2008; Shu et al. 2008; Boutant et al. 2012; Heller et al. 2011). Notably, as mentioned above and discussed in detail later, TCF7L2 is a major

susceptibility factor for the development of T2D manifested by diminished insulin production (Jin 2008; Lyssenko 2008; Hattersley 2007; Chiang et al. 2012; Florez 2007; Schafer et al. 2007).

Noncanonical Wnt Signaling

Wnt signaling via Frizzled receptors can also lead to the activation of noncanonical pathways that are independent of β-catenin and TCF/LEF complexes (Semenov et al. 2007). Two of the several recognized (Semenov et al. 2007) β-catenin-independent pathways are considered (Fig. 2). One such noncanonical pathway consists of the release of intracellular calcium. Other intracellular second messengers associated with this pathway include heterotrimeric G-proteins, phospholipase C (PLC), and protein kinase C (PKC). The Wnt/Ca^{2+} pathway is important for cell adhesion and cell movements during gastrulation (Komiya and Habas 2008). Wnt/Ca^{2+} pathway is also known to control cell migration and involved in regulating endothelial cell migration. Interestingly, The Wnt/Ca^{2+} pathway may antagonize the canonical Wnt/β-catenin pathway. The canonical and noncanonical Wnt pathways are likely to have opposing effect on endothelial cells and probably antagonize each other in order to finely balance endothelial cell growth.

The Wd/planar cell polarity (PCP) signaling pathway is a second noncanonical Wnt signaling pathway (Komiya and Habas 2008; Wada and Okamoto 2009; Veeman et al. 2003). PCP controls tissue polarity and cell movement through the activation of RhoA, c-Jun N-terminal kinase (JNK), and nemo-like kinase (NLK) signaling cascades. In the planar cell polarity pathway, Wnt signaling through Frizzled receptors mediates asymmetric cytoskeletal organization and polarization of cells by inducing modifications to the actin cytoskeleton.

Wnt Signaling in Pancreas Development and Regeneration

Expression of components of the Wnt signaling pathway, including Wnt ligand family members and various Frizzled receptors, is well documented in the developing mouse, rat, chick, fish, and human pancreas (Heller et al. 2002, 2003; Pedersen and Heller 2005; Kim et al. 2005; Wang et al. 2006). A description of the subsets of the dozen or so Wnt ligands, Frizzled receptors, and the Wnt/FZ regulators, secreted frizzle-related proteins, and Dickkopfs is provided in Heller et al. (Liu and Habener 2008). Endogenous Wnt signaling also occurs in mouse and rat β cell lines (Liu and Habener 2008). Detailed information on the cellular distributions of expression of the various Wnt ligands, receptors, and regulators is not available. From the findings of Heller et al. (2002), it is clear that Wnt signaling factors are expressed both in epithelium and in mesenchyme. Several studies confirm that functional Wnt signaling is active in islets throughout development. A Wnt reporter strain of mice, in which lacZ was inserted into the locus of the Wnt target gene conductin/axin2, expressed β-galactosidase, the product of the

lacZ gene, throughout the islets (Dessimoz et al. 2005). Expression of the conductin gene is transcriptionally activated by the canonical Wnt pathway via TCF binding sites in its promoter. Furthermore, the β-galactosidase (lacZ) reporter activity is maintained in islets of mice up to 6 weeks after birth. A monoclonal antibody specific for the non-phosphorylated form of β-catenin revealed a strong immunoreactivity in the pancreatic epithelium of the mouse at embryonic day 13 (Papadopoulou and Edlund 2005). Taken together, human and rodent islets and rodent β cell lines are known to express members of the Wnt ligand and Frizzled receptor families, along with modulators of Wnt signaling, the LRP co-receptors, and secreted Dkk (Dickkopf) proteins. Another source of Wnt ligands is adipose tissue (Schinner et al. 2008). Adipocytes secrete a wide range of signaling molecules including Wnt proteins. Fat-cell-conditioned media from human adipocytes increases the proliferation of INS-1 β cells and induces Wnt signaling, which could contribute to the β cell hyperplasia that occurs in humans and rodents in response to obesity. Interestingly inhibitory noncanonical Wnt ligand Wnt5b gene is associated strongly with obesity and type 2 diabetes (Schinner et al. 2008). Expression of Wnt5b in preadipocytes increases adipogenesis and expression of adipokine genes through the inhibition of canonical Wnt signaling (Schinner et al. 2008). Thusly, alterations in Wnt5b levels in humans could alter adipogenesis and, consequently, affect the risk of diabetes onset.

Wnt signaling loss-of-function studies. Following early pancreas specification, Wnt signaling appears to be indispensable for pancreas development, although its precise role remains controversial. The majority of studies have shown that Wnt signaling is essential in the development of the exocrine pancreas. Disruption of the Wnt signaling pathway results in an almost complete lack of exocrine cells (Dessimoz et al. 2005; Papadopoulou and Edlund 2005; Murtaugh et al. 2005; Wells et al. 2007). However, its role in endocrine cell development is still uncertain. Several studies in which Wnt signaling is abolished by conditional β-catenin knockout in the developing mouse pancreas have revealed that the endocrine component of the pancreas develops normally and is functionally intact. In the studies of Murtaugh et al. (2005) and Wells et al. (2007) in which the β-catenin gene in the epithelium of the pancreas and duodenum was specifically deleted, pancreatic islets are intact and contain all lineages of endocrine cells. In contrast, using a different β-catenin knockout approach, Dessimoz et al. (2005) found a reduction in endocrine islet numbers. Selective deletion of β-catenin in β cells of mice resulted in defective insulin secretion, reduced endocrine tissue, and perinatal mortality (Dabernat et al. 2009). Knockdown of β-catenin by administration of antisense RNA to normal and diabetic rats altered the normal and compensatory growth of β cells, mainly through the inhibition of proliferation (Figeac et al. 2010). It is worth noting that knockout studies should be interpreted with some caution because of the potential occurrence of adaptive compensatory mechanisms that could alter the phenotype. Furthermore, the use of different strains of mice expressing PDX-Cre, which have different recombination efficiencies, is expressed at different stages of development and is shown to have mosaic expression in the pancreata of transgenic mice (Heiser et al. 2006). It seems possible that β-catenin

and Wnt signaling have several different roles throughout the development of the pancreas. Since the timing of the activation or inactivation of Wnt signaling is crucial for its effects on pancreas development, the currently available Cre-based recombinant technology might not be adequate to fully explore the role of Wnt signaling. Collectively, the loss-of-function studies have not yet provided a definitive role for β-catenin in the development and/or maintenance of function of adult islets. Nonetheless, these results underscore the possible dual nature of Wnt signaling in pancreas growth and development. Excessive Wnt signaling activation prevents proper differentiation and expansion of early pancreatic progenitor cells during early, first transition, specification. During the second transition, β-catenin acts as a pro-proliferative cue that induces gross enlargement of the exocrine and/or endocrine pancreas.

Wnt signaling gain-of-function studies. Gain-of-function experiments suggest an inhibitory role for Wnt pathway in pancreas specification, a stage when cells at the appropriate regions of the foregut begin to form a bud. Heller et al. (2002) showed that forced mis-expression of Wnt1 driven by PDX-1 promoter in mice induces a block in the expansion and differentiation of PDX-1 positive cells and causes ensuing reduction in endocrine cell number and a lack of organized islet formation. Excessive Wnt signaling in the epithelia limits the expansion of both the mesenchyme and epithelium and inhibits growth of the pancreas and islets. Using a different approach, the Heiser et al. (2006) study reached a similar conclusion. The conditional knockin of stable β-catenin in early pancreatic development of mice using PDX-1-driven Cre recombinase efficiently targets all three pancreatic lineages – the endocrine, exocrine, and duct – and results in upregulation of Hedgehog and leads to a loss of PDX-1 expression in early pancreatic progenitor cells (Heiser et al. 2006).

This genetic model of forced overexpression of β-catenin prevents normal formation of the exocrine and endocrine compartments of the pancreas. Using a Xenopus model, McLin et al. (2007) found forced Wnt/β-catenin signaling in the anterior endoderm, between gastrula and early somite stages, inhibits foregut development. By contrast, blocking β-catenin activity in the posterior endoderm is sufficient to initiate ectopic pancreas development (Heiser et al. 2006). These genetic manipulations of Wnt signaling in mice suggest a contribution of both inhibitory and facilitating roles of Wnt signaling during pancreas development. The gain-of-function studies by Dessimoz et al. (2005) show a distinctive role of Wnt signaling in endocrine development. Wnt3a induces the proliferation of islet and MIN-6 cells (Rulifson et al. 2007). The addition of the soluble Wnt inhibitor, Fz 8-cysteine-rich domain (Fz8-CRD), eliminated this stimulatory effect of Wnt3a on cell proliferation (Rulifson et al. 2007). The treatment of islets with Wnt3a significantly increased mRNA levels of cyclin D1, cyclin D2, and CDK4, all of which have Wnt responsive elements in the promoter regions of their genes (Wang et al. 2006). Conditional knockin of active β-catenin in mice promotes the expansion of functional β cells (Heiser et al. 2006), whereas the conditional knock-in of the Wnt inhibitor, axin, impaired proliferation of neonatal β cells (Rulifson et al. 2007).

Surprisingly, recent studies found that Wnt signaling may play a role in regulating the secretory function of mature β cells (Fujino et al. 2003). The Wnt

co-receptor, LRP5, is required for glucose-induced insulin secretion from the pancreatic islets. The knockout of LRP5 in mice resulted in glucose intolerance (Fujino et al. 2003). Treatment of isolated mouse islets with purified Wnt3a and Wnt5a ligands causes potentiation of glucose-stimulated insulin secretion. Thus, LRP5 together with Wnt proteins appears to modulate glucose-induced insulin secretion. Furthermore, Schinner et al. (2008) reported that activating Wnt signaling increases insulin secretion in primary mouse islets and activates transcription of the glucokinase gene in both islets and INS-1 cells. The consummate evidence came in isolated mouse and human islets, in which reducing levels of TCF7L2 by siRNA decreases glucose-stimulated insulin secretion, expression of insulin and PDX-1, and insulin content (Shu et al. 2008; Loder et al. 2008; da Silva et al. 2009).

Role of Wnt Signaling in β Cell Growth and Survival

In addition to its potential role in regulating glucose-stimulated insulin secretion, the Wnt pathway is involved in β cell growth and survival. The activation of Wnt signaling in β cell lines or primary mouse islets results in an expansion of the functional β cell mass, with findings consistent with the upregulation of pro-proliferative genes including cyclin D1 and D2 (Liu and Habener 2008). Furthermore, the mis-expression of a negative regulator of Wnt signaling, axin, impairs the proliferation of neonatal β cells, demonstrating a requirement for Wnt signaling during β cell expansion (Rulifson et al. 2007). Axin expression impaired normal expression of islet cyclin D2 and pitx2, a transcriptional activator that directly associates with promoter regions of the cyclin D2 gene. The inhibition of GSK3β activity in normal and diabetic rats by the administration of antisense oligonucleotides, or lithium chloride, increased the stability of β-catenin and enhanced β cell proliferation (Figeac et al. 2010). Shu et al. (2008) provide further evidence in support of a role for Wnt signaling in β cell growth and survival in both mouse and human islets. Depletion of TCF7L2 in human islets causes a decrease in β cell proliferation, an increase in levels of apoptosis, and a decline in levels of active Akt, an important β cell survival factor (Liu and Habener 2008). Similarly, in INS-1 cells, expression of dominant-negative TCF7L2 decreases proliferation rates (Liu and Habener 2008). Furthermore, overexpression of TCF7L2 in both mouse and human islets protects β cells against glucotoxicity or cytokine-induced apoptosis (Liu and Habener 2009).

Roles of Non-Wnt Hormonal Ligands in the Activation of the Wnt Signaling Pathway in Islets

Several hormones and growth factors, such as insulin, insulin-like growth factor-1, platelet-derived growth factor, parathyroid hormone, and prostaglandins, are known to activate the canonical and noncanonical Wnt signaling pathways. However, these observations have been made in non-islet tissues such as the

intestine, cancer cell lines, osteoblasts, and fibroblasts (Yi et al. 2008). It has been proposed that a primary function of Wnt signaling is to maintain stem cells in a pluripotent state and that growth factors such as FGF and EGF augment their proliferation (Nusse 2008). Very little is known, however, about the hormonal activation of Wnt signaling in pancreatic islets. Recent studies of glucagon-like peptide-1 (GLP-1) and stromal cell-derived factor-1 (SDF-1) actions on islet β cells demonstrate that both hormones activate downstream Wnt signaling via β-catenin/TCF7L2-regulated gene transcription and that downstream Wnt signaling is required for the pro-proliferative actions of GLP-1 (Liu and Habener 2008; Boutant et al. 2012) and the anti-apoptotic actions of SDF-1 (Liu and Habener 2009).

Downstream Wnt Signaling Requirement for GLP-1-Induced Stimulation of β Cell Proliferation

Glucagon-like peptide-1 (GLP-1) is a glucoincretin hormone released from the intestines in response to meals and stimulates glucose-dependent insulin secretion from pancreatic β cells (Kieffer and Habener 1999; Drucker 2006). GLP-1 also stimulates both the growth and the survival of β cells. GLP-1 is produced in the enteroendocrine L-cells that reside within the crypts of the intestinal mucosa by selective posttranslational enzymatic cleavages of the prohormonal polypeptide, proglucagon, the protein product of the expression of the glucagon gene (Gcg). Notably, the same proglucagon expressed from Gcg in the α cells of the pancreas is alternatively cleaved to yield the hormone glucagon in mature, fully differentiated α cells and is induced to produce GLP-1 and glucagon in response to β cell injuries or loss of glucagon signaling (Liu et al. 2011; Habener and Stanojevic 2012, 2013). Glucagon functions as an insulin counter-regulatory hormone to stimulate hepatic glucose production and thereby to maintain blood glucose levels in the postabsorptive, fasted state.

Genes expressed in Wnt signaling in β cells were examined using a focused Wnt signaling gene microarray and the clonal β cell line INS-1 (Liu and Habener 2008). Of the 118 probes represented on the Wnt signaling gene array, 37 were expressed above background in cultured INS-1 cells. Exposure of the cells to GLP-1 enhanced the expression of 14 of the genes, including cyclin D1 and c-Myc, strongly suggesting that GLP-1 agonists activate components and target genes of the Wnt signaling pathway. GLP-1 agonists activate β-catenin- and TCF7L2-dependent Wnt signaling in isolated mouse islets and INS-1 β cells, and antagonism of β-catenin by siRNAs and of TCF7L2 by a dominant-negative form of TCF7L2 inhibited GLP-1-induced proliferation (Liu and Habener 2008). These findings suggest that Wnt signaling is required for GLP-1-stimulated proliferation of β cells. Although INS-1 cells maintain high basal levels of Wnt signaling via Wnt ligands and Frizzled receptors, GLP-1 agonists specifically enhance Wnt signaling through their binding to the GLP-1 receptor (GLP-1R), a G-protein-coupled receptor coupled to GαS and the activation of cAMP-dependent protein kinase A (PKA).

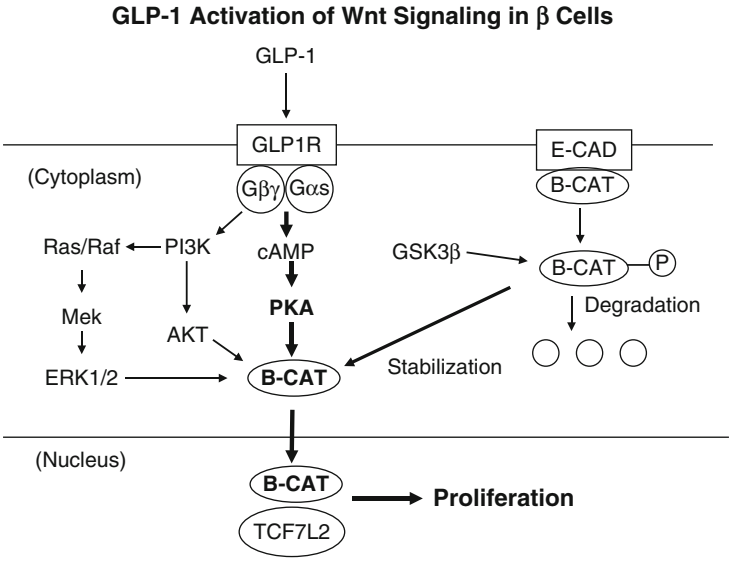

Fig. 3 Diagram summarizing the signaling pathway in pancreatic β cells by which GLP-1 actions couple to the downstream Wnt signaling pathway (Weedon 2007). The interaction of GLP-1 with the GLP-1 receptor (GLP-1R) activates G-protein α S (GαS) resulting in cAMP formation and activation of the cAMP-dependent protein kinase A (PKA). Remarkably, by the GLP-1-activated pathway, β-catenin is stabilized by direct phosphorylation by PKA, rendering it resistant to degradation in response to phosphorylations by GSK3β. This stabilization of β-catenin by PKA-mediated phosphorylation is a distinct departure from the canonical Wnt pathway in which phosphorylation of β-catenin by GSK3β results in its degradation. β-catenin thusly stabilized by PKA-mediated phosphorylation is resistant to degradation in response to phosphorylation by GSK3β, accumulates in the cytoplasm, and is translocated to the nucleus where it associates with TCF7L2 to form a productive transcriptional activation complex. β-catenin/TCF7L2 complexes activate the expression of target genes involved in β cell proliferation

Although PKA is not involved in maintaining basal levels of Wnt signaling, it is essential for the enhancement of Wnt signaling by GLP-1 (Liu and Habener 2008). In addition, the prosurvival protein kinase Akt, along with active MEK/ERK signaling, is required for maintaining both basal and GLP-1-induced Wnt signaling (Liu and Habener 2008; Fig. 3). In summary, both β-catenin and TCF7L2 appear to be required for GLP-1-mediated transcriptional responses and cell proliferation.

Downstream Wnt Signaling Requirement for SDF-1-Induced Promotion of β Cell Survival

SDF-1 is a chemokine originally identified as a bone marrow (BM) stromal cell-secreted factor and now recognized to be expressed in stromal tissues in multiple organs (Burger and Kipps 2006; Kucia et al. 2005; Ratajczak et al. 2006; Kryczek et al. 2007).

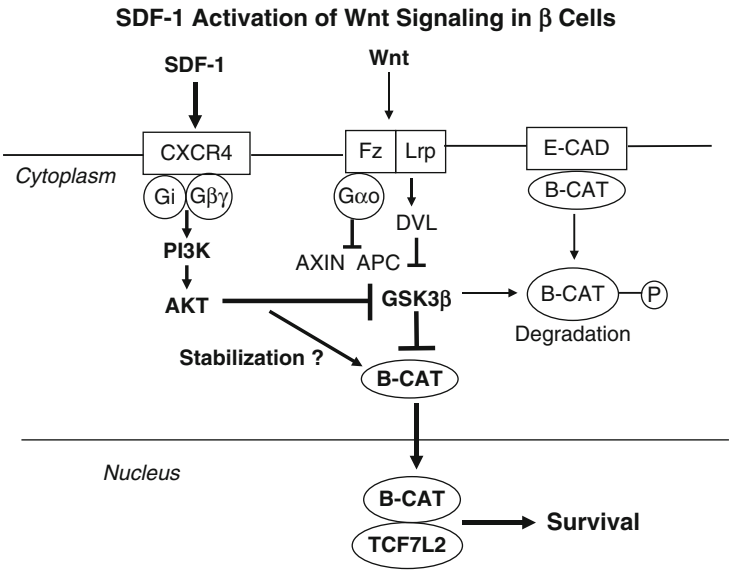

Fig. 4 Schematic model of signaling pathways utilized by SDF-1/CXCR4 in the activation of β catenin-/TCF7L2-mediated transcriptional expression of genes involved in β cell survival. Interactions of SDF-1 with its G-protein-coupled receptor CXCR4 activates G-protein i/o that activates the phosphoinositol kinase 3 (PI3K) and the downstream prosurvival kinase Akt. Akt is a potent inhibitor of the Wnt signaling destruction complex composed of axin, APC, and GSK3β. Inhibition of GSK3β by Akt results in the inhibition of phosphorylation of β-catenin by GSK3, prevents the degradation of β-catenin, and thereby results in the stabilization of β-catenin which accumulates in the cytoplasm and enters the nucleus, where it associates with TCF7L2. The β-catenin/TCF7L2 forms a transcriptional activation complex that activates the expression of genes that promote β cell survival. A direct action of Akt on the stabilization of β-catenin remains conjectural

The most extensively studied function of the SDF-1/receptor CXCR4 axis is that of chemoattraction involved in leukocyte trafficking and stem cell homing in which local tissue gradients of SDF-1 attract circulating stem/progenitor cells. SDF-1/CXCR4 signaling in the pancreas remains relatively unexplored. Kayali and co-workers reported expression of SDF-1 and CXCR4 in the fetal mouse pancreas and CXCR4 in the proliferating duct epithelium of the regenerating pancreas of the nonobese diabetic mouse (Kayali et al. 2003). The cross talk between the SDF-1-CXCR4 axis and Wnt signaling pathway was first demonstrated by Luo et al. (2006) in studies of rat neural progenitor cells. Transgenic mice expressing SDF-1 in their β cells (RIP-SDF-1 mice) are protected against streptozotocin-induced diabetes through activation of the prosurvival protein kinase Akt and resulting downstream prosurvival, antiapoptotic signaling pathways (Yano et al. 2007). An examination of SDF-1-activated Wnt signaling in both isolated islets and INS-1 cells using a β-catenin-/TCF-activated reporter gene assay revealed enhanced Wnt signaling through the Gαi/o-PI3K-Akt axis, suppression of GSK3β, and stabilization of β-catenin (Liu and Habener 2009; Fig. 4). Phosphorylation of GSK3 by Akt

represses its phosphorylating activities on β-catenin and thereby reduces the degradation of β-catenin. Moreover, SDF-1 signaling in INS-1 β cells stimulates the accumulation of β-catenin mRNA, likely due to an enhancement in the transcription of the β-catenin gene (Liu and Habener 2009). Recent evidence also suggests that active Wnt signaling mediates, and is required for, the cytoprotective, survival actions of SDF-1 on β cells (Liu and Habener 2009).

Potential Mechanisms by Which GLP-1 and SDF-1 May Act Cooperatively on Wnt Signaling to Enhance β Cell Growth and Survival

There appear to be differences in the mechanisms of the interactions of SDF-1/CXCR4 signaling and GLP-1/GLP-1R signaling with the Wnt signaling pathway in β cells. Although both SDF-1 and GLP-1 activate the downstream pathway of Wnt signaling, consisting of β-catenin-/TCF7L2-mediated gene expression, they do so by way of different pathways of interactions with the more upstream components of the Wnt signaling pathway. These proposed different upstream pathways of signaling utilized by GLP-1 and SDF-1 raise the possibility of additive or synergistic effects on downstream Wnt signaling in the promotion of β cell growth and survival. SDF-1 inhibits the destruction complex of the canonical Wnt signaling pathway consisting of axin, APC, and the protein kinases, glycogen synthase kinase-3 (GSK3) and casein kinase-1 (CSNK1). This inhibition of GSK3 and CSNK1 by SDF-1 is likely mediated by the well-known actions of Akt to inhibit these kinases, resulting in the stabilization and accumulation of β-catenin. In marked contrast to the actions of SDF-1 on β cells, GLP-1 activates β-catenin/TCF7L2 complexes via the stabilization of β-catenin by a different mechanism involving the phosphorylation and stabilization of β-catenin by the cAMP-dependent protein kinase A (PKA). PKA activated by GLP-1/GLP-1R phosphorylates β-catenin on serine-675 resulting in its stabilization and accumulation. Thusly, unlike SDF-1, GLP-1-induced activation of gene expression by β-catenin/TCF7L2 in β cells occurs independently of the destruction box and the activities of GSK3. It also remains possible that β-catenin may be stabilized by its direct phosphorylation by Akt.

β-catenin is the activation domain, and TCF7L2 is the DNA-binding domain of the transactivator. It is tempting to speculate that different phosphorylations of β-catenin provided by SDF-1 signaling versus GLP-1 signaling result in different conformations of β-catenin. When different conformers of β-catenin interact with TCF7L2, they confer different conformations to the DNA-binding domains of TCF7L2 resulting in differing affinities of TCF7L2 for its cognate enhancer binding sites on the promoters of various Wnt signaling target genes. Such a combinatorial mechanism could account for the difference in genes regulated by β-catenin/TCF7L2 in β cells in response to SDF-1 compared to GLP-1.Wnt signaling may be a final downstream pathway for both SDF-1 and GLP-1 signaling in β cells. However, gene expression targets diverge so that SDF-1 predominately

regulates genes involved in cell survival, whereas GLP-1 regulates genes involved in cell cycle control (proliferation). If this circumstance proves to be valid, our findings raise the possibility of a dual therapeutic approach for increasing β cell mass. GLP-1 is predominantly pro-growth and SDF-1 is predominantly prosurvival. Thereby the two peptides may act synergistically to promote both the growth and survival of β cells and to conserve, or even enhance, β cell mass in response to injury.

Type 2 Diabetes Genes

Genome-wide scans in several large populations have uncovered associations of specific genetic loci with the development of type 2 diabetes (Lyssenko et al. 2008; Florez 2008a; Van Hoek et al. 2008; Florez 2008b; Ahlqvist et al. 2011; Marchetti et al. 2012; Zeggini et al. 2007; Saxena et al. 2007; Sladek et al. 2007; Scott et al. 2007; Grarup et al. 2007; Hayes et al. 2007; Cauchi et al. 2008; Ruchat et al. 2009; Owen and McCarthy 2007b; Moore et al. 2008; Steinthorsdottir et al. 2007; Palmer et al. 2008). At least 90 genes have associations with diabetes that are consistent among various population studies (Table 1). Of note, the majority of these genes (55 of 99) are expressed in pancreatic β cells. Further, several of the genes (20) appear to be involved in the Wnt signaling pathway. TCF7L2, the DNA-binding component of the downstream transcription factor complex, appears to have a particularly strong association with type 2 diabetes. Brief descriptions and specific references to the 20 genes implicated in Wnt signaling in the pancreas are given below. References to the remaining genes in Table 1 can be found in the recent review articles (References #25 and #26).

Genes Associated with Islet Development/Function and Wnt Signaling

TCF7L2 (transcription factor 7-like 2). Grant and co-workers provided the index report on an association of polymorphisms in TCF7L2 with type 2 diabetes (Grant et al. 2006). Epidemiology studies from Icelandic, Danish, and US cohorts reported that the inheritance of specific single nucleotide polymorphisms (SNPs), at the region DG10S478, within the intron 3 region of TCF7L2 gene is related to an increased risk of type 2 diabetes (Lyssenko 2008; Jin and Liu 2008; Perry and Frayling 2008; Cauchi and Froguel 2008; Bordonaro 2009; Hattersley 2007; Weedon 2007; Chiang et al. 2012; Florez 2007; Xiong et al. 2012; Owen and McCarthy 2007a; Smith 2007). Then two other SNPs within introns 4 and 5 of TCF7L2, namely, rs12255372 and rs7903146, were found in strong linkage disequilibrium with DG10S478 and showed similarly robust associations with type 2 diabetes patients with glucose intolerance. In Asian populations, the frequencies of SNPs rs7903146 and rs12255372 are quite low, but two novel SNPs – rs290487 and rs11196218 – are associated with the risk of type 2 diabetes in a Chinese population.

Table 1 Type 2 diabetes genes identified by candidate gene approach and genome-wide association studies (99)

Genes associated with islet development/function and Wnt signaling			
Gene symbol	Name(s)	β cell functions	Wnt signaling
TCF7L2	HMG transcription factor-7L2	β cell proliferation and survival, insulin secretion, impaired GLP-1 responses, increased hepatic glucose production	Canonical Wnt signaling, regulates target genes in association with β-catenin
FTO	Fatso. Fused toes locus. Includes FTS, FTM	Pancreas development, obesity	FTS, a target gene for Wnt signaling
NOTCH 2	Delta/notch signaling	Pancreas development	Wnt signaling interaction via phosphorylation by GSK3
IGF2BP2	Insulin growth factor 2 mRNA-binding protein 2	Islet growth	Expression induced by β-catenin and TCF7L2
HHEX	Hematopoietic homeobox transcription factor	Early pancreas development	Repressed by β-catenin and TCF7L2
CDKN2A/N2B	Cyclin-dependent kinase inhibitor, P16, INK4A	Islet regeneration regulates CDK4 in β cells	Cross talk with Wnt signaling, induced by β-catenin
HNF1B	HNF1 homeobox B	MODY 5, TCF2, islet development	β catenin, Wnt signaling
PROX1	Prospero homeobox 1	Pancreas development, islet endocrine development	Target Tcf/Lef/Ctnnb1 neural
PTPRD	Protein tyrosine phosphatase receptor type D	Growth, autism, diabetes	Target in PC12 cells
TLE4	Transducin-like enhancer of split 4, Xgrg4, Groucho	α cell development Nkx2.2	Hex β-catenin, co-repressor
ZBED3	Zinc finger BED-type containing protein 3	β cell function	Axin-interacting protein
TSPAN8/LGR5/GPR49	Tetraspanin 8. Leucine-rich G-protein-coupled receptor 5. G-protein-coupled receptor 49	Impaired GSIS	Wnt signaling target gene in intestinal crypt stem cells
TP53INP1	Tumor protein p53 inducible nuclear protein 1	β cell cytoprotection	Wnt signaling? Tcf7L2 regulated
WFS1	Wolfram syndrome 1 transmembrane protein	Insulin secretion, endoplasmic reticulum, protein trafficking	Strong target locus for TCF7L2 binding [Zhao 2010]
CDKAL1	Cyclin-dependent kinase 5 homolog inhibitor	Islet glucotoxicity, impaired insulin secretion	Strong target locus for TCF7L2 binding [Zhao 2010]
ADAMTS9	Metallopeptidase with thrombospondin 9	β cell survival and cytoprotection	Strong target locus for TCF7L2 binding [Zhao 2010]

(*continued*)

Table 1 (continued)

GLIS3	Kruppel zinc finger protein	Impaired GSIS	Regulates Ngn3 in β cells
GRK5	G-protein receptor kinase 5	Elevated plasma insulin levels	Regulates LRP6 Wnt signaling
SPRY1	Sprouty 1	Influences insulin secretion	Target for β-catenin/LEF
HNF4A	Hepatocyte nuclear factor 4	Impaired insulin secretion (MODY 1)	Liver zonation, intestinal development – Wnt signaling
Genes associated with islet development/function, Wnt signaling unknown (Bordonaro 2009)			
Gene symbol	Name(s)	β cell functions	
CDC123/CAMK1D	Cell division cycle 123/calcium/calmodulin dependent	β cell apoptosis? Impaired GSIS	
PPARgamma	Peroxisome proliferator receptor gamma	Insulin resistance, insulin secretion	
KCNJ11	Inward-rectifying potassium channel	Regulates insulin secretion and Sur1 (ABCC8)	
SLC30A8	Solute carrier 30a8 zinc transporter	Insulin granules, secretion	
KCNQ1	Potassium channel	Insulin secretion	
MTNR1B	Melatonin receptor 1b increased in response to GLP-1	Insulin secretion	
JAZF1	Nuclear zinc finger transcriptional repressor	β cell apoptosis? Impaired GSIS	
ADAM30	ADAM metallopeptidase domain 30	β cell oxidative stress?	
ADCY5	Adenylate cyclase 5	Increases conversion of proinsulin to insulin	
AIF1	Allograft inflammatory factor 1	Modulates insulin production and GSIS. Role in T1D	
ARAP1/CENTD2	ArfGAP with RhoGAP domain, ankyrin repeat with PH domain 1	Modulates GSIS	
THADA	Thyroid adenoma associated	Lower β cell response to GLP-1, arginine, decreased β cell mass?	
BCL11A	β-cell CLL/lymphoma zinc finger protein 11A	Altered GSIS	
CAPN10	Calpain 10 Ca^{++}-activated neutral proteinase	β cell exocytosis apoptosis, metabolism increased in T2D β cells	
DGKB/DGKG	Diacylglycerol kinases	Insulin secretion and β cell metabolism	

(*continued*)

Table 1 (continued)

ETV5	Ets variant 5	Mediates mesenchymal to epithelial signaling in pancreas development
GCK	Glucokinase hexokinase 4	MODY 2, impaired insulin secretion
HNF1A	HNF1 homeobox A	Insulin secretion, MODY 3
RASGRP1	RAS guanyl nucleotide-releasing protein	Influences insulin secretion
GCKR	Glucokinase regulator	Insulin resistance, obesity, metabolic syndrome
TMEM195	Transmembrane protein 195	Reduced GSIS
GIPR	Gastric inhibitory peptide receptor	Increased proinsulin production, blunted insulin response
C2CD4B	C2 calcium-dependent domain-containing 4B	Impaired GSIS
FADS1	Fatty acid desaturase-1	Abnormal early insulin secretion
G6PC2	Glucose-6-phosphatase catalytic 2	Abnormal early insulin secretion
MADD	MAP kinase-activating death domain protein	Abnormal insulin processing
CRY2	Cryptochrome 2	Associated with fasting insulin
SLC2A2	Solute carrier family 2 Member 2, glucose carrier	Impaired GSIS
VPS13C	Vacuolar protein sorting 13 homolog C	Abnormal insulin processing and impaired secretion
ADRA2A	α 2A adrenergic receptor	Impaired GSIS
ANK1	Ankyrin 1, erythrocytic	Integral membrane protein
BCL2	β-cell CLL/lymphoma 2	Anti-apoptosis protein
ST6GAL1	ST6 β-galactosamine α-like 1	Associated with β cell function
CMIP	C-Maf-inducing protein	Modulates Maf and PI3K signaling
COBLL1-GRB14	Cordon bleu-like 1 growth factor receptor bound 14	Actin nucleator
Genes not known to be involved in either islet development/function or Wnt signaling (Willert and Jones 2006)		
Gene symbol	Name(s)	Cell functions
DUSP9	Dual specificity phosphatase 9	Altered proinsulin conversion to insulin?

(*continued*)

Table 1 (continued)

BCDIN3D	BCDIN3 domain containing	BMI, obesity
CHCHD9	Coiled-coil-helix-coiled-coil-helix domain 9	Unknown
FAIM2	Fas apoptotic inhibitory molecule 2	Obesity related
GNPDA2	Glucosamine-6-P-deaminase 2	Obesity related
HHCA2	YY-associated protein1 HCCA2	Chromatin, oncogene insulin resistance
HMGA2	High-mobility group AT hook 2	Oncoprotein
IRS1	Insulin receptor substrate 1	Increased insulin resistance
KLF14	Kruppel-like factor 14	Increased insulin resistance
MCR4	Melanocortin 4 receptor	Obesity, hypothalamic
MTCH2	Mitochondrial carrier homolog 2	Obesity, mitochondrial death pathways
NCR3	Natural cytotoxicity triggering receptor 3	Autoimmunity modulation
NEGR1	Neuronal growth regulator 1	Obesity
PRC1	Protein regulator of cytokinesis 1	Cancer
RBMS1	RNA-binding motif single-stranded-interacting protein 1	DNA synthesis and gene transcription
SFRS10	Transformer 2 β homolog	Obesity, oxidative stress
SH2B1	SH2B adaptor protein 1	Obesity
SSR	Serine racemase	D-serine synthesis from L-serine
TMEM18	Transmembrane protein 18	Obesity DNA-binding protein
ZFAND6	Zinc finger AN1-type domain	Peroxisome biogenesis
HK1	Hexokinase 1 enzyme	Tissue ubiquitous, high-affinity glucose-phosphorylating enzyme
PEPD	Peptidase D, prolidase	Cytosolic dipeptidase, hydrolyzes dipeptides with proline or hydroxyproline at the carboxy terminus
UBE2E2	Ubiquitin-conjugating enzyme E2E2	Accepts ubiquitin and catalyzes its covalent attachment to proteins

(*continued*)

Table 1 (continued)

IGF-1	Insulin-like growth factor 1	Endocrine growth-promoting hormone and paracrine regulator. Akt activator and apoptosis inhibitor
FN3K	Fructosamine-3-kinase	Phosphorylates fructosamines resulting in deglycation of glycated proteins
SPTA1	Spectrin α erythrocytic 1	Actin cross-linking and molecular scaffold protein
ATP11A	ATPase, type 11A	Integral membrane ATPase
GATAD2A	GATA zinc finger domain-containing A	Transcriptional repressor. Enhances MBD2-mediated repression
SREBF1	Sterol regulatory element-binding transcription factor	Insulin-regulated transcription factor. Glucose and lipid production
TH/INS	Tyrosine hydroxylase/ insulin	Obesity related to polymorphisms in the chromosome 11p15 locus containing TH/INS/IGF2
PCSK1	Prohormone convertase subtilisin kexin, type 1	Involved in the cleavages of proinsulin and proglucagon to the active hormones insulin and GLP-1
LARP6	La ribonucleoprotein domain, family, member 6	Translational regulator. Associated with fasting glucose traits, type 2 diabetes, and obesity
SGSM2	Small G-protein signaling modulator 2	Modulates small G-protein (RAP and RAB)-mediated signaling pathway. Associated with type 2 diabetes
SNX7	Sorting nexin 7	Contains a phox (PX) domain phosphoinositide-binding domain involved in intracellular trafficking
VPS26A	Vacuolar sorting protein 26A	Retrograde transport of proteins from endosomes to the trans-Golgi network
HMG20A	High-mobility group 20A	Transcription factor induces expression of neuronal genes
AP3S2	Adaptor-related protein complex	Facilitates protein trafficking from Golgi vesicles

(*continued*)

Table 1 (continued)

GRB14	Growth factor receptor-bound protein 14	Adaptor protein inhibits tyrosine kinase and insulin receptor signaling
ADIPOQ	Adiponectin, CTQ, and collagen domain containing	Adipokine involved in the control of fat metabolism and insulin sensitivity
ZNF664	Zinc finger protein 664	Transcription factor associated with obesity and diabetes
GNL3	Guanine nucleotide-binding protein-like 3	Nucleolar protein involved in stem cell proliferation. Stabilizes MDM2
LYPLAL1	Lysophospholipase-like 1	Hydrolyzes fatty acids from G-proteins and HRAS
PPP1R3B	Protein phosphatase 1 regulatory subunit 3B	Increases basal and insulin-stimulated glycogen synthesis
UHRF1BP1	UHRF1-binding protein 1	Ubiquitin-like containing PHD and ring finger domains 1-binding protein Function unknown

The most likely candidate is the rs7903146 single nucleotide polymorphism that has a strong association with type 2 diabetes (Gloyn et al. 2009). This polymorphism resides in an intronic, noncoding region of the gene, and the mechanisms for its effects on TCF7L2 expression are unclear. The TT risk allele in humans with T2D impairs β cell functions manifested in reduced glucose-stimulated insulin secretion (GSIS) and glucoincretin actions and increased hepatic glucose production (Schafer et al. 2007; Nauck and Meier 2007; Lyssenko et al. 2007).

The mechanisms involved in the regulation of β cell functions by Tcf7l2 have proved to be complex. In initial studies mRNA levels for Tcf7l2 were increased by 5-fold in donor islets obtained from T2D subjects, particularly in carriers of the TT genotype, and overexpression of Tcf7l2 in isolated human islets reduced GSIS (Lyssenko et al. 2007). However, depleting Tcf7l2 mRNA by siRNA in human islets resulted in a decrease in GSIS, proliferation, and an increase in apoptosis (Shu et al. 2008; da Silva et al. 2009; Le Bacquer et al. 2011) attributed in part to a reduction in the expression of glucoincretin (GLP-1 and GIP) receptors (Shu et al. 2009). Analyses of Tcf7l2 transcripts and protein levels in islets obtained from diabetic donors uncovered several alternatively spliced mRNAs and that the Tcf7l2 mRNAs and translated proteins are unstable (Le Bacquer et al. 2011). A role for Tcf7l2 in β cell proliferation and regeneration is provided by findings of an association of increased expression of Tcf7l2 with pancreatic duct proliferation and neogenesis of endocrine cells (Shu et al. 2012). Evidence obtained from genetic manipulations of the expression of Tcf7l2 in mice appears to be contradictory. Loss of Tcf7l2 function by deletion of Tcf7l2 in β cells impairs GSIS and β cell expansion in response to HFD (da Silva et al. 2012), whereas hemizygous

(Yang et al. 2012) Tcf7l2 mice and mice with a loss-of-function allele (Savic et al. 2011) improves glucose tolerance. Further, mice overexpressing Tcf7l2 in β cells display glucose intolerance (Savic et al. 2011). Notwithstanding the contradictory findings in mice, a major characteristic of islets of diabetic individuals harboring polymorphisms in Tcf7l2 is high Tcfl2 mRNA and low protein levels (Lyssenko et al. 2007; Le Bacquer et al. 2011; Shu et al. 2009), associated with impaired glucose-stimulated and glucoincretin-potentiated insulin secretion (Schafer et al. 2007; Nauck and Meier 2007; Lyssenko et al. 2007).

It is tempting to speculate that the discrepancies observed in mice with Tcf7l2 gene knockout or knockdown of expression, or forced overexpression of Tcf7l2, might reflect a necessary requirement for precise amounts of Tcfl2 protein within nuclei of cells to optimally activate and modulate gene transcription. Different-sized transcripts of the Tcf7l2 gene might translate different isoforms of Tcf7l2 protein with differing transactivation activities (activation and/or suppression), depending on its association with cofactors such as β-catenin. Since the polymorphisms, including the T mutation, are located in intronic regions of the gene locus, alternative RNA splicing (Le Bacquer et al. 2011) and/or intron-encoded microRNAs could regulate mRNA stability and translation efficiency.

The glucoincretin hormone GLP-1 appears to be involved in the pathogenesis of diabetes in individuals who carry TCF7L2 risk alleles. These carriers of TCF7L2 risk alleles have impaired insulin secretion as a major contributor to impaired glucose tolerance or diabetes (Lyssenko 2008; Jin and Liu 2008; Perry and Frayling 2008; Cauchi and Froguel 2008; Bordonaro 2009; Hattersley 2007; Weedon 2007; Chiang et al. 2012; Florez 2007; Xiong et al. 2012; Owen and McCarthy 2007a; Smith 2007). Glucose clamp studies on a large cohort of carriers of TCF7L2 polymorphisms revealed both reduced insulin secretion in response to oral glucose tolerance tests and impaired GLP-1-induced insulin secretion (Schafer et al. 2007). However, in these studies plasma GLP-1 levels were not influenced by the TCF7L2 variants (Schafer et al. 2007). These findings are of interest because two pathogenetic mechanisms involving GLP-1 have been proposed: impaired GLP-1 production in the intestine (Bordonaro 2009; Yi et al. 2008) and impaired GLP-1 actions on pancreatic β cells (Liu and Habener 2008). The studies of Schafer et al. (2007) suggest that the defect in the enteroinsular axis in individuals with defective TCF7L2 functions lies at the level of impaired actions of GLP-1 on insulin secretion from pancreatic β cells, rather than the level of impaired production of GLP-1 by intestinal L-cells. Evidence is reported from studies in vitro that support an important role for β-catenin-/TCF7L2-mediated Wnt signaling in both the expression of the proglucagon gene in intestinal cells (Korinek Barker et al. 1998) and in the regulation of insulin secretion (Shu et al. 2008; Loder et al. 2008; da Silva et al. 2009) and β cell proliferation (Liu and Habener 2008). Interestingly, there is some reported evidence that TCF7L2 may be expressed at low levels (Korinek Barker et al. 1998; Barker et al. 2007), or not at all (Yi et al. 2005) in β cells. These reports conflict with those of the Rutter (da Silva et al. 2009) and Maeder (Shu et al. 2008) laboratories, and our own observations

(Liu and Habener 2008). Based on the findings currently available, the contributions of TCF7L2 functions to the enteroinsular axis may occur at the levels of both the production of GLP-1 by intestinal L-cells and the actions of GLP-1 on pancreatic β cells. The two levels of involvement of TCF7L2 actions are not necessarily mutually exclusive.

FTO (Fat Mass and Obesity-Associated Protein). FTO encodes a protein that is homologous to the DNA repair AlkB family of proteins that are involved in the repair of alkylated nucleobases in DNA and RNA (Jia et al. 2008). The FTO gene is upregulated in orexigenic neurons in the feeding center of the hypothalamus (Frederiksson et al. 2008). Genetic variants in FTO result in excessive adiposity and insulin resistance as well as a markedly increased predisposition to the development of diabetes (Do et al. 2008). A 1.6 mb deletion mutation in the mouse results in the deletion of a locus containing FTO, FTS (fused toes), FTM, and three members of the Iroquois gene family, Irx3, Irx5, and Irx6 (Anselme et al. 2007), resulting in multiple defects in the patterning of the body plan during development (Anselme et al. 2007; Peters et al. 2002). The Irx (Iroquois) proteins are homeodomain transcription factors. The FTO, FTS, IRX locus is implicated in Wnt signaling. FTS is a small ubiquitin-like protein with conjugating protein ligase activity that is known to interact with the protein kinase Akt, a potent inhibitor of GSK3β activity in the Wnt signaling pathway. Moreover, Wnt signaling is reported to induce the expression of Irx3 (Braun et al. 2003). Irx1 and Irx2 are expressed in the endocrine pancreas of the mouse under the control of neurogenin-3 (Ngn3) expression (Petri et al. 2006).

Notch2. The delta/notch signaling pathway is an important cell-cell interactive signaling pathway (lateral inhibition) involved in embryonic stem cell amplification, differentiation, and determination of organogenesis. Notch2 is expressed in pancreatic ductal progenitor cells and may be involved in early branching morphogenesis of the pancreas (Lee et al. 2005). The conditional ablation of Notch2 signaling in mice moderately disturbed the proliferation of epithelial cells during early pancreas development (Nakhai et al. 2008). Evidence is presented linking Notch2 to Wnt signaling (Espinosa et al. 2003). GSK3β phosphorylates Notch2 thereby inhibiting the activation of Notch target genes.

IGF2BP2 (Insulin-Like Growth Factor 2-Binding Protein 2). IGF2BP2 is a paralog of IGF2BP1, which binds to the 5′ UTR of the insulin-like growth factor 2 (IGF2) mRNA and regulates IGF2 translation (Nielsen et al. 1999). IGF2 is a member of the insulin family of polypeptide growth factors involved in development, growth, and stimulation of insulin action. Wnt1 is reported to induce the expression of IGF2 in preadipocytes (Longo et al. 2002).

Hhex (Hematopoietically Expressed Homeobox). Hhex is a homeodomain protein that regulates cell proliferation and tissue specification underlying vascular, pancreatic, and hepatic differentiation (Bort et al. 2004, 2006; Hallaq et al. 2004). Variants in the Hhex gene manifest in impaired β cell function (Pascoe et al. 2007). Hhex is associated with Wnt signaling during pancreas development as it acts with β-catenin to serve as a corepressor of Wnt signaling (Foley and Mercola 2005; Zamparnini et al. 2006).

HNF1β (Hepatocyte Nuclear Factor 1 β, TCF2, and MODY 5 Gene). Tcf2 is a critical regulator of a transcriptional network that controls the specification, growth, and differentiation of the embryonic pancreas (Maestro et al. 2007a). Mutations in the TCF2 gene result in hypoplasia of the pancreas resulting in exocrine pancreas dysfunction to varying degrees (Maestro et al. 2007a; Haldorsen et al. 2008; Haumaitre et al. 2006). Some mutations manifest as a form of maturity onset diabetes of the young (MODY 5).

CDKN2A/B (Cyclin-Dependent Kinase Inhibitor 2A/B, ARF, and p16INK4a). The CDKN2A/B gene generates several transcript variants which differ in their first exons. CDKN2A is a known tumor suppressor, and its product, p16INK4a, inhibits CDK4 (cyclin-dependent kinase 4), a powerful regulator of pancreatic β cell replication (Rane et al. 1999; Mettus and Rane 2003; Marzo et al. 2004). Overexpression of CDKN2A leads to decreased islet proliferation in ageing mice (Krishnamurthy et al. 2006). CDKN2B overexpression is also causally related to islet hypoplasia and diabetes in murine models (Moritani et al. 2005). p16(IND4a) is linked to the Wnt signaling pathway as stabilized β-catenin silences the p16(INK4a) promoter in melanoma cells (Delmas et al. 2007).

PROX1 (Prospero-Related Homeobox Gene). Prospero-related homeobox gene (Prox1) is a target of β catenin-TCF/LEF signaling involved in the differentiation of neural stem cells towards the neuronal lineage in hippocampal neurogenesis (Karalay et al. 2011).

PTPRD (Protein Tyrosine Phosphatase Receptor D). PTPRD (Pcp-2) is a protein tyrosine phosphatase that inhibits β-catenin signaling in the Wnt pathway (Yan et al. 2006). PTPRD prevents tyrosine phosphorylation and release of β-catenin bound to E-cadherin in the plasma membrane. By this action β-catenin remains sequestered and cannot translocate to the nucleus and partner with TCF/LEF DNA-binding proteins and activate transcription. The role of β-catenin bound to E-cadherin is to stabilize intercellular membrane adhesions and to maintain epithelial integrity.

TLE4/Groucho (Gro). TLE4/Groucho (Gro) is a member of a family of transcriptional corepressors that bind to TCF/LEF factors and prevent their activation by β-catenin (Hanson et al. 2012; Zamparini et al. 2006).

ZBED3 (Zinc Finger BED Domain-Containing Protein 3). ZBED3 is a zinc finger BED domain-containing protein that interacts with axin and activates β-catenin/Wnt signaling (Chen et al. 2009a). The interaction of ZBED3 with axin disrupts the destruction box and prevents phosphorylation and degradation of β-catenin, augmenting the activation of Wnt signaling.

TSPAN8/LGR5/GPR49. The protein encoded by this gene is a member of the transmembrane 4 superfamily, also known as the tetraspanin family. Most of these members are cell-surface proteins that have a role in the regulation of cell development, activation, growth, and motility. LGR5/GPR49 is a leucine-rich repeat-containing G-protein-coupled receptor. A role for TSPAN8 in the pancreas is as yet unknown. However, Tspan8/LGR5 is a recognized Wnt signaling target gene in small intestinal and colonic stem cells (Barker et al. 2007).

TP53INP1. TP53-induced nuclear protein-1 is induced by TP53 in response to depletion of Tcf7l2 and is involved in apoptosis (Zhou et al. 2012). Inhibition of TP53INP1 protects β cells from Tcf7l2 depletion-induced apoptosis suggesting that TP53INP1 is at least partially responsible for activating proapoptotic pathways in β cells deficient in Tcf7l2.

WFS1 (Wolfram Syndrome 1). WFS1 encodes a transmembrane protein of 890 amino acids that is highly expressed in the endoplasmic reticulum of neurons and pancreatic β cells (Takeda et al. 2001). Mutations in WFS1 result in Wolfram syndrome, an autosomal recessive neurodegenerative disorder. Disruption of the WFS1 gene in mice causes progressive β cell loss and impaired stimulus-secretion coupling in insulin secretion (Ishihara et al. 2004). The reduction in β cell mass is likely a consequence of enhanced endoplasmic reticulum stress resulting in the apoptosis of β cells (Riggs et al. 2005; Yamada et al. 2006; Fonseca et al. 2005). Impaired proinsulin processing to insulin and insulin transport through the secretory pathway may also be involved in the impaired insulin secretion. To date no information is available on the mechanisms that regulate WFS1 expression or of an involvement of Wnt signaling in its expression.

CDKAL1 (CDK5 Regulatory Subunit-Associated Protein-1-Like (1). CDKAL1 encodes a protein of unknown functions. However, the protein is similar to CDK5 regulatory subunit-associated protein-1 (encoded by CDK5RAP1), expressed in neuronal tissues. CDKAL1 inhibits cyclin-dependent kinase 5 (CDK5) activity by binding to the CDK5 regulatory subunit p35 (Ching et al. 2002). Variants in the CDKAL1 gene in humans are associated with decreased pancreatic β cell functioning (Pascoe et al. 2007). CDK5 has a role in the loss of β cell function in response to glucotoxicity as the inhibition of the CDK5/p35 complex prevents a decrease of insulin gene expression that results from glucotoxicity (Ubeda et al. 2006). Therefore, it seems possible that CDKAL1 may have a role in the inhibition of the CDK5/p35 complex in pancreatic β cells similar to that of CDK5RAP1 in neuronal tissue. One may conjecture that a reduced expression and inhibitory function of CDKAL1 or reduced inhibitory function could exacerbate β cell impairment in response to glucotoxicity.

ADAMTS9. The ADAMTS9 gene encodes a member of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) protein family (http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=retrieve&dopt=full_report&list_uids=56999&log$=databasead&logdbfrom=protein). Members of the ADAMTS family have been implicated in the cleavage of proteoglycans, the control of organ shape during development, and the inhibition of angiogenesis. ADAMTS8 is widely expressed during mouse embryo development (Jungers et al. 2005). Functions for ADAMTS9 in the pancreas or in Wnt signaling are heretofore unrecognized.

GLIS3. GLIS3 is a Kruppel-like zinc finger transcription factor that regulates neurogenin-3 (Ngn3) through its distal promoter (Kim et al. 2012). GLIS3 and hepatic nuclear factor-6 (HNF6) bind close together to the distal promoter of Ngn3 and activate transcription, suggesting that cross talk between GLIS3 and HNF6

might be involved in the regulation of Ngn3 during pancreatic endocrine cell specification and development.

GRK5 (G-protein Coupled Receptor Kinase-5). G-protein-coupled receptor kinase-5, known to phosphorylate and inhibit the activities of G-protein-coupled receptors, also is found to phosphorylate the Wnt receptor LRP6 resulting in the disruption of the destruction box, stabilization of β-catenin, and activation of Wnt signaling (Chen et al. 2009b).

SPRY1 (Sprouty 1). Sprouty 1, a Wnt signaling target gene (Colli et al. 2013), is a member of conserved proteins involved in the modulation of receptor tyrosine kinases (RTKs) and branching morphogenesis during development (Edwin et al. 2009; Guy et al. 2009). The inducible expression of SPRY4 in pancreatic β cells of mice during development results in a reduction in islet size, an increased number of α cells, and an impaired islet cell type segregation (Jäggi et al. 2008).

HNF4A (Hepatic Nuclear Factor 4 α, MODY 1). Hepatic nuclear factor 4 α is a transcription factor involved in Wnt signaling by its interactions with lymphocyte enhancer factor-1 (LEF1) in controlling the zonation of hepatocytes in the liver (Colletti et al. 2009). HNF4A deficiency in mice causes abnormal insulin secretion and impaired expansion of β cell mass during pregnancy (Maestro et al. 2007b). Mutations in the HNF4A gene can lead to MODY 1, one of the several genetic causes of maturity onset diabetes of the young (McDonald and Ellard 2013) and, rarely, congenital hyperinsulinemia (James et al. 2009).

Future Directions

Continued studies of the involvement of the Wnt signaling pathway in islet development and function may reveal novel factors important in β cell growth and survival. A prerequisite for understanding the potential importance of Wnt signaling in islets is the identification of the specific Wnt signaling factors that are expressed in islets. Identification of these factors may provide opportunities for development of small molecules that target specific components of the pathways to promote growth and survival. Ongoing high-throughput screening studies of hundreds of thousands of compounds using islet tissues containing fluorescence reporter genes and growth or apoptosis-responsive promoters may uncover such small molecules.

Antidiabetogenic therapies consisting of combinations of GLP-1 and SDF-1 agonists may provide additive benefits in promoting both the growth and survival of β cells, thereby preserving or enhancing β cell mass. Recent findings suggest that both the pro-proliferative actions of GLP-1 and the anti-apoptosis actions of SDF-1 are mediated by the activation of β-catenin and TCF7L2 in β cells. Although the GLP-1/GLP-1R and SDF-1/CXCR4 axes both converge on downstream Wnt signaling at the level of the formation of transcriptionally productive complexes of β-catenin/TCF7L2, the target genes activated by GLP-1 and by SDF-1 differ. GLP-1-mediated activation of β-catenin/TCF7L2 results in the expression of genes involved in the cell division cycle, whereas SDF-1 actions result in the activation of the expression of genes engaged in cell survival. Furthermore,

downstream β-catenin/TCF7L2 activation is a requisite for the pro-proliferative actions of GLP-1 and the anti-apoptotic actions of SDF-1. The two hormones, GLP-1 and SDF-1, acting together may provide additive benefits in promoting the regeneration and maintenance of β cell mass in diabetes.

Genome-wide association studies in search of risk alleles for type 2 diabetes are just beginning. It is estimated that 80–90 % of the human genome remains yet to be explored for the existence of diabetes-associated genes in the population. Predictably, further genome-wide scans in the future will uncover even more than the current 19 genes, and many will likely be involved in islet and β cell development and functions. It is tempting to speculate that the additional risk genes for type 2 diabetes that remain to be discovered in the future will include genes encoding components of the Wnt signaling pathway.

Intriguing current evidence warrants further investigations of Wnt ligands and Wnt signaling in the cross talk between adipose tissue and islets. Possibilities arise suggesting that Wnt ligands produced and secreted by adipocytes act on β cells to stimulate Wnt signaling.

Acknowledgments We thank Michael Rukstalis and Melissa Thomas for their helpful comments on this chapter and Sriya Avadhani, Violeta Stanojevic, and Karen McManus for their expert experimental assistance. Effort was supported in part by grants from the US Public Health Service, the American Diabetes Association, and the Juvenile Diabetes Research Foundation.

References

Ahlqvist E, Ahluwalia TS, Groop L (2011) Genetics of type 2 diabetes. Clin Chem 57:241–254, PMID: 21119033

Al-Hasani K, Pfeifer A, Courtney M, Ben-Othman N, Gjernes E, Vieira A, Druelle N, Avolio F, Ravassard P, Leuckx G, Lacas-Gervais S, Ambrosetti D, Benizri E, Hecksher-Sorensen J, Gounon P, Ferrer J, Gradwohl G, Heimberg H, Mansouri A, Collombat P (2013) Adult duct-lining cells can reprogram into β-like cells able to counter repeated cycles of toxin-induced diabetes. Dev Cell 26:86–100

Anselme I, Lacief C, Lanaud M, Ruther U, Schneider-Maunoury S (2007) Defects in brain patterning and head morphogenesis in the mouse mutant fused toes. Dev Biol 304:208–220

Barker N et al (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449:1003–1007

Bonner-Weir S, Sharma A (2006) Are their pancreatic progenitor cells from which new islets form after birth? Nat Clin Pract Endocrinol Metab 2:240–241

Bordonaro M (2009) Role of Wnt signaling in the development of type 2 diabetes. Vitam Horm 80:563–581

Bort R, Martinez-Barbera JP, Beddington RS, Zaret KS (2004) Hex homeobox gene-dependent tissue positioning is required for organogenesis of the ventral pancreas. Development 131:797–806

Bort R, Signore M, Tremblay K, Martinez-Barbera JP, Zaret KS (2006) Hex homeobox gene controls the transition of the endoderm to a pseudostratified, cell emergent epithelium for liver bud development. Dev Biol 290:44–56

Boutant M, Ramos OH, Tourrel-Cuzin C, Movassat J, Ilias A, Vallois D, Planchais J, Pégorier JP, Schuit F, Petit PX, Bossard P, Maedler K, Grapin-Botton A, Vasseur-Cognet M (2012) COUP-TFII controls mouse pancreatic β-cell mass through GLP-1-β-catenin signaling pathways. PLoS One 7:e30847

Braun MM, Etheridge A, Bernard A, Robertson CP, Roelink H (2003) Wnt signaling is required at distinct stages of development for the induction of the posterior forebrain. Development 130:5579–5587

Burger JA, Kipps TJ (2006) CXCR4 a key receptor in the crosstalk between tumor cells and their microenvironment. Blood 107:1761–1767

Cano DA, Rulifson IC, Heiser PW, Swigart LB, Pelengaris S, German M, Evan GI, Bluestone JA, Hebrok M (2008) Regulated β-cell regeneration in the adult mouse pancreas. Diabetes 57:958–966

Cauchi S, Froguel P (2008) TCF7L2 genetic defect and type 2 diabetes. Curr Diab Rep 8:149–155, Review

Cauchi S et al (2008) Post genome-wide association studies of novel genes associated with type 2 diabetes show gene-gene interaction and high predictive value. PLoS One 3:e2031

Chen T, Li M, Ding Y, Zhang LS, Xi Y, Pan WJ, Tao DL, Wang JY, Li L (2009a) Identification of zinc-finger BED domain-containing 3 (Zbed3) as a novel Axin-interacting protein that activates Wnt/β-catenin signaling. J Biol Chem 284:6683–6689

Chen M, Philipp M, Wang J, Premont RT, Garrison TR, Caron MG, Lefkowitz RJ, Chen W (2009b) G Protein-coupled receptor kinases phosphorylate LRP6 in the Wnt pathway. J Biol Chem 284:35040–35048

Cheta D (1998) Animal models of type 1 (insulin-dependent) diabetes mellitus. J Pediatr Endocrinol Metab 11:11–19

Chiang YT, Ip W, Jin T (2012) The role of the Wnt signaling pathway in incretin hormone production and function. Front Physiol 12(3):273

Ching YP, Pang AS, Lam WH, Qi RZ, Wang JH (2002) Identification of a neuronal Cdk5 activator-binding protein as Cdk5 inhibitor J. Biol Chem 277:15237–15240

Colletti M, Cicchini C, Conigliaro A, Santangelo L, Alonzi T, Pasquini E, Tripodi M, Amicone L (2009) Convergence of Wnt signaling on the HNF4α-driven transcription in controlling liver zonation. Gastroenterology 137:660–672

Colli LM, Saggioro F, Serafini LN, Camargo RC, Machado HR, Moreira AC, Antonini SR, de Castro M (2013) Components of the canonical and non-canonical Wnt pathways are not mis-expressed in pituitary tumors. PLoS One 8:e62424

Collombat P, Xu X, Ravassard P, Sosa-Pineda B, Dussaud S, Billestrup N, Madsen OD, Serup P, Heimberg H, Mansouri A (2009) The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into α and subsequently β cells. Cell 138:449–462

Courtney M, Gjernes E, Druelle N, Ravaud C, Vieira A, Ben-Othman N, Pfeifer A, Avolio F, Leuckx G, Lacas-Gervais S, Hecksher-Sorensen J, Ravassard P, Heimberg H Mansouri A, Patrick Collombat P. The inactivation of Arx in pancreatic α-cells triggers their neogenesis and conversion into functional β-like cells. PLoS Genetics 9(10):e1003934

da Silva XG, Loder MK, McDonald A, Tarasov AI, Carzaniga R, Kronenberger K, Barg S, Rutter GA (2009) TCF7L2 regulates late events in insulin secretion from pancreatic islet β cells. Diabetes 58(4):894–905, Jan 23 ahead of print

da Silva XG, Mondragon A, Sun G, Chen L, McGinty JA, French PM, Rutter GA (2012) Abnormal glucose tolerance and insulin secretion in pancreas-specific Tcf7l2-null mice. Diabetologia 55:2667–2676

Dabernat S, Secrest P, Peuchant E, Moreau-Gaudry F, Dubus P, Sarvetnick N (2009) Lack of β-catenin in early life induces abnormal glucose homeostasis in mice. Diabetologia 52:1608–1617

Delmas V, Beermann F, Martinozzi S, Carreira S, Ackermann J, Kumasaka M, Denat L, Goodall J, Luciani F, Viros A, Demirkan N, Bastian BC, Goding CR, Larue L (2007) β-catenin induces immortalization of melanocytes by suppressing p16INK4a expression and cooperates with N-Ras in melanoma development. Genes Dev 21:2923–2935

Dessimoz J, Bonnard C, Huelsken J, Grapin-Botton A (2005) Pancreas-specific deletion of β-catenin reveals Wnt-dependent and Wnt-independent functions during development. Curr Biol 15:1677–1683

Do R, Bailey SD, Desbiens K, Belisle A, Montpetite, Bouchard C, Perusse L, Vohl MC, Engert JC (2008) Genetic variants of FTO influence adiposity, insulin sensitivity, leptin levels, and resting metabolic rate in the Quebec family diabetes. Diabetes 57:1147–1150

Dor Y, Brown J, Martinez OI, Melton DA (2004) Adult pancreatic β cells are formed by self-duplication rather than stem-cell differentiation. Nature 429:41–46

Drucker DJ (2006) The biology of incretin hormones. Cell Metab 3:153–165

Edwin F, Anderson K, Ying C, Patel TB (2009) Intermolecular interactions of Sprouty proteins and their implications in development and disease. Mol Pharmacol 76:679–691

Espinosa L, Engles-Esteve J, Aguilera C, Bigas A (2003) Phosphorylation by glycogen synthase kinase 3 β down-regulates Notch activity, a link for Notch and Wnt pathways. J Biol Chem 278:32227–32235

Figeac F, Uzan B, Faro M, Chelali N, Portha B, Movassat J (2010) Neonatal growth and regeneration of β-cells are regulated by the Wnt/β-catenin signaling in normal and diabetic rats. Am J Physiol Endocrinol Metab 298(2):E245–E256

Florez JC (2007) The new type 2 diabetes gene TCF7L2. Curr Opin Clin Nutr Metab Care 10:391–396

Florez J (2008a) Clinical review: the genetics of type 2 diabetes: a realistic appraisal in 2008. J Clin Endocrinol Metab 93:4633–4642

Florez J (2008b) Newly identified loci highlight β cell dysfunction as a key cause of type 2 diabetes: where are the insulin resistance genes? Diabetologia 51:1100–1110

Foley AC, Mercola M (2005) Heart induction by Wnt antagonists depends on the homeodomain transcription factor Hex. Genes Dev 19:387–396

Fonseca SG, Fukuma M, Lipson KL et al (2005) WFS1 is a novel component of the unfolded protein response and maintains homeostasis of the endoplasmic reticulum in pancreatic β cells. J Biol Chem 280:39609–39615

Frederiksson R, Hagglund M, Olszewski PK, Sstephansson O, Jacobsson JA, Olszewska AM, Levine AS, Lindblom J, Schioth HB (2008) The obesity gene, FTO, is of ancient origin, up-regulated during food deprivation and expressed in neurons of feeding-related nuclei of the brain. Endocrinology 149:2062–2071

Fujino T et al (2003) Low-density lipoprotein receptor-related protein 5 (LRP5) is essential for normal cholesterol metabolism and glucose-induced insulin secretion. Proc Natl Acad Sci USA 100:229–234

Gloyn AL, Braun M, Rorsman P (2009) Type 2 diabetes susceptibility gene TCF7L2 and its role in β cell function. Diabetes 58:832–834

Gordon MD, Nusse R (2006) Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors. J Biol Chem 281:22429–22433

Gradwohl G, Dierich A, LeMeur M, Guillemot F (2000) Neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. Proc Natl Acad Sci USA 9:1607–1611

Grant SF et al (2006) Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nature Genet 38:320–323

Grarup N et al (2007) Studies of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects: validation and extension of genome-wide association studies. Diabetes 56:3105–3111

Guy GR, Jackson RA, Yusoff P, Chow SY (2009) Sprouty proteins: modified modulators, matchmakers or missing links? J Endocrinol 203:191–202

Habener JF, Stanojevic V (2012) α-cell role in β-cell generation and regeneration. Islets 4(3):188–198

Habener JF, Stanojevic V (2013) α cells come of age. Trends Endocrinol Metab 24:153–163

Haldorsen IS, Vesterhus M, Raeder H, Jensen DK, Sovik O, Molven A, Njelstad PR (2008) Lack of pancreatic body and tail in HNF1B mutation carriers. Diabet Med 25:782–787

Hallaq H et al (2004) A null mutation of Hhex results in abnormal cardiac development, defective vasculogenesis and elevated Vegfa levels. Development 131:5197–5209

Hanson AJ, Wallace HA, Freeman TJ, Beauchamp RD, Lee LA, Lee E (2012) XIAP monoubiquitylates Groucho/TLE to promote canonical Wnt signaling. Mol Cell 45(5):619–628

Hattersley AT (2007) Prime suspect: the TCF7L2 gene and type 2 diabetes risk. J Clin Invest 117:2077–2079

Haumaitre C, Fabre M, Cormier S, Baumann C, Delezoide AL, Ceereghini S (2006) Severe pancreas hypoplasia and multicystic renal dysplasia in two human fetuses carrying novel HNF1β/MODY5 mutations. Hum Mol Genet 15:2363–2375

Hayes MG et al (2007) Identification of type 2 diabetes genes in Mexican Americans through genome-wide association studies. Diabetes 56:3033–3044

Heiser PW, Lalu J, Taketo MM, Herrera PL, Hebrok M (2006) Stabilization of β-catenin impacts pancreatic growth. Development 133:2023–2033

Heller RS, Dichmann DS, Jensen J, Miller C, Wong G, Madsen OD, Serup P (2002) Expression patterns of Wnts, Frizzleds, sFRPs and misexpression in transgenic mice suggesting a role for Wnts in pancreas and foregut pattern formation. Dev Dyn 225:260–270

Heller RS, Klein T, Ling Z, Heimberg H, Katoh M, Madsen OD, Serup (2003) Expression of Wnt, Frizzled, sFRP, and DKK genes in adult human pancreas. Gene Expr 11:141–147

Heller C, Kühn MC, Mülders-Opgenoorth B, Schott M, Willenberg HS, Scherbaum WA, Schinner S (2011) Exendin-4 upregulates the expression of Wnt-4, a novel regulator of pancreatic β-cell proliferation. Am J Physiol Endocrinol Metab 301:E864–E872

Ishihara H, Takeda S, Tamura A et al (2004) Disruption of the WFS1 gene in mice causes progressive β cell loss and impaired stimulus-secretion coupling in insulin secretion. Hum Mol Genet 13:1159–1170

Jäggi F, Cabrita MA, Perl AK, Christofori G (2008) Modulation of endocrine pancreas development but not β-cell carcinogenesis by Sprouty4. Mol Cancer Res 6:468–482

James C, Kapoor RR, Ismail D, Hussain K (2009) The genetic basis of congenital hyperinsulinism. J Med Genet 46:289–299

Jensen JM, Cameron E, Baray MV, Starkev TW, Gianani R, Jensen J (2005) Recapitulation of elements on embryonic development in adult mouse pancreatic regeneration. Gastroenterology 128:728–741

Jia G, Yano CG, Yang S, Jian X, Yi C, Zhou ZA, He C (2008) Oxidative demethylation of 3-methylthymidine and 3-methyluracil in single-stranded DNA and RNA by mouse and human FTO. FEBS Lett 582:331319

Jin T (2008) The WNT, signalling pathway and diabetes mellitus. Diabetologia 51:1771–1780

Jin T, Liu L (2008) The Wnt signaling pathway effector TCF7L2 and type 2 diabetes mellitus. Mol Endocrinol 22:2383–2392

Jin W, Patti ME (2009) Genetic determinants and molecular pathways in the pathogenesis of type 2 diabetes. Clin Sci 116:99–111

Jungers KA, Le Goff C, Sommerville RP, Apte SS (2005) Adamts9 is widely expressed during mouse embryo development. Gene Expr Patterns 5:609–617

Karalay O, Doberauer K, Vadodaria KC, Knobloch M, Berti L, Miquelajauregui A, Schwark M, Jagasia R, Taketo MM, Tarabykin V, Lie DC, Jessberger S (2011) Prospero-related homeobox 1 gene (Prox1) is regulated by canonical Wnt signaling and has a stage-specific role in adult hippocampal neurogenesis. Proc Natl Acad Sci USA 108:5807–5812

Kayali AG, Van Gunst K, Campbell IL, Stotland A, Kritzik M, Liu G, Flodstrom-Tullberg M, Zhang YQ, Sarvetnick N (2003) The stromal cell-derived factor-1α/CXCR4 ligand-receptor axis is critical for progenitor survival and migration in the pancreas. J Cell Biol 163:859–869

Kieffer TJ, Habener JF (1999) The glucagon-like peptides. Endocr Rev 20:876–913

Kikuchi A, Kishido S, Yamamoto H (2006) Regulation of Wnt signaling by protein-protein interaction and post-translational modifications. Exp Mol Med 38:1–10

Kim HJ, Schieffarth JB, Jessurun J, Sumanas S, Petryk A, Lin S, Ekker SC (2005) Wnt5 signaling in vertebrate pancreas development. BMC Biol 24:3–23

Kim YS, Kang HS, Takeda Y, Hom L, Song HY, Jensen J, Jetten AM (2012) Glis3 regulates neurogenin 3 expression in pancreatic β-cells and interacts with its activator, Hnf6. Mol Cells 34:193–200

Komiya Y, Habas R (2008) Wnt signal transduction pathways. Organogenesis 4:68–75

Korinek V, Barker N, Moerer P, van Donselaar E, Huls G, Peters PJ, Clevers H (1998) Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. Nat Genet 19:379–383

Krishnamurthy J, Ramsey MR, Ligon KL, Torrice C, Koh A, Bonner-Weir S, Sharpless NE (2006) p16INK4a induces an age-dependent decline in islet regenerative potential. Nature 443:453–457

Kryczek I, Wei S, Keller E, Liu R, Zou W (2007) Stroma-derived factor (SDF-1/CXCL12) and human tumor pathogenesis. Am J Physiol Cell Physiol 292:C987–C995

Kucia M, Ratajczak J, Ratajczak MZ (2005) Bone marrow as a source of circulating $CXCR4^+$ tissue-committed stem cells. Biol Cell 97:133–146

Le Bacquer O, Shu L, Marchand M, Neve B, Paroni F, Kerr Conte J, Pattou F, Froguel P, Maedler K (2011) TCF7L2 splice variants have distinct effects on β-cell turnover and function. Hum Mol Genet 20:1906–1915

Lee KM, Yasuda H, Hollingsworth MA, Ouellette MM (2005) Notch2-positive progenitors with the intrinsic ability to give rise to pancreatic duct cells. Lab Invest 85:1003–1012

Lee SH, Demeterco C, Geron I, Abrahamsson A, Levine F, Itkin-Ansari P (2008) Islet specific Wnt activation in human type 2 diabetes. Exp Diabetes Res 2008:728–763

Liu Z, Habener JF (2008) Glucagon-like peptide-1 activation of TCF7L2-dependent Wnt signaling enhances pancreatic β cell proliferation. J Biol Chem 283:8723–8735

Liu Z, Habener JF (2009) Stromal cell-derived factor-1 promotes survival of pancreatic β cells by the stabilisation of β-catenin and activation of transcription factor 7-like 2 (TCF7L2). Diabetologia 52:1589–1598

Liu Z, Stanojevic V, Avadhani S, Yano T, Habener JF (2011) Stromal cell-derived factor-1 (SDF-1)/chemokine (C-X-C motif) receptor 4 (CXCR4) axis activation induces intra-islet glucagon-like peptide-1 (GLP-1) production and enhances β cell survival. Diabetologia 54:2067–2076

Loder MK, da Silva XG, McDonald A, Rutter GA (2008) TCF7L2 controls insulin gene expression and insulin secretion in mature pancreatic β cells. Biochem Soc Trans 36:357–359

Logan CY, Nusse R (2004) The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 20:781–810

Longo KA, Kennell JA, Ochocinska MJ, Ross SE, Wright WS, McDougald OA (2002) Wnt signaling protects 3 T3-L1 preadipocytes from apoptosis through induction of insulin-like growth factors. J Biol Chem 277:38239–38244

Luo Y, Cai J, Xue H, Mattson MP, Rao MS (2006) SDF-1α/CXCR4 signaling stimulates β-catenin transcriptional activity in rat neural progenitors. Neurosci Lett 398:291–295

Lyssenko V (2008) The transcription factor 7-like 2 gene and increased risk of type 2 diabetes: an update. Curr Opin Clin Nutr Metab Care 11:385–392

Lyssenko V, Lupi R, Marchetti P, Del Guerra S, Orho-Melander M, Almgren P, Sjögren M, Ling C, Eriksson KF, Lethagen AL, Mancarella R, Berglund G, Tuomi T, Nilsson P, Del Prato S, Groop L (2007) Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. J Clin Invest 117:2155–2163

Lyssenko V, Jonsson A, Almgren P, Pulizzi N, Isomaa B, Tusomi T, Gerglund G, Altshuler D, Nisson P, Groop L (2008) Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med 359:2220–2232

MacDonald BT, Semenov MV, He X (2007) SnapShot: Wnt/β-catenin signaling. Cell 131:1204

Maestro MA, Cardaida C, Boj SF, Luco RF, Servitja JM, Ferrer J (2007a) Distinct roles of HNF1β, HNF1α, and HNF4α in regulating pancreas development, β cell function, and growth. Endocr Rev 12:33–45

Maestro MA, Cardalda C, Boj SF, Luco RF, Servitja JM, Ferrer J (2007b) Distinct roles of HNF1β, HNF1α, and HNF4α in regulating pancreas development, β-cell function and growth. Endocr Dev 12:33–45

Marchetti P, Syed F, Suleiman M, Bugliani M, Marselli L (2012) From genotype to human β cell phenotype and beyond. Islets 4:323–332

Marzo N, Mora C, Fabregat ME, Martín J, Usac EF, Franco C, Barbacid M, Gomis R (2004) Pancreatic islets from cyclin-dependent kinase 4/R24C (Cdk4) knockin mice have significantly increased β cell mass and are physiologically functional, indicating that Cdk4 is a potential target for pancreatic β cell mass regeneration in Type 1 diabetes. Diabetologia 47:686–694

McDonald TJ, Ellard S (2013) Maturity onset diabetes of the young: identification and diagnosis. Ann Clin Biochem 50:403–415

McLin VA, Rankin SA, Zorn AM (2007) Repression of Wnt/β-catenin signaling in the anterior endoderm is essential for liver and pancreas development. Development 134:2207–2217

Mettus RV, Rane SG (2003) Characterization of the abnormal pancreatic development, reduced growth and infertility in Cdk4 mutant mice. Oncogene 22:8413–8422

Moon RT, Kohn AD, De Ferrari GV, Kaykas A (2004) WNT and β-catenin signalling: diseases and therapies. Nat Rev Genet 5:691–701

Moore AF et al (2008) Extension of type 2 diabetes genome-wide association scan results in the diabetes prevention program. Diabetes 57:2503–2510

Moritani M, Yamasaki S, Kagami M, Suzuki T, Yamaoka T, Sano T, Hata J, Itakura M (2005) Hypoplasia of endocrine and exocrine pancreas in homozygous transgenic TGF-β1. Mol Cell Endocrinol 229:175–184

Murtaugh LC (2008) The what, where, when and how of Wnt/β-catenin signaling in pancreas development. Organogenesis 4:81–86

Murtaugh LC, Law AC, Dor Y, Melton DA (2005) β-catenin is essential for pancreatic acinar but not islet development. Development 132:4663–4674

Nakhai H et al (2008) Conditional ablation of Notch signaling in pancreatic development. Development 135:2757–2765

Nauck MA, Meier JJ (2007) The enteroinsular axis may mediate the diabetogenic effects of TCF7L2 polymorphisms. Diabetologia 50:2413–2416

Nelson WJ, Nusse R (2004) Convergence of Wnt, β-catenin, and cadherin pathways. Science 303:1483–1487

Nielsen J, Christiansen J, Lykke-Andersen J, Johnsen AH, Wewer UM, Nielsen FC, Elsen J et al (1999) A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. Mol Cell Biol 19:1262–1269

Nusse R (2008) Wnt signaling and stem cell control. Cell Res 18:523–527

Owen KR, McCarthy MI (2007a) Genetics of type 2 diabetes. Curr Opin Genet Dev 17:239–244

Owen KR, McCarthy MI (2007b) Genetics of type 2 diabetes. Curr Opin Genet Develop 17:239–244

Palmer ND et al (2008) Quantitative trait analysis of type 2 diabetes susceptibility loci identified from whole genome association studies in the insulin resistance atherosclerosis family study. Diabetes 57:1093–1100

Papadopoulou S, Edlund H (2005) Attenuated Wnt signaling perturbs pancreatic growth but not pancreatic function. Diabetes 54:2844–2851

Pascoe L, Tura A, Patel SK, Ibrahim IM, Ferrannini E, Zeggini E, Weedon MN, Mari A, Hattersley AT, McCarthy MI, Frayling TM, Walker M (2007) RISC Consortium; U.K. Type 2 Diabetes Genetics Consortium. Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic β cell function. Diabetes 56:3101–3104

Pauls F, Bancroft RW (1950) Production of diabetes in the mouse by partial pancreatectomy. Am J Physiol 160:103–106

Pedersen AH, Heller RS (2005) A possible role for the canonical Wnt pathway in endocrine cell development in chicks. Biochem Biophys Res Commun 333:961–968

Perry JR, Frayling TM (2008) New gene variants alter type 2 diabetes risk predominantly through reduced β cell function. Curr Opin Clin Nutr Metab Care 11:371–377

Peters T, Ausmeier K, Dildrop R, Ruther U (2002) The mouse Fused toes (Ft) mutation is the result of a 1.6 Mb deletion including the entire Iroquois B gene locus. Mamm Genome 13:186–188

Petri A, Anfelt-Ronne J, Fredericksen RS, Edwards DG, Madsen D, Serup P, Fleckner J, Heller RS (2006) The effect of neurogenin3 deficiency on pancreatic gene expression in embryonic mice. J Mol Endocrinol 37:301–316

Ruchat SM et al (2009) Association between insulin secretion, insulin sensitivity and type 2 diabetes susceptibility variants identified in genome-wide association studies. Acta Diabetol. 46:217–226

Rane SG, Dubus P, Mettus RV, Galbreath EJ, Boden G, Reddy EP, Barbacid M (1999) Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in β-islet cell hyperplasia. Nat Genet 22:44–52

Ratajczak MZ, Zuba-Surma E, Kucia M, Reca R, Wojakowski W, Ratajczak J (2006) The pleiotropic effects of the SDF-1-CXCR4 axis in organogenesis, regeneration and tumorigenesis. Leukemia 20:1915–1924

Rees DA, Alcolado JC (2005) Animal models of diabetes mellitus. Diabet Med 22:359–370

Riggs AC, Bernal-Mizrachi E, Ohsugi M et al (2005) Mice conditionally lacking the Wolfram gene in pancreatic islet β cells exhibit diabetes as a result of enhanced endoplasmic reticulum stress and apoptosis. Diabetologia 48:2313–2321

Rulifson JC, Karnik SK, ten Heiser PW, Berge D, Chen H, Gu X, Taketo MM, Nusse R, Hebrok M, Kim SK (2007) Wnt signaling regulates pancreatic β cell proliferation. Proc Natl Acad Sci USA 104:6247–6252

Sakaguchi Y, Inaba M, Kusafuka K, Okazaki K, Ikehara S (2006) Establishment of animal models for three types of pancreatic and analyses of regeneration mechanisms. Pancreas 33:371–381

Savic D, Ye H, Aneas I, Park SY, Bell GI, Nobrega MA (2011) Alterations in TCF7L2 expression define its role as a key regulator of glucose metabolism. Genome Res 21(9):1417–1425

Saxena R et al (2007) Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 316:1332–1336

Schafer SA, Tschritter O, Machicao F, Thamer C, Stefan N, Gallwitz B, Holst JJ, Dekker JM, 't Hart LM, Nipeis G, van Haeften TW, Haring HU, Fritsche A (2007) Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (TCF7L2) gene polymorphisms. Diabetologia 59:2443–2450

Schinner S, Ulgen F, Papewalis C, Schott M, Woelk A, Vidal-Puig A, Scherbaurm WA (2008) Regulation of insulin secretion, glucokinase gene transcription and β cell proliferation by adipocyte-derived Wnt signalling molecules. Diabetologia 51:147–154

Scott LJ et al (2007) A genome-wide association study of type 2 diabetes in France detects multiple susceptibility variants. Science 316:1341–1345

Semenov MV, Habas R, Macdonald BT, He X (2007) SnapShot: Noncanonical Wnt signaling pathways. Cell 131:1738

Serafimidis I, Rakatzi I, Episkopou V, Gouti M, Gavalas A (2008) Novel effectors of directed and Ngn3-mediated differentiation of mouse embryonic stem cells into endocrine pancreas progenitors. Stem Cells 26:3–16

Shu L, Sauter NS, Schulthess FT, Matvevenko AV, Oberholzer J, Maedler K (2008) Transcription factor 7-like 2 regulates β cell survival and function in human pancreatic islets. Diabetes 57:645–653

Shu L, Matveyenko AV, Kerr-Conte J, Cho JH, McIntosh CH, Maedler K (2009) Decreased TCF7L2 protein levels in type 2 diabetes mellitus correlate with downregulation of GIP- and GLP-1 receptors and impaired β-cell function. Hum Mol Genet 18:2388–2399

Shu L, Zien K, Gutjahr G, Oberholzer J, Pattou F, Kerr-Conte J, Maedler K (2012) TCF7L2 promotes β cell regeneration in human and mouse pancreas. Diabetologia 55:3296–3307

Sladek R et al (2007) A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 445:881–885

Smith U (2007) TCF7L2 and diabetes – what we Wnt to know. Diabetologia 50:5–7
Steinthorsdottir V et al (2007) CDKAL1 influences insulin response and risk of type 2 diabetes. Nature Genet 39:770–775
Takeda K, Inoue H, Tanizawa Y et al (2001) WFS1 (Wolfram syndrome 1) gene product: predominant subcellular localization to endoplasmic reticulum in cultured cells and neuronal expression in rat brain. Hum Mol Genet 10:477–484
Talchai C, Xuan S, Lin HV, Sussel L, Accili D (2012) Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. Cell 150:1223–1234
Ubeda M, Rukstalis JM, Habener JF (2006) Inhibition of cyclin-dependent kinase 5 activity protects pancreatic β cells from glucotoxicity. J Biol Chem 281:28858–28864
Van Hoek M, Dehghan A, Witteman JC, Van Dulin CM, Utterfinden AG, Oostra BA, Hofman A, Sijbrands BA, Janssens AC (2008) Predicting type 2 diabetes based on polymorphisms from genome-wide association studies: a population-based study. Diabetes 57:3122–3128
Veeman MT, Axelrod JD, Moon RT (2003) A second canon. Functions and mechanisms of β-catenin-independent Wnt signaling. Dev Cell 5:367–377
Wada H, Okamoto H (2009) Roles of planar cell polarity pathway genes for neural migration and differentiation. Dev Growth Differ 51:233–240
Wang OM, Zhang Y, Yang KM, Zhou HY, Yano HJ (2006) Wnt/β-catenin signaling pathway is active in pancreatic development of rat embryo. World J Gastroenterol 12:2615–2619
Weedon MN (2007) The importance of TCF7L2. Diabet Med 24:1062–1066
Wells JM, Esni F, Bolvin GP, Aronow BJ, Stuart W, Combs C, Sklenka A, Leach SD, Lowy AM (2007) Wnt/β-catenin signaling is required for development of the exocrine pancreas. BMC Dev Biol 7:4
Welters HJ, Kulkarni RN (2008) Wnt signaling: relevance to β cell biology and diabetes. Trends Endocrinol Metab 19:349–355
Willert K, Jones KA (2006) Wnt signaling: is the party in the nucleus? Genes Dev 20:1394–1404
Xiong X, Shao W, Jin T (2012) New insight into the mechanisms underlying the function of the incretin hormone glucagon-like peptide-1 in pancreatic β-cells: the involvement of the Wnt signaling pathway effector β-catenin. Islets 4:359–365
Xu X, D'Hoker J, Stangé G, Bonné S, De Leu N, Xiao X, Van de Casteele M, Mellitzer G, Ling Z, Pipeleers D, Bouwens L, Scharfmann R, Gradwohl G, Heimberg H (2008) β cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 132:197–207
Yamada T, Ishihara H, Tamura A et al (2006) WFS1-deficiency increases endoplasmic reticulum stress, impairs cell cycle progression and triggers the apoptotic pathway specifically in pancreatic β cells. Hum Mol Genet 15:1600–1609
Yan HX, Yang W, Zhang R, Chen L, Tang L, Zhai B, Liu SQ, Cao HF, Man XB, Wu HP, Wu MC, Wang HY (2006) Protein-tyrosine phosphatase PCP-2 inhibits β-catenin signaling and increases E-cadherin-dependent cell adhesion. J Biol Chem 281:15423–15433
Yang H, Li Q, Lee JH, Shu Y (2012) Reduction in Tcf7l2 expression decreases diabetic susceptibility in mice. Int J Biol Sci 8(6):791–801
Yano T, Liu Z, Donovan J, Thomas MK (2007) Habener JF (2007) Stromal cell derived factor-1 (SDF-1)/CXCL12 attenuates diabetes in mice and promotes pancreatic β cell survival by activation of the prosurvival kinase Akt. Diabetes 56:2946–2957
Yi F, Brubaker PL, Jin T (2005) TCF-4 mediates cell type-specific regulation of proglucagon gene expression by β-catenin and glycogen synthase kinase 3 β. J Biol Chem 280:1457–1464
Yi F, Sun J, Lim GE, Fantus IG, Brubaker PL, Jin T (2008) Cross talk between the insulin and Wnt signaling pathways: evidence from intestinal endocrine L cells. Endocrinology 149:2341–2351
Zamparini AL, Watts T, Gardner CE, Tomlinson SR, Johnston GI, Brickman JM (2006) Hex acts with β-catenin to regulate anteroposterior patterning via a Groucho-related co-repressor and Nodal. Development 133:3709–3722
Zamparnini AL, Watts T, Gardner CE, Tomlinson SR, Johnston GI, Brickman JM (2006) Hex acts with β-catenin to regulate anteroposterior patterning via a Groucho-related co-repressor and Nodal. Development 133:3709–3722

Zeggini et al (2007) Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 316:1336–1341

Zhao J, Schug J, Li M, Kaestner KH, Grant SF (2010) Disease-associated loci are significantly over-represented among genes bound by transcription factor 7-like 2 (TCF7L2) in vivo. Diabetologia 53:2340–2346

Zhou Y, Zhang E, Berggreen C, Jing X, Osmark P, Lang S, Cilio CM, Göransson O, Groop L, Renström E, Hansson O (2012) Survival of pancreatic β cells is partly controlled by a TCF7L2-p53-p53INP1-dependent pathway. Hum Mol Genet 21:196–207

Islet Structure and Function in the GK Rat

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Contents

Abstract

Type 2 diabetes mellitus (T2D) arises when the endocrine pancreas fails to secrete sufficient insulin to cope with the metabolic demand because of β-cell secretory dysfunction and/or decreased β-cell mass. Defining the nature of the pancreatic islet defects present in T2D has been difficult, in part because human islets are inaccessible for direct study. This review is aimed to illustrate to what extent the Goto–Kakizaki rat, one of the best characterized animal models of spontaneous T2D, has proved to be a valuable tool offering sufficient

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_24,

commonalities to study this aspect. A comprehensive compendium of the multiple functional GK islet abnormalities so far identified is proposed in this perspective. The pathogenesis of defective β-cell number and function in the GK model is also discussed. It is proposed that the development of T2D in the GK model results from the complex interaction of multiple events: (i) several susceptibility loci containing genes responsible for some diabetic traits (distinct loci encoding impairment of β-cell metabolism and insulin exocytosis, but no quantitative trait locus for decreased β-cell mass); (ii) gestational metabolic impairment inducing an epigenetic programming of the offspring pancreas (decreased β-cell neogenesis and proliferation) transmitted over generations; and (iii) loss of β-cell differentiation related to chronic exposure to hyperglycemia/hyperlipidemia, islet inflammation, islet oxidative stress, islet fibrosis, and perturbed islet vasculature.

Keywords
Type 2 diabetes • GK rat • Islet cells • β-cell development • Differentiation and survival • Insulin release

The Goto–Kakizaki (GK) Wistar Rat as Model of Spontaneous T2D

Type 2 diabetes (T2D) arises when the endocrine pancreas fails to secrete sufficient insulin to cope with the metabolic demand (Butler et al. 2003; Donath and Halban 2004) because of β-cell secretory dysfunction and/or decreased β-cell mass. Hazard of invasive sampling and lack of suitable noninvasive methods to evaluate β-cell mass and β-cell functions are strong limitations for studies of the living pancreas in human. In such a perspective, appropriate rodent models are essential tools for identification of the mechanisms that increase the risk of abnormal β-cell mass/function and of T2D. Some answers to these major questions are available from studies using the endocrine pancreas of the Goto–Kakizaki (GK) rat model of T2D. It is the aim of this chapter to review the common features that make studies of the GK β-cell so compelling.

The GK line was established by repeated inbreeding from Wistar (W) rats selected at the upper limit of normal distribution for glucose tolerance (Goto et al. 1975, 1988; Portha et al. 2001, 2007; Östenson 2001; Portha 2005).

Until the end of the 1980s, GK rats were bred only in Sendai (Goto et al. 1975). Colonies were then initiated with breeding pairs from Japan, in Paris, France (GK/Par) (Portha et al. 1991); Dallas, TX, USA (GK/Dal) (Ohneda et al. 1993); Stockholm, Sweden (GK/Sto) (Östenson 2001); Cardiff, UK (GK/Card) (Lewis et al. 1996); Coimbra, Portugal (GK/Coi) (Duarte et al. 2004); and Tampa, USA (GK/Tamp) (Villar-Palasi and Farese 1994). Some other colonies existed for shorter periods during the 1990s in London, UK (GK/Lon) (Hughes et al. 1994); Aarhus, Denmark; and Seattle, USA (GK/Sea) (Metz et al. 1999). There are also GK rat colonies derived from Paris in Oxford, UK (GK/Ox) (Wallis et al. 2004),

and Brussels, Belgium (GK/Brus) (Sener et al. 2001). Also, GK rats are available commercially from Japanese breeders Charles River Japan, Yokohama; Oriental Yeast, Tokyo; Clea Japan Inc, Osaka (GK/Clea); Japan SLC, Shizuoka (GK/SLC); and Takeda Lab Ltd, Osaka (GK/Taked) and from Taconic, USA (GK/Mol/Tac).

In our colony (GK/Par subline) maintained since 1989, the adult GK/Par body weight is 10–30 % lower than that of age- and sex-matched control animals. In male GK/Par rats, non-fasting plasma glucose levels are typically 10–14 mM (6–8 mM in age-matched Wistar (W) outbred controls). Despite the fact that GK rats in the various colonies bred in Japan and outside over 20 years have maintained rather stable degree of glucose intolerance, other characteristics such as β-cell number, insulin content, and islet metabolism and secretion have been reported to differ between some of the different colonies, suggesting that different local breeding environments and/or newly introduced genetic changes account for contrasting phenotypic properties.

Presently it is not clear whether the reported differences are artifactual or true. Careful and extensive identification of GK phenotype within each local subline is therefore necessary when comparing data from different GK sources. For further details concerning the pathogenic sequence culminating in the chronic hyperglycemia at adult age in the GK/Par rat, please refer to recent reviews (Östenson 2001; Portha 2005; Portha et al. 2007).

A Perturbed Islet Architecture, with Signs of Progressive Fibrosis, Inflammatory Microenvironment, Microangiopathy, and Increased Oxidative Stress

The adult GK/Par pancreas exhibits two different populations of islets in situ: large islets with pronounced fibrosis (Portha et al. 2001) and heterogeneity in the staining of their β-cells and small islets with heavily stained β-cells and normal architecture. One striking morphologic feature of GK rat islets is the occurrence of these big islets characterized by connective tissue separating strands of endocrine cells (Goto et al. 1988; Suzuki et al. 1992; Guenifi et al. 1995). Accordingly, the mantle of glucagon and somatostatin cells is disrupted and these cells are found intermingled between β-cells. These changes increase in prevalence with ageing (Suzuki et al. 1992).

No major alteration in pancreatic glucagon content, expressed per pancreatic weight, has been demonstrated in GK/Sto rats (Abdel-Halim et al. 1993), although the total α-cell mass was decreased by about 35 % in adult GK/Par rats (Movassat et al. 1997). The peripheral localization of glucagon-positive cells in W islets was replaced in GK/Sto rats with a more random distribution throughout the core of the islets (Guest et al. 2002). Pancreatic somatostatin content was slightly but significantly increased in GK/Sto rats (Abdel-Halim et al. 1993).

Chronic inflammation at the level of the GK/Par islet has recently received demonstration, and it is now considered as a pathophysiological contributor in

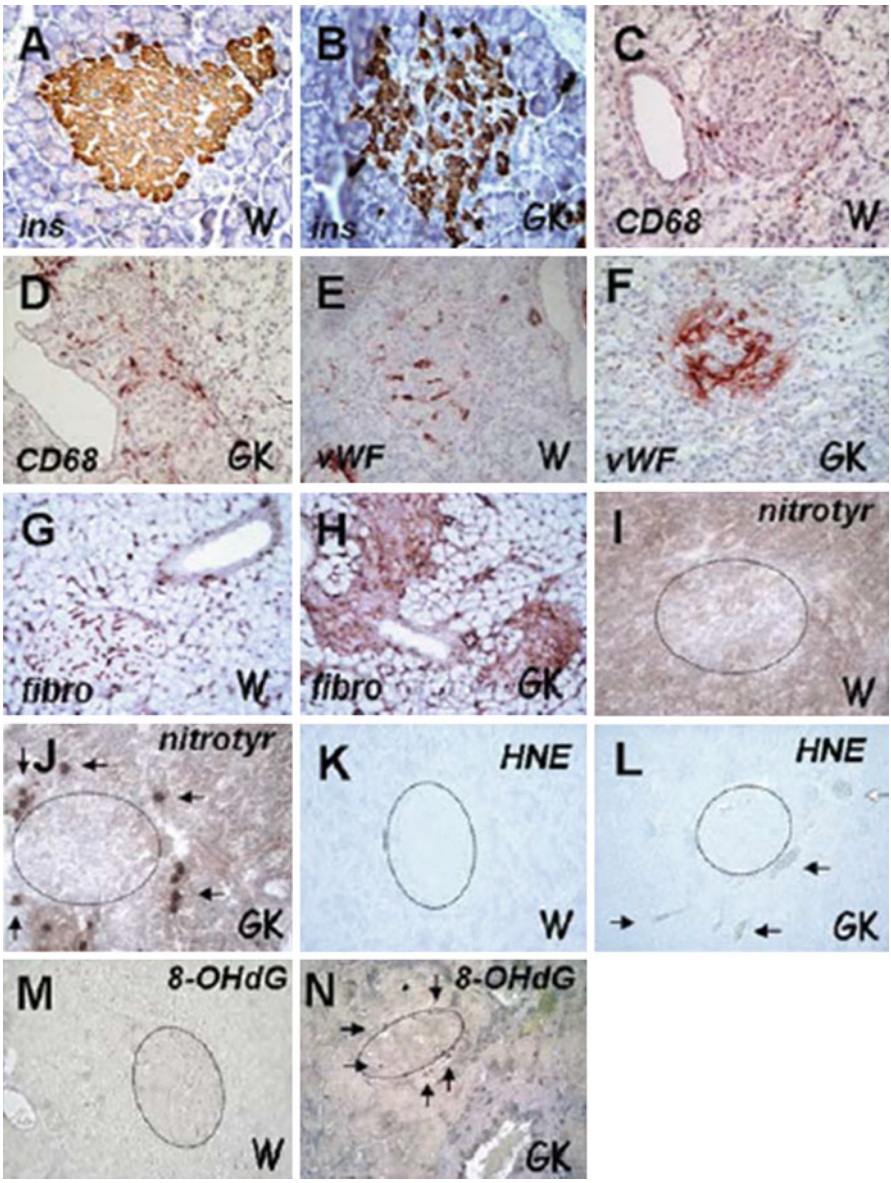

Fig. 1 Insulin labelling demonstrates the concomitant presence of large fibrotic islets (*B*) in adult GK/Par pancreas as compared with age-matched control Wistar (W) pancreas (*A*) (×500). Fibrosis is extensive in large GK/Par islets, as shown by fibronectin (*H*) labelling (×250). Small intact islets coexist with large fibrotic islets (not shown). Inflammatory cells infiltrate the islets of adult GK/Par rats. Compared with adult W rats, numerous macrophages are present in/around GK/Par islets, as shown by CD68 (*D* vs. *C*; ×500) and MHC class II (not shown) labellings. The concomitant presence of macrophages and granulocytes together with the quasi-absence of T and B cells and ED3 macrophages that are involved in autoimmune reaction (data not shown)

type 2 diabetes (Ehses et al. 2007a, b). Using an Affymetrix microarray approach to evaluate islet gene expression in freshly isolated adult GK/Par islets, we found that 34 % of the 71 genes found to be overexpressed belong to inflammatory/immune response gene family and 24 % belong to extracellular matrix (ECM)/cell adhesion gene family (Homo-Delarche et al. 2006) Numerous macrophages ($CD68^+$ and MHC class II^+) and granulocytes were found in/around adult GK/Par islets (Homo-Delarche et al. 2006). Upregulation of the MHC class II gene was also reported in a recent study of global expression profiling in GK/Takonic islets (Ghanaat-Pour et al. 2007). Immunolocalization with anti-fibronectin and anti-vWF antibodies indicated that ECM deposition progresses from intra- and peri-islet vessels, as it happens in microangiopathy (Homo-Delarche et al. 2006). These data demonstrate that a marked inflammatory reaction accompanies GK/Par islet fibrosis and suggest that islet alterations develop in a way reminiscent of microangiopathy (Ehses et al. 2007b). The previous reports by our group and others that increased blood flow and altered vascularization are present in the GK/Par and GK/Sto models (Atef et al. 1994; Svensson et al. 1994, 2000) are consistent with such a view. The increased islet blood flow in GK rats may be accounted for by an altered vagal nerve regulation mediated by nitric oxide, since vagotomy as well as inhibition of NO synthase normalized GK/Sto islet flow (Svensson et al. 1994). In addition, islet capillary pressure was increased in GK/Sto rats (Carlsson et al. 1997); this defect was reversed after 2 weeks of normalization of glycemia by phlorizin treatment. The precise relationship between islet microcirculation and β-cell secretory function remains to be established.

\Immunohistochemistry on diabetic GK/Par pancreases (Fig. 1) showed, unlike Wistar islets, the presence of nitrotyrosine and HNE labellings, which identify ROS and lipid peroxidation, respectively. Marker-positive cells were predominantly localized at the GK/Par islet periphery or along ducts and were accompanied by inflammatory infiltrates. Intriguingly, no marker-positive cell was detected within the islets in the same GK/Par pancreases (Lacraz et al. 2009). Such was not apparently the case in GK/Taked pancreases, as 8-OHdG and HNE-modified proteins accumulation was described within the islets. In this last study, the animals were older as compared to our study and accumulation of markers was correlated to hyperglycemia duration (Ihara et al. 1999). This suggests that the lack of OS-positive cells within islets as found in the young adult diabetic GK/Par is only transient and represents an early stage for a time-dependent evolutive islet adaptation.

Fig. 1 (continued) suggests a pure inflammatory process. Islet vascularization is altered in adult GK/Par rats. Labelling for vWF, a factor known to be produced by endothelial cells, shows the normal organization of islet vascularization in adult W rats (*E*). Islet vascularization differs markedly in age-matched GK/Par rats and appears to be hypertrophied (*F*) (×500). Nitrotyrosine, 4-hydroxy-2-nonenal (HNE)-modified proteins, and 8-hydroxy-2′-deoxyguanosine (8-OHdG) accumulate in the peri-islet vascular and inflammatory compartments of the adult GK/Par pancreas. Immunolabelling of nitrotyrosine, HNE adducts, or 8-OHdG (*arrows*) in pancreatic tissues of GK/Par (*J*, *L*, *N*) and *W* rats (*I*, *K*, *M*). An islet is encircled in each image (×250)

Less β-Cells Within the Pancreas with Less Replicative Activity but Intact Survival Capacity

In the adult hyperglycemic GK/Par rats (males or females), total pancreatic β-cell mass is decreased (by 60 %) (Portha et al. 2001; Movassat et al. 1997). This alteration of the β-cell population cannot be ascribed to increased β-cell apoptosis but is related, at least partly, to significantly decreased β-cell replication as measured in vivo, in situ (Portha et al. 2001). The islets isolated by standard collagenase procedure from adult GK/Par pancreases show limited decreased β-cell number (by 20 % only) and low insulin content compared with control islets (Giroix et al. 1999). The islet DNA content was decreased to a similar extent, consistent with our morphometric data, which indicates that there is no major change in the relative contribution of β-cells to total endocrine cells in the GK islets. In addition, the insulin content, when expressed relative to DNA, remains lower in GK islets than in control (inbred W/Par) islets, which supports some degranulation in the β-cells of diabetic animals (Giroix et al. 1999). Electron microscopy observation of β-cell in GK/SLC pancreas revealed that the number of β granules is decreased and that of immature granules increased. The Golgi apparatus was developed and the cisternae of the rough endoplasmic reticulum were dilated, indicating cell hyperfunction (Momose et al. 2006).

The distribution of various GK islet cell types appears to differ between some of the GK rat colonies. Thus, in the Stockholm colony, β-cell density and relative volume of insular cells were alike in adult GK/Sto rats and control W rats (Östenson 2001; Guenifi et al. 1995; Abdel-Halim et al. 1993). Similar results were reported in the Dallas colony (GK/Dal) (Ohneda et al. 1993). Reduction of adult β-cell mass, to an extent similar to that we reported in GK/Par rats, was however mentioned in GK rats from Sendai original colony (Goto et al. 1988), in GK/Taked (Koyama et al. 1998), in GK/Clea (Goda et al. 2007), and in GK/Coi (Seiça et al. 2003). Another element of heterogeneity between the different GK sources is related to the time of appearance of significant β-cell mass reduction when it is observed: It varies from fetal age in GK/Par to neonatal age in GK/Coi (Duarte et al. 2004) or young adult age (8 weeks) in GK/Taked (Koyama et al. 1998) and GK/SLC (Momose et al. 2006). The reason for such discrepancies in the onset and the severity of the β-cell mass reduction among colonies is not identified, but can be ascribed to differences in islet morphometric methodologies and/or characteristics acquired within each colony and arising from different nutritional and environmental conditions.

A meaningful set of data from our group (Movassat and Portha 1999; Miralles and Portha 2001; Plachot et al. 2001; Calderari et al. 2007) suggest that the permanently reduced β-cell mass in the GK/Par rat reflects a limitation of β-cell neogenesis during early fetal life and thereafter. Follow-up of the animals after delivery revealed that GK/Par pups become overtly hyperglycemic for the first time after 3–4 weeks of age only (i.e., during the weaning period). Despite normoglycemia, total β-cell mass was clearly decreased (by 60 %) in the GK/Par pups when compared with age-related W pups (Movassat et al. 1997). Since this early β-cell growth retardation in the prediabetic GK/Par rat pups can be ascribed neither to decreased β-cell replication nor to increased apoptosis (Movassat et al. 1997), we postulated that the recruitment

of new β-cells from the precursor pool (β-cell neogenesis) was defective in the young prediabetic GK/Par rat. A comparative study of the development of GK/Par and W pancreases indicates that the β-cell deficit (reduced by more than 50 %) starts as early as fetal age 16 days (E16) (Miralles and Portha 2001). During the time window E16–E20, we detected an unexpected anomaly of proliferation and apoptosis of undifferentiated ductal cells in the GK/Par pancreatic rudiments (Miralles and Portha 2001; Calderari et al. 2007). Therefore, the decreased cell proliferation and survival in the ductal compartment of the pancreas, where the putative endocrine precursor cells localize, suggest that the impaired development of the β-cell in the GK/Par fetus could result from the failure of the proliferative and survival capacities of the endocrine precursor cells. Data from our group indicate that defective signaling through the IGF2/IGF1-R pathway is involved in this process at this stage. Importantly this represents a primary anomaly since Igf2 and IGF1-R protein expressions are already decreased within the GK/Par pancreatic rudiment at E13.5, at a time when β-cell mass (first wave of β-cell expansion) is in fact normal (Calderari et al. 2007). Low levels of pancreatic Igf2 associated with β-cell number deficiency are maintained thereafter in the GK/Par fetuses until delivery (Serradas et al. 2002). We have also published data illustrating a poor proliferation and/or survival of the endocrine precursors also during neonatal and adult life (Movassat and Portha 1999; Plachot et al. 2001). Altogether these arguments support the notion that an impaired capacity of β-cell neogenesis (either primary in the fetus or compensatory in the newborn and the adult) results from the permanently decreased pool of endocrine precursors in the GK/Par pancreas (Movassat et al. 2007).

Which Etiology for the β-Cell Mass Abnormalities?

During the last few years, some important information concerning the determinants (morbid genes vs. environment impact) for the low β-cell mass in the GK/Par model has been supplied. Hyperglycemia experienced during the fetal and/or early postnatal life may contribute to programming of the endocrine pancreas (Simmons 2006). Such a scenario potentially applies to the GK/Par rat, as GK/Par mothers are slightly hyperglycemic through their gestation and during the suckling period (Serradas et al. 1998). We have preliminary data using an embryo transfer strategy first described by Gill-Randall et al. (2004) suggesting that GK/Par embryos transferred in the uterus of euglycemic W mother still develop deficiency of β-cell mass when adults, to the same extent as the GK/Par rats from our stock colony (Chavey et al. 2008). While this preliminary conclusion rather favors a major role for inheritance of morbid genes, additional studies are needed to really eliminate the option that the gestational diabetic pattern of the GK/Par mothers does not contribute to establish and/or maintain the transmission of endocrine pancreas programming from one GK/Par generation to the next one. Moreover, studies on the offspring in crosses between GK/Par and W rats demonstrated that F1 hybrid fetuses, regardless of whether the mother was a GK or a W rat, exhibit decreased β mass and glucose-induced insulin secretion closely resembling those in

GK/GK fetuses (Serradas et al. 1998). This finding indicates that conjunction of GK genes from both parents is not required for defective β-cell mass to be fully expressed. We have also shown that to have one GK parent is a risk factor for a low β-cell mass phenotype in young adults, even when the other parent is a normal W rat (Calderari et al. 2006).

Search for identification of the morbid genes using a quantitative trait locus (QTL) approach has led to identification of six independently segregating loci containing genes regulating fasting plasma glucose and insulin levels, glucose tolerance, insulin secretion, and adiposity in GK/Par rats (Gauguier et al. 1996). The same conclusion was drawn by Galli et al. (1996) using GK/Sto rats. This established the polygenic inheritance of diabetes-related parameters in the GK rats whatever their origin. Both studies found the strongest evidence of linkage between glucose tolerance and markers spanning a region on rat chromosome 1, called Niddm1 locus. Recent works using congenic technology have identified a short region on the Niddm1i locus of GK/Sto rats that may contribute to defective insulin secretion (Lin et al. 2001). It has been recently reported that β-cell mass is intact in Niddm1i subcongenics (Granhall et al. 2006). These results are however inconsistent with the enhanced insulin release and increased islet size described in a GK/Ox congenic strain targeting a similar short region of the GK QTL Niddm1 (Wallis et al. 2008). Finally, no QTL association with β-cell mass or β-cell size could be found in the GK/Par rat (Ktorza and Gauguier, personal communication of unpublished data). Therefore, the likelihood that a genotype alteration directly contributes to the low β-cell mass phenotype in the GK/Par rat is reduced. The raised question to be answered now is whether or not epigenetic perturbation of gene expression occurs in the developing GK/Par pancreas and programs a durable alteration of the β-cell mass as seen in the adult. *igf2* and *igf1r* genes are good candidates for such a perspective.

Finally, since the loss of GK/Taked β-cells was mitigated by in vivo treatment with the α-glucosidase inhibitor voglibose (Koyama et al. 2000) or miglitol (Goda et al. 2007) or enhanced when the animals are fed with sucrose (Koyama et al. 1998; Mizukami et al. 2008), pathological progression (β-cell number, fibrosis) of the GK β-cell mass is also dependent on the metabolic (glycemic) control.

Multiple β-Cell Functional Defects Mostly Targeting Insulin Release

Insulin Biosynthesis Is Grossly Preserved

As for total pancreatic β-cell mass, there is some controversy regarding the content of pancreatic hormones in GK rats. In the adult hyperglycemic GK/Par rats, total pancreatic insulin stores are decreased by 60–40 % (Portha et al. 2001). In other GK rat colonies (Takeda, Stockholm, Seattle), total insulin store values have been found similarly or more moderately decreased, compared with control rats (Metz et al. 1999; Abdel-Halim et al. 1993; Keno et al. 1994; Östenson et al. 1993a;

Suzuki et al. 1997; Salehi et al. 1999). The islets isolated by standard collagenase procedure from adult GK/Par pancreases show lower insulin content compared with control islets (Giroix et al. 1999). In addition, when expressed relative to DNA, the GK/Par islet insulin content remains lower (by 30 %) than in that of the control (inbred W/Par) islets, therefore supporting some degranulation in the diabetic β-cells (Giroix et al. 1999).

Glucose-stimulated insulin biosynthesis in freshly isolated GK/Par, GK/Jap, or GK/Sto islets has been reported grossly normal (Guest et al. 2002; Giroix et al. 1993a; Nagamatsu et al. 1999). The rates of biosynthesis, processing, and secretion of newly synthesized (pro)insulin were comparable (Guest et al. 2002). This is remarkable in the face of markedly lower prohormone convertase PC2 immunoreactivity and expression in the GK/Sto islets, while the expression patterns of insulin, PC1, and carboxypeptidase E (CPE) remained normal (Guest et al. 2002). Circulating insulin immunoreactivity in GK/Sto rats was predominantly insulin 1 and 2 in the expected normal ratios with no (pro)insulin evident. The finding that proinsulin biosynthesis and processing of proinsulin appeared normal in adult GK rats suggests that the defective insulin release by β-cells does not arise from a failure to recognize glucose as an activator of prohormone biosynthesis and granule biogenesis. Rather it points to an inability of the β-cell population as a whole to meet the demands on insulin secretion imposed by chronic hyperglycemia in vivo. Although basal circulating GK insulin levels were similar or slightly elevated as compared to W rats, they were always inappropriate for the level of glycemia, indicative of a secretory defect.

Glucose-Induced Activation of Insulin Release Is Lost

Impaired glucose-stimulated insulin secretion has been repeatedly demonstrated in GK rats (whatever the colony), in vivo (Portha et al. 1991; Gauguier et al. 1994, 1996; Galli et al. 1996; Salehi et al. 1999), in the perfused isolated pancreas (Östenson 2001; Portha et al. 1991; Abdel-Halim et al. 1993, 1996; Östenson et al. 1993a; Kimura et al. 1982), and in freshly isolated islets (Hughes et al. 1994; Östenson et al. 1993a; Giroix et al. 1993a, b). A number of alterations or defects have been shown in the stimulus secretion coupling for glucose in GK islets. GLUT2 is underexpressed, but not likely to the extent that it could explain the impairment of insulin release (Ohneda et al. 1993). This assumption is supported by the fact that glucokinase/hexokinase activities are normal in GK rat islets (Östenson et al. 1993b; Tsuura et al. 1993; Giroix et al. 1993c). In addition, glycolysis rates in GK rat islets are unchanged or increased compared with control islets (Hughes et al. 1994; Östenson et al. 1993a; Giroix et al. 1993a, b, c; Ling et al. 1998, 2001; Fradet et al. 2008). Furthermore, oxidation of glucose has been reported decreased (Giroix et al. 1993a), unchanged (Hughes et al. 1994, 1998; Koyama et al. 2000; Östenson et al. 1993a; Giroix et al. 1993c; Fradet et al. 2008), or even enhanced (Ling et al. 1998). There exists however a common message between these data: The ratio of oxidized to glycolysed glucose was always reduced

in GK islets compared to W islets. Also, lactate dehydrogenase gene expression (Lacraz et al. 2009) and lactate production (Ling et al. 1998) are increased and pyruvate dehydrogenase activity is decreased (Zhou et al. 1995) in GK rat islets. In GK/Par islets, we showed that mitochondria exhibit a specific decrease in the activities of FAD-dependent glycerophosphate dehydrogenase (Giroix et al. 1993a, c) and branched-chain ketoacid dehydrogenase (Giroix et al. 1999). Similar reduction of the FAD-linked glycerol phosphate dehydrogenase activity was reported in GK/Sto islets (Östenson et al. 1993b; MacDonald et al. 1996). These enzymatic abnormalities could work in concert to depress glucose oxidation. An inhibitory influence of islet fatty acid oxidation on glucose oxidation can be eliminated since the islet triglyceride content was found normal and etomoxir, an inhibitor of fatty acid oxidation, failed to restore glucose-induced insulin release in GK/Sto islets (Zhou et al. 1995).

We also found that the β-cells of adult GK/Par rats had a significantly smaller mitochondrial volume compared to control β-cells (Serradas et al. 1995). No major deletion or restriction fragment polymorphism could be detected in mtDNA from adult GK/Par islets (Serradas et al. 1995); however, they contained markedly less mtDNA than control islets. The lower islet mtDNA was paralleled by decreased content of some islet mt mRNAs such as cytochrome b (Serradas et al. 1995). In accordance with this, insufficient increase in ATP generation in response to high glucose was shown by our group (Giroix et al. 1993c). This supports the hypothesis that the defective insulin response to glucose in GK islet is accounted for by an impaired ATP production, closure of the ATP-regulated K^+ channels (Tsuura et al. 1993), and impaired elevation of intracellular [Ca^{2+}] (Hughes et al. 1998; Marie et al. 2001; Dolz et al. 2005).

Such a view validated in the GK/Par β-cell is however contradictory to the reports in GK/Sto and GK/Sea islets that the rate of ATP production is unimpaired (Metz et al. 1999; Ling et al. 1998). Other energy metabolism defects identified in GK/Sto islets include increased glucose cycling due to increased glucose-6-phosphatase activity (Östenson et al. 1993a; Ling et al. 1998) and decreased pyruvate carboxylase activity (MacDonald et al. 1996). It is possible that these alterations may affect ATP concentrations locally. However, the enzyme dysfunctions were restored by normalization of glycemia in GK/Sto rats (MacDonald et al. 1996; Ling et al., unpublished observations), but with only partial improvement of glucose-induced insulin release. Hence, it is likely that these altered enzyme activities result from a glucotoxic effect rather than being primary causes behind the impaired secretion. Also, lipotoxic effects leading to defective insulin release have been observed in GK rats on high-fat diet (Shang et al. 2002; Briaud et al. 2002), possibly mediated by a mechanism partly involving modulation of UCP-2 expression.

Insulin Secretion Amplifying Mechanisms Are Altered

Phosphoinositide (Dolz et al. 2005) and cyclic AMP metabolism (Dolz et al. 2005, 2006) are also affected in GK/Par islets. While carbachol was able to promote

normal inositol generation in GK/Par islets, high glucose failed to increase inositol phosphate accumulation. The inability of glucose to stimulate IP production is not related to defective phospholipase C activity per se (total activity in islet homogenates is normal) (Dolz et al. 2005). It is rather linked to abnormal targeting of the phosphorylation of phosphoinositides: The activity of phosphatidylinositol kinase, which is the first of the two phosphorylating activities responsible for the generation of phosphatidylinositol biphosphate, is clearly reduced (5, Giroix, unpublished data). Moreover, deficient calcium handling and ATP supply in response to glucose probably also contribute to abnormal activation of PI kinases and phospholipase C. A marked decrease in SERCA3 expression has also been described in the GK/Sto islets (Váradi et al. 1996).

Concerning cAMP, it is remarkable that its intracellular content is very high in GK/Par β-cells already at low glucose (Dolz et al. 2005). This is related to increased expression (mRNA) of the adenylyl cyclase isoforms 2 and 3, and of the GαS and Gαolf, while AC8 and phosphodiesterases PDE3B and PDE1C isoforms remain normal (Lacraz, unpublished data 2009). Furthermore, cAMP is not further enhanced at increasing glucose concentrations (at variance with the situation in normal β-cells) (Dolz et al. 2005, 2006). This suggests that there exists a block in the steps linking glucose metabolism to activation of adenylate cyclase in the GK/Par β-cell. In the GK/Sto rat, it has been shown that increased AC3 is due to functional mutations in the promoter region of the Ac3 gene (Abdel-Halim et al. 1998). We do not retain this hypothesis in the GK/Par islet since we found that the expressions (mRNA) of AC 2 and AC 3, and of GαS and Gαolf, are not increased in the prediabetic GK/Par islets (Lacraz, unpublished data 2009).

The increased cAMP production has also offered the possibility to fully restore the β-cell secretory competence to glucose in GK/Par as well as GK/Sto islets (Abdel-Halim et al. 1996; Dolz et al. 2006) with a clear biphasic response (Dolz et al. 2006). This also proves that the glucose incompetence of the GK/Par β-cell is not irreversible and emphasizes the usefulness of GLP-1 as a therapeutic agent in T2D. Also, cholinergic stimulation has been demonstrated to restore glucose-induced insulin secretion from GK/Sto as well as GK/Par islets (Dolz et al. 2005; Guenifi et al. 2001). We have proposed that such a stimulation is not mediated through activation of the PKC pathway, but via a paradoxical activation of the cAMP/PKA pathway to enhance Ca^{2+}-stimulated insulin release in the GK/Par β-cell (Dolz et al. 2005).

Other intriguing aspects of possible mechanisms behind defective glucose-induced insulin release in GK/Sto rat islets are the findings of dysfunction of islet lysosomal enzymes (Salehi et al. 1999), as well as excessive NO generation (Mosén et al. 2008; Salehi et al. 2008) or marked impairment of the glucose–heme oxygenase–carbon monoxide signaling pathway (Mosén et al. 2005). Islet activities of classical lysosomal enzymes, such as acid phosphatase, *N*-acetyl-β-D-glucosaminidase, β-glucuronidase, and cathepsin D, were reduced by 20–35 % in the GK rat. In contrast, the activities of the lysosomal α-glucosidehydrolases (acid glucan-1,4-α-glucosidase and acid α-glucosidase) were increased by 40–50 %. Neutral α-glucosidase (endoplasmic reticulum) was unaffected. Comparative

analysis of liver tissue did not display such a difference. Since no sign of an acarbose effect on GK α-glucosidehydrolase activity (contrarily to Wistar islet) was seen, it was proposed that dysfunction of the islet lysosomal/vacuolar system participates to impairment of glucose-induced insulin release in the GK/Sto rat (Salehi et al. 1999). An abnormally increased NO production in the GK/Sto islets might also be an important factor in the pathogenesis of β-cell dysfunction, since it was associated with abnormal iNOS expression in insulin and glucagon cells, increased ncNOS activity, impaired glucose-stimulated insulin release, glucagon hypersecretion, and impaired glucose-induced glucagon suppression. Moreover, pharmacological blockade of islet NO production by the NOS inhibitor NG-nitro-L-arginine methyl ester greatly improved hormone secretion from GK/Sto islets, and GLP-1 suppressed iNOS and ncNOS expression and activity with almost full restoration of insulin release and partial restoration of glucagon release (Mosén et al. 2008; Salehi et al. 2008).

Also carbon monoxide (CO) derived from β-cell heme oxygenase (HO) might be involved in the secretory dysfunction. GK/Sto islets displayed a markedly decreased HO activity measured as CO production and immunoblotting revealed a 50 % reduction of HO-2 protein expression (Mosén et al. 2005). Furthermore, a prominent expression of inducible HO (HO-1) was found in GK/Sto (Mosén et al. 2005) as well as GK/Par (Lacraz et al. 2009) islets. The glucose-stimulated CO production and the glucose-stimulated insulin response were considerably reduced in GK/Sto islets. Since addition of the HO activator hemin or gaseous CO to incubation media brought about a normal amplification of glucose-stimulated insulin release in GK/Sto islets, it was proposed that distal steps in the HO–CO signaling pathway are not affected (Mosén et al. 2005).

A diminished pattern of expression and glucose-stimulated activation of several PKC isoenzymes (α, θ, and ζ) has been reported in GK/Sto islets, while the novel isoenzyme PKC ε not only showed a high expression level but also lacked glucose activation (Warwar et al. 2006; Rose et al. 2007). Since broad-range inhibition of the translocation of PKC isoenzymes by BIS increased the exocytotic efficacy of Ca^{2+} to trigger secretion in isolated GK/Sto β-cells (Rose et al. 2007), perturbed levels and/or activation of some PKC isoforms may be part of the defective signals downstream to glucose metabolism, involved in the GK insulin secretory lesion.

Peroxovanadium, an inhibitor of islet protein tyrosine phosphatase (PTP) activities, was shown to enhance glucose-stimulated insulin secretion from GK/Sto islets (Abella et al. 2003; Chen and Ostenson 2005). One possible target for this effect could be PTP sigma that is overexpressed in GK/Sto islets (Östenson et al. 2002). At present it is not known which exocytosis-regulating proteins are affected by the increased PTPase activity. In addition, defects in islet protein histidine phosphorylation have been proposed to contribute to impaired insulin release in GK/Sea islets (Kowluru 2003).

Lastly, an increased storage and secretion of amylin relative to insulin was found in the GK/Sto rat (Leckström et al. 1996), and GLP1 treatment in vivo was recently reported to exert a beneficial effect on the ratio of amylin to insulin mRNA in GK pancreas besides improvement of glucose-induced insulin release

(Weng et al. 2008). This is consistent with hypersecretion of amylin being one of the factors contributing to the impairment of glucose-induced insulin release.

Insulin Exocytotic Machinery Is Abnormal

In addition to these upstream abnormalities, important defects reside late in signal transduction, i.e., in the exocytotic machinery. Indeed, glucose-stimulated insulin secretion was markedly impaired in GK/Taked, GK/Sto, GK/Sea, and GK/Par islets also when the islets were depolarized by a high concentration of potassium chloride and the ATP-regulated K^+ channels kept open by diazoxide (Metz et al. 1999; Abdel-Halim et al. 1996; Okamoto et al. 1995, Szkudelski and Giroix, unpublished data). Similar results were obtained when insulin release was induced by exogenous calcium in electrically permeabilized GK/Jap islets (Okamoto et al. 1995). In fact, markedly reduced expressions of the SNARE complex proteins (α-SNAP, SNAP-25, syntaxin-1, Munc13-1, Munc18-1, *N*-ethylmaleimide-sensitive fusion protein and synaptotagmin 3) have been demonstrated in GK/Sto and GK/Taked islets (Nagamatsu et al. 1999; Gaisano et al. 2002; Zhang et al. 2002). We also recently found similar results in the GK/Par islets (Tourrel-Cuzin, unpublished data 2009). Thus, a reduced number of docking granules may account for impaired β-cell secretion (Ohara-Imaizumi et al. 2004), and this defect should partly be related to glucotoxicity (Gaisano et al. 2002). Actin cytoskeleton has also been implicated in regulated exocytosis. It has been proposed that in secretory cells, actin network under the plasma membrane acts as a physical barrier preventing the access of secretory granules to the membrane. However, the role of the subcortical actin is certainly more complex as it is also required for final transport of vesicles to the sites of exocytosis. The level of total actin protein evaluated by western blotting has been found similar in GK/Par and W islets (Movassat et al. 2005), at variance with reports in other GK rat lines (Nagamatsu et al. 1999; Gaisano et al. 2002). However, confocal analysis of the distribution of phalloidin-stained cortical actin filaments revealed a higher density of the cortical actin web nearby the plasma membrane in GK/Par islets as compared to W. Moreover, preliminary functional results suggest that the higher density of actin cortical web in the GK/Par islets contributes to the defects in glucose-induced insulin secretion exhibited by GK islets (Movassat et al. 2005).

Secretory Response to Non-glucose Stimuli Is Partly Preserved

Among the non-glucidic insulin stimulators, arginine has been shown to induce a normal or even augmented insulin response from perfused pancreases or isolated islets of GK/Clea, GK/Par, GK/Sto, and GK/Lon (Portha et al. 1991; Hughes et al. 1994; Kimura et al. 1982). Since preperfusion for 50–90 min in the absence of glucose reduced the insulin response to arginine in the GK/Par but not in the control pancreas (Portha et al. 1991), it is likely that previous exposure to glucose in vivo or during the perfusion experiment potentiates arginine-induced insulin

secretion. Insulin responses to another amino acid, leucine, and its metabolite, ketoisocaproate (KIC), were diminished in GK/Par and GK/Sto rats (Östenson 2001; Giroix et al. 1993a, 1999). This was attributed to defective mitochondrial oxidative decarboxylation of KIC operated by the branched-chain 2-ketoacid dehydrogenase (BCKDH) complex (Giroix et al. 1999). However, in GK/Lon and GK/Taked islets, KIC induced normal insulin responses (Hughes et al. 1994; Tsuura et al. 1993). Finally it is of interest that GK islets are duly responsive to non-nutrient stimuli such as the sulfonylureas gliclazide (GK/Par) (Giroix et al. 1993a) and mitiglinide (GK/Sto) (Kaiser et al. 2005), the combination of Ba^{2+} and theophylline (GK/Par) (Giroix et al. 1993a), or high external K^+ concentrations (GK/Lon, GK/Sto, GK/Seat, GK/Par) (Metz et al. 1999; Abdel-Halim et al. 1996; Katayama et al. 1995, Dolz and Portha, unpublished data). However, this does not support the assumption from the molecular biology data that there exists a defect in the late steps of insulin secretion. As a tentative to elucidate this apparent contradiction, exocytosis assessment with high time-resolution membrane capacitance measurement in GK/Sto pancreatic slices showed a decreased efficacy of depolarization-evoked Ca^{2+} influx to trigger rapid vesicle release, contrasting with a facilitation of vesicle release in response to strong sustained Ca^{2+} stimulation (Rose et al. 2007).

Islet ROS Scavenging Capacity Is Increased

Considerable interest has recently been focused on the putative role of oxidative stress (OS) upon deterioration of β-cell function/survival in diabetes. Recent data from our group indicate that paradoxically GK/Par islets revealed protected against OS since they maintained basal ROS accumulation similar to or even lower than nondiabetic islets. Remarkably, GK/Par insulin secretion also exhibited strong resistance to the toxic effect of exogenous H_2O_2 or endogenous ROS exposures. Such adaptation was associated to both high glutathione content and overexpression of a large set of genes encoding antioxidant proteins as well as UCP2 (Portha et al. 2007; Lacraz et al. 2009).

Figure 2 illustrates a compendium of the abnormal intracellular sites so far identified in the diabetic GK islets from the different sources.

Which Etiology for the Islet Functional Abnormalities?

There are several arguments indicating that the GK β-cell secretory failure is, at least partially, related to the abnormal metabolic environment (gluco-lipotoxicity). When studied under in vitro static incubation conditions, islets isolated from normoglycemic (prediabetic) GK/Par pups amplified their secretory response to high glucose, leucine, or leucine plus glutamine to the same extent as age-related W islets (Portha et al. 2001). This suggests that there does not exist a major intrinsic secretory defect in the prediabetic GK/Par β-cells which can be considered as

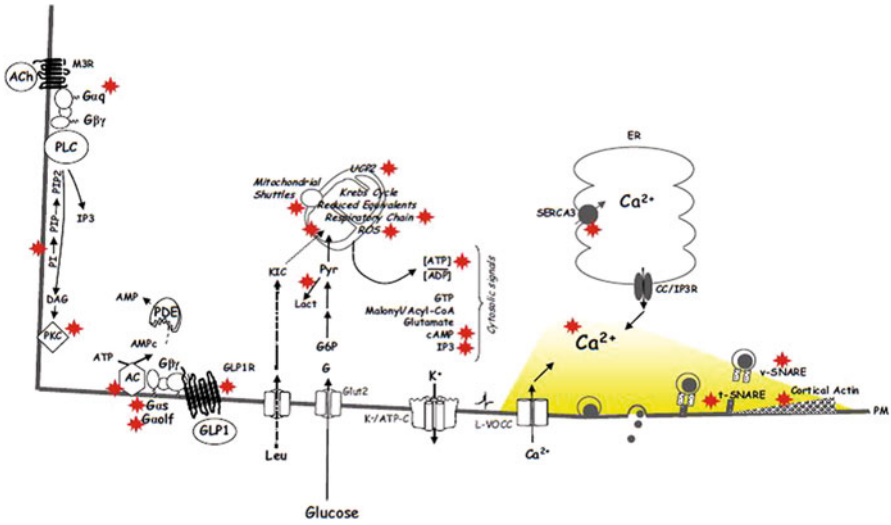

Fig. 2 Model for defective glucose-induced insulin release and the abnormal intracellular sites so far identified in the β-cell of the diabetic GK rats from the different sources (mostly the GK/Par and the GK/Sto sources). Where data are available, the impaired sites in the β-cell are indicated with the symbol ✦. Abbreviations: *Glut2* glucose transporter isoform 2, *Leu* leucine, *KIC* ketoisocaproate, *AC* adenylate cyclase isoforms, *Gαs, Gαolf, Gαq* α subunits of heterotrimeric G proteins, *Gβγ* β and γ subunits of heterotrimeric G proteins, *PI, PIP, PIP2* phosphoinositides, *PLC* phospholipase C, *PKC* protein kinase C, *DAG* diacylglycerol, *IP3* inositol-3-phosphate, *UCP2* uncoupling protein 2, *ROS* reactive oxygen species, *tSNARE, v-SNARE* SNARE proteins (syntaxin-1A, SNAP-25, VAMP-2, Munc-18), *SERCA-3* endoplasmic reticulum Ca^{2+}-ATPase isoform 3, *L-VOCC* L-type calcium channel modulated by the membrane polarization, *CC/IP3R* calcium channel modulated by receptor to IP3, K^+/*ATP-C* potassium channel modulated by the ATP/ADP ratio, *Ach* acetylcholine, *M3-R* muscarinic receptor isoform 3, *GLP-1* glucagon-like peptide 1, *GLP1-R* GLP1 receptor, *PDE* cAMP-dependent phosphodiesterase isoforms

normally glucose competent at this stage, at least when tested in vitro. In the GK/Par rat, basal hyperglycemia and normal to very mild hypertriglyceridemia are observed only after weaning (Portha et al. 2001). The onset of a profound alteration in glucose-stimulated insulin secretion by the GK/Par β-cell (after weaning) is time correlated with the exposure to the diabetic milieu. These changes in islet function could be ascribed, at least in part, to a loss of differentiation of β-cells chronically exposed to even mild chronic hyperglycemia and elevated plasma non-esterified fatty acids. This view is supported by the reports that chronic treatments of adult GK rats with phlorizin (Portha et al. 2007; Nagamatsu et al. 1999; Ling et al. 2001; Gaisano et al. 2002), T-1095 (Yasuda et al. 2002), glinides (Kaiser et al. 2005; Kawai et al. 2008), glibenclamide (Kawai et al. 2008), gliclazide (Dachicourt et al. 1998), JTT-608 (Ohta et al. 1999, 2003), voglibose (Ishida et al. 1998), or insulin (Kawai et al. 2008) partially improved glucose-induced insulin release, while hyperlipidemia induced by high-fat feeding markedly impaired their insulin secretion (Briaud et al. 2002).

The recent identification of TCF7L2 as a major predisposition gene for T2D and the predominant association of TCF7L2 variants with impaired insulin secretion have highlighted the importance of Wnt signaling in glucose homeostasis. In fact, two studies in human diabetic islets have reported that the expression of TCF7L2 is increased at mRNA (Lyssenko et al. 2007; Lee et al. 2008) and at protein levels (Lee et al. 2008), and it has been found that TCF7L2 overexpression in pancreatic β-cells is associated with reduced insulin secretion (Lyssenko et al. 2007). Islet TCF7L2 mRNA and protein levels revealed higher in GK/Par islets (Tourrel-Cuzin and Movassat, unpublished). Similar observation was reported in GK/Sto islets (Granhall et al. 2006). The functional link between the upregulation of TCF7L2 and the impairment of β-cell growth and function in the GK model remains to be uncovered.

Besides, there are indications in the GK/Sto rat that two distinct loci encode separately defects in β-cell glucose metabolism and insulin exocytosis (Granhall et al. 2006). Generation of congenic rat strains harboring different parts of GK/Sto-derived Niddm1i has recently enabled fine mapping of this locus. Congenic strains carrying the GK genotype distally in Niddm1i displayed reduced insulin secretion in response to both glucose and high potassium, as well as decreased single-cell exocytosis. By contrast, the strain carrying the GK genotype proximally in Niddm1i exhibited both intact insulin release in response to high potassium and intact single-cell exocytosis, but insulin secretion was suppressed when stimulated by glucose. Islets from this strain also failed to respond to glucose by increasing the cellular ATP to ADP ratio. Since the congenics had not developed overt hyperglycemia and their β-cell mass was found normal, it was concluded that their functional defects in glucose metabolism and insulin exocytosis were encoded by two distinct loci within Niddm1i (Granhall et al. 2006). These results in the GK/Sto are however inconsistent as previously mentioned (see Section 21.4), with the conclusion of a similarly designed congenic study indicating that the corresponding short region of the QTL Nidd/gk1 in GK/Ox congenics contributes to enhanced (and not decreased) insulin release (Wallis et al. 2008). Interestingly, the gene encoding for transcription factor TCF7L2 is also located in this locus and has recently been identified as a candidate gene for T2D in humans (Grant et al. 2006). However, Tcf7l2 RNA levels were not different in the GK/Sto congenics displaying reduced insulin secretion compared with controls (Granhall et al. 2006).

In conclusion, taking into account the diverse information so far available from the GK model through its different phenotype variants, it is proposed that the reduction of GK β-cell number and function reflects the complex interactions of different pathogenic items: multiple morbid genes causing impaired insulin secretion, early epigenetic programming of the pancreas by gestational diabetes (decreased β-cell neogenesis and/or proliferation) which is transmitted from one generation to the other, and acquired loss of β-cell differentiation due to chronic exposure to hyperglycemia/hyperlipidemia, inflammatory mediators, and oxidative stress and to perturbed islet microarchitecture. Last but not least, careful comparison of the alterations so far detected in the diabetic GK β-cell population and those found in the T2D human β-cell population put into the front stage a number of striking commonalities (Portha et al. 2009). Of course, the GK β-cell is not a

blueprint for the diseased β-cell in human. There are however sufficient similarities with high value, to justify more efforts to understand the etiopathogenesis of T2D in this rat model now widely used and, more specifically, the central role played by the GK islet cells.

Acknowledgments The GK/Par studies done at Lab B2PE, BFA Unit, have been funded by the CNRS, the French ANR (programme Physio 2006 – Prograbeta), the EFSD/MSD European Foundation, MERCK-SERONO, French Diabetes Association, and NEB Research Foundation. G. Lacraz and F. Figeac received a doctoral fellowship from the Ministere de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche (Ecole Doctorale 394, Physiologie/Physiopathologie). A. Chavey was the recipient of a CNRS postdoctoral fellowship and a NESTLE-France grant.

Cross-References

▶ Apoptosis in Pancreatic β-Islet Cells in Type 1 and Type 2 Diabetes
▶ β-Cell Function in Obese-hyperglycaemic Mice [*ob*/*ob* mice]
▶ The β-Cell in Human Type 2 Diabetes

References

Abdel-Halim SM, Guenifi A, Efendic S, Östenson CG (1993) Both somatostatin and insulin responses to glucose are impaired in the perfused pancreas of the spontaneously non-insulin dependent diabetic GK (Goto-Kakizaki) rat. Acta Physiol Scand 148:219–226

Abdel-Halim SM, Guenifi A, Khan A, Larsson O, Berggren PO, Östenson CG, Efendic S (1996) Impaired coupling of glucose signal to the exocytotic machinery in diabetic GK rats; a defect ameliorated by cAMP. Diabetes 45:934–940

Abdel-Halim SM, Guenifi A, He B, Yang B, Mustafa M, Höjeberg B, Hillert J, Bakhiet M, Efendic S (1998) Mutations in the promoter of adenylyl cyclase (AC)-III gene, overexpression of AC-III mRNA, and enhanced cAMP generation in islets from the spontaneously diabetic GK rat model of type 2 diabetes. Diabetes 47:498–504

Abella A, Marti L, Camps M, Claret M, Fernández-Alvarez J, Gomis R, Guma A, Viguerie N, Carpéné C, Palacin M, Testar X, Zorzan A (2003) Semicarbazide-sensitive amine oxidase/vascular adhesion protein-1 activity exerts an antidiabetic action in Goto–Kakizaki rats. Diabetes 52:1004–1013

Atef N, Portha B, Penicaud L (1994) Changes in islet blood flow in rats with NIDDM. Diabetologia 37:677–680

Briaud I, Kelpe CL, Johnson LM, Tran PO, Poitout V (2002) Differential effects of hyperlipidemia on insulin secretion in islets of Langerhans from hyperglycemic versus normoglycemic rats. Diabetes 51:662–668

Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC (2003) β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 52:102–110

Calderari S, Gangnerau MN, Meile MJ, Portha B, Serradas P (2006) Is defective pancreatic β-cell mass environmentally programmed in Goto Kakizaki rat model of type 2 diabetes: insights from cross breeding studies during suckling period. Pancreas 33:412–417

Calderari S, Gangnerau MN, Thibault M, Meile MJ, Kassis N, Alvarez C, Kassis N, Portha B, Serradas P (2007) Defective IGF-2 and IGFR1 protein production in embryonic pancreas

precedes β cell mass anomaly in Goto-Kakizaki rat model of type 2 diabetes. Diabetologia 50:1463–1471

Carlsson PO, Jansson L, Östenson CG, Källskog O (1997) Islet capillary blood pressure increase mediated by hyperglycemia in NIDDM GK rats. Diabetes 46:947–952

Chavey A, Gangnerau MN, Maulny L, Bailbe D, Movassat J, Renard JP, Portha B (2008) Intrauterine programming of β-cell development and function by maternal diabetes. What tell us embryo-transfer experiments in GK/Par rats? (Abstract). Diabetologia 51[Suppl 1]:A151

Chen J, Ostenson CG (2005) Inhibition of protein-tyrosine phosphatases stimulates insulin secretion in pancreatic islets of diabetic Goto–Kakizaki rats. Pancreas 30:314–317

Dachicourt N, Bailbé D, Gangnerau MN, Serradas P, Ravel D, Portha B (1998) Effect of gliclazide treatment on insulin secretion and β-cell mass in non-insulin dependent diabetic Goto-Kakizaki rats. Eur J Pharmacol 361:243–251

Dolz M, Bailbé D, Giroix MH, Calderari S, Gangnerau MN, Serradas P, Rickenbach K, Irminger JC, Portha B (2005) Restitution of defective glucose-stimulated insulin secretion in diabetic GK rat by acetylcholine uncovers paradoxical stimulatory effect of β cell muscarinic receptor activation on cAMP production. Diabetes 54:3229–3237

Dolz M, Bailbé D, Movassat J, Le Stunff H, Kassis K, Giroix MH, Portha B (2006) Pivotal role of cAMP in the acute restitution of defective glucose-stimulated insulin release in diabetic GK rat by GLP-1. Diabetes 55(Suppl 1):A371

Donath MY, Halban PA (2004) Decreased β-cell mass in diabetes: significance, mechanisms and therapeutic implications. Diabetologia 47:581–589

Duarte AI, Santos MS, Seiça R, Oliveira CR (2004) Oxidative stress affects synaptosomal γ-aminobutyric acid and glutamate transport in diabetic rats. The role of insulin. Diabetes 53:2110–2116

Ehses JA, Perren A, Eppler E, Ribaux P, Pospisilik JA, Maor-Cahn R, Gueripel X, Ellingsgaard H, Schneider MKJ, Biollaz G, Fontana A, Reinecke M, Homo-Delarche F, Donath MY (2007a) Increased number of islet-associated macrophages in type 2 diabetes. Diabetes 56:2356–2370

Ehses JA, Calderari S, Irminger JC, Serradas P, Giroix MH, Egli A, Portha B, Donath MY, Homo-Delarche F (2007b) Islet Inflammation in type 2 diabetes (T2D): from endothelial to β-cell dysfunction. Curr Immunol Rev 3:216–232

Fradet M, Giroix MH, Bailbé D, El Bawab S, Autier V, Kergoat M, Portha B (2008) Glucokinase activators modulate glucose metabolism and glucose-stimulated insulin secretion in islets from diabetic GK/Par rats (Abstract). Diabetologia 51 [Suppl 1]:A198–9

Gaisano HY, Östenson CG, Sheu L, Wheeler MB, Efendic S (2002) Abnormal expression of pancreatic islet exocytotic soluble N-ethylmaleimide-sensitive factor attachment protein receptors in Goto-Kakizaki rats is partially restored by phlorizin treatment and accentuated by high glucose treatment. Endocrinology 143:4218–4226

Galli J, Li LS, Glaser A, Östenson CG, Jiao H, Fakhrai-Rad H, Jacob HJ, Lander E, Luthman H (1996) Genetic analysis of non-insulin dependent diabetes mellitus in the GK rat. Nat Genet 12:31–37

Gauguier D, Nelson I, Bernard C, Parent V, Marsac C, Cohen D, Froguel P (1994) Higher maternal than paternal inheritance of diabetes in GK rats. Diabetes 43:220–224

Gauguier D, Froguel P, Parent V, Bernard C, Bihoreau MT, Portha B, James MR, Penicaud L, Lathrop M, Ktorza A (1996) Chromosomal mapping of genetic loci associated with non-insulin dependent diabetes in the GK rat. Nat Genet 12:38–43

Ghanaat-Pour H, Huang Z, Lehtihet M, Sjohölm A (2007) Global expression profiling of glucose-regulated genes in pancreatic islets of spontaneously diabetic Goto-Kakizaki rats. J Mol Endocrinol 39:135–150

Gill-Randall R, Adams D, Ollerton RL, Lewis M, Alcolado JC (2004) Type 2 diabetes mellitus – genes or intrauterine environment? An embryo transfer paradigm in rats. Diabetologia 47:1354–1359

Giroix MH, Vesco L, Portha B (1993a) Functional and metabolic perturbations in isolated pancreatic islets from the GK rat, a genetic model of non-insulin dependent diabetes. Endocrinology 132:815–822

Giroix MH, Sener A, Portha B, Malaisse WJ (1993b) Preferential alteration of oxidative relative to total glycolysis in pancreatic islets of two rats models of inherited or acquired type 2 (non-insulin dependent) diabetes mellitus. Diabetologia 36:305–309

Giroix MH, Sener A, Bailbé D, Leclercq-Meyer V, Portha B, Malaisse WJ (1993c) Metabolic, ionic and secretory response to D-glucose in islets from rats with acquired or inherited non-insulin dependent diabetes. Biochem Med Metab Biol 50:301–321

Giroix MH, Saulnier C, Portha B (1999) Decreased pancreatic islet response to L-leucine in the spontaneously diabetic GK rat: enzymatic, metabolic and secretory data. Diabetologia 42:965–977

Goda T, Suruga K, Komori A, Kuranuki S, Mochizuki K, Makita Y, Kumazawa T (2007) Effects of miglitol, an α-glucosidase inhibitor, on glycaemic status and histopathological changes in islets in non-obese, non-insulin-dependent diabetic Goto-Kakizaki rats. Br J Nutr 98:702–710

Goto Y, Kakizaki M, Masaki N (1975) Spontaneous diabetes produced by selective breeding of normal Wistar rats. Proc Jpn Acad 51:80–85

Goto Y, Suzuki KI, Sasaki M, Ono T, Abe S (1988) GK rat as a model of nonobese, noninsulin-dependent diabetes. Selective breeding over 35 generations. In: Shafrir E, Renold AE (eds) Lessons from animal diabetes. Libbey, London, pp 301–303

Granhall C, Rosengren AH, Renstrom E, Luthman H (2006) Separately inherited defects in insulin exocytosis and β-cell glucose metabolism contribute to type 2 diabetes. Diabetes 55:3494–3500

Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadottir A, Styrkarsdottir U, Magnusson KP, Walters GB, Palsdottir E, Jonsdottir T, Gudmundsdottir T, Gylfason A, Saemundsdottir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdottir U, Gulcher JR, Kong A, Stefansson K (2006) Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet 38:320–323

Guenifi A, Abdel-Halim SM, Höög A, Falkmer S, Östenson CG (1995) Preserved β-cell density in the endocrine pancreas of young, spontaneously diabetic Goto–Kakizaki (GK) rats. Pancreas 10:148–153

Guenifi A, Simonsson E, Karlsson S, Ahren B, Abdel-Halim SM (2001) Carbachol restores insulin release in diabetic GK rat islets by mechanisms largely involving hydrolysis of diacylglycerol and direct interaction with the exocytotic machinery. Pancreas 22:164–171

Guest PC, Abdel-Halim SM, Gross DJ, Clark A, Poitout V, Amaria R, Östenson CG, Hutton JC (2002) Proinsulin processing in the diabetic Goto–Kakizaki rat. J Endocrinol 175:637–647

Homo-Delarche F, Calderari S, Irminger JC, Gangnerau MN, Coulaud J, Rickenbach K, Dolz M, Halban P, Portha B, Serradas S (2006) Islet inflammation and fibrosis in a spontaneous model of type 2 diabetes, the GK rat. Diabetes 55:1625–1633

Hughes SJ, Suzuki K, Goto Y (1994) The role of islet secretory function in the development of diabetes in the GK Wistar rat. Diabetologia 37:863–870

Hughes SJ, Faehling M, Thorneley CW, Proks P, Ashcroft FM, Smith PA (1998) Electrophysiological and metabolic characterization of single β-cells and islets from diabetic GK rats. Diabetes 47:73–81

Ihara Y, Toyokuni S, Uchida K, Odaka H, Tanaka T, Ikeda H, Hiai H, Seino Y, Yamada Y (1999) Hyperglycemia causes oxidative stress in pancreatic β-cells of GK rats, a model of type 2 diabetes. Diabetes 48:927–932

Ishida H, Kato S, Nishimura M, Mizuno N, Fujimoto S, Mukai E, Kajikawa M, Yamada Y, Odaka H, Ikeda H, Seino Y (1998) Beneficial effect of long-term combined treatment with vogli-bose and pioglitazone on pancreatic islet function of genetically diabetic GK rats. Horm Metab Res 30:673–678

Kaiser N, Nesher R, Oprescu A, Efendic S, Cerasi E (2005) Characterization of the action of S21403 (mitiglinide) on insulin secretion and biosynthesis in normal and diabetic β-cells. Br J Pharmacol 146:872–881

Katayama N, Hughes SJ, Persaud SJ, Jones PM, Howell SL (1995) Insulin secretion from islets of GK rats is not impaired after energy generating steps. Mol Cell Endocrinol 111:125–128

Kawai J, Ohara-Imaizumi M, Nakamichi Y, Okamura T, Akimoto Y, Matsushima S, Aoyagi K, Kawakami H, Watanabe T, Watada H, Kawamori R, Nagamatsu S (2008) Insulin exocytosis in Goto-Kakizaki rat β-cells subjected to long-term glinide or sulfonylurea treatment. Biochem J 412:93–101

Keno Y, Tokunaga K, Fujioka S, Kobatake T, Kotani K, Yoshida S, Nishida M, Shimomura I, Matsuo T, Odaka H, Tarui S, Matsuzawa Y (1994) Marked reduction of pancreatic insulin content in male ventromedial hypothalamic-lesioned spontaneously non-insulin-dependent diabetic (Goto-Kakizaki) rats. Metabolism 43:32–37

Kimura K, Toyota T, Kakizaki M, Kudo M, Takebe K, Goto Y (1982) Impaired insulin secretion in the spontaneous diabetes rats. Tohoku J Exp Med 137:453–459

Kowluru A (2003) Defective protein histidine phosphorylation in islets from the Goto– Kakizaki diabetic rat. Am J Physiol 285:E498–E503

Koyama M, Wada R, Sakuraba H, Mizukami H, Yagihashi S (1998) Accelerated loss of islet β-cells in sucrose-fed Goto-Kakizaki rats, a genetic model of non-insulin-dependent diabetes mellitus. Am J Pathol 153:537–545

Koyama M, Wada R, Mizukami H, Sakuraba H, Odaka H, Ikeda H, Yagihashi S (2000) Inhibition of progressive reduction of islet β-cell mass in spontaneously diabetic Goto-Kakizaki rats by α-glucosidase inhibitor. Metabolism 49:347–352

Lacraz G, Figeac F, Movassat J, Kassis N, Coulaud J, Galinier A, Leloup C, Bailbé D, Homo-Delarche F, Portha B (2009) Diabetic Rat β-cells can achieve self-protection against oxidative stress through an adaptive up-regulation of their antioxidant defenses. PLoS ONE 4(8):e6500

Leckström A, Östenson CG, Efendic S, Arnelo U, Permert J, Lundquist I, Westermark P (1996) Increased storage and secretion of islet amyloid polypeptide relative to insulin in the spontaneously diabetic GK rat. Pancreas 13:259–267

Lee SH, Demeterco C, Geron I, Abrahamsson A, Levine F, Itkin-Ansari P (2008) Islet specific Wnt activation in human type 2 diabetes. Exp Diabetes Res Article ID 728763, doi: 10.1155/2008/728763

Lewis BM, Ismail IS, Issa B, Peters JR, Scanlon MF (1996) Desensitisation of somatostatin, TRH and GHRH responses to glucose in the diabetic Goto-Kakizaki rat hypothalamus. J Endocrinol 151:13–17

Lin JM, Ortsäter H, Fakhraid-Ra H, Galli J, Luthman H, Bergsten P (2001) Phenotyping of individual pancreatic islets locates genetic defects in stimulus secretion coupling to Niddm1i within the major diabetes locus in GK rats. Diabetes 50:2737–2743

Ling ZC, Efendic S, Wibom R, Abdel-Halim SM, Östenson CG, Landau BR, Khan A (1998) Glucose metabolism in Goto–Kakizaki rat islets. Endocrinology 139:2670–2675

Ling ZC, Hong-Lie C, Östenson CG, Efendic S, Khan A (2001) Hyperglycemia contributes to impaired insulin response in GK rat islets. Diabetes 50(Suppl 1):108–112

Lyssenko V, Lupi R, Marchetti P, Del Guerra S, Orho-Melander M, Almgren P, Sjögren M, Ling C, Eriksson KF, Lethagen AL, Mancarella R, Berglund G, Tuomi T, Nilsson P, Del Prato S, Groop L (2007) Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. J Clin Invest 117:2155–2163

MacDonald MJ, Efendic S, Östenson CG (1996) Normalization by insulin treatment of low mitochondrial glycerol phosphate dehydrogenase and pyruvate carboxylase in pancreatic islets of the GK rat. Diabetes 45:886–890

Marie JC, Bailbé D, Gylfe E, Portha B (2001) Defective glucose-dependent cytosolic Ca^{2+} handling in islets of GK and nSTZ rat models of type2 diabetes. J Endocrinol 169:169–176

Metz SA, Meredith M, Vadakekalam J, Rabaglia ME, Kowluru A (1999) A defect late in stimulus secretion coupling impairs insulin secretion in Goto-Kakizaki diabetic rats. Diabetes 48:1754–1762

Miralles F, Portha B (2001) Early development of β-cells is impaired in the GK rat model of type 2 diabetes. Diabetes 50(Suppl 1):84–88

Mizukami H, Wada R, Koyama M, Takeo T, Suga S, Wakui M, Yagihashi S (2008) Augmented β-cell loss and mitochondrial abnormalities in sucrose-fed GK rats. Virchows Arch 452:383–392

Momose K, Nunomiya S, Nakata M, Yada T, Kikuchi M, Yashiro T (2006) Immunohistochemical and electron-microscopic observation of β-cells in pancreatic islets of spontaneously diabetic Goto-Kakizaki rats. Med Mol Morphol 39:146–153

Mosén H, Salehi A, Alm P, Henningsson R, Jimenez-Feltström J, Östenson CG, Efendic S, Lundquist I (2005) Defective glucose-stimulated insulin release in the diabetic Goto-Kakizaki (GK) rat coincides with reduced activity of the islet carbon monoxide signaling pathway. Endocrinology 146:1553–1558

Mosén H, Östenson CG, Lundquist I, Alm P, Henningsson R, Jimenez-Feltstrom J, Guenifi A, Efendic S, Salehi A (2008) Impaired glucose-stimulated insulin secretion in the GK rat is associated with abnormalities in islet nitric oxide production. Regul Pept 151:139–146

Movassat J, Portha B (1999) β-cell growth in the neonatal Goto-Kakizaki rat and regeneration after treatment with streptozotocin at birth. Diabetologia 42:1098–1106

Movassat J, Saulnier C, Serradas P, Portha B (1997) Impaired development of pancreatic β-cell mass is a primary event during the progression to diabetes in the GK rat. Diabetologia 40:916–925

Movassat J, Deybach C, Bailbé D, Portha B (2005) Involvement of cortical actin cytoskeleton in the defective insulin secretion in Goto-Kakizaki rats (Abstract). Diabetes 54:A1

Movassat J, Calderari S, Fernández E, Martín MA, Escrivá F, Plachot C, Gangnerau MN, Serradas P, Álvarez C, Portha B (2007) Type 2 diabetes – a matter of failing β-cell neogenesis? Clues from the GK rat model. Diabetes Obes Metab 9(Suppl 2):187–195

Nagamatsu S, Nakamichi Y, Yamamura C, Matsushima S, Watanabe T, Azawa S, Furukawa H, Ishida H (1999) Decreased expression of t-SNARE, syntaxin 1, and SNAP-25 in pancreatic β-cells is involved in impaired insulin secretion from diabetic GK rat islets: restoration of decreased t-SNARE proteins improves impaired insulin secretion. Diabetes 48:2367–2373

Ohara-Imaizumi M, Nishiwaki C, Kikuta T, Nagai S, Nakamichi Y, Nagamatsu S (2004) TIRF imaging of docking and fusion of single insulin granule motion in primary rat pancreatic β-cells: different behaviour of granule motion between normal and Goto–Kakizaki diabetic rat β-cells. Biochem J 381:13–18

Ohneda M, Johnson JH, Inman LR, Chen L, Suzuki KI, Goto Y, Alam T, Ravazzola M, Orci L, Unger RH (1993) GLUT2 expression and function in β-cells of GK rats with NIDDM. Diabetes 42:1065–1072

Ohta T, Furukawa N, Komuro G, Yonemori F, Wakitani K (1999) JTT-608 restores impaired early insulin secretion in diabetic Goto-Kakizaki rats. Br J Pharmacol 126:1674–1680

Ohta T, Miyajima K, Komuro G, Furukawa N, Yonemori F (2003) Antidiabetic effect of chronic administration of JTT-608, a new hypoglycaemic agent, in diabetic Goto-Kakizaki rats. Eur J Pharmacol 476:159–166

Okamoto Y, Ishida H, Tsuura Y, Yasuda K, Kato S, Matsubara H, Nishimura M, Mizuno N, Ikeda H, Seino Y (1995) Hyperresponse in calcium-induced insulin release from electrically permeabilized pancreatic islets of diabetic GK rats and its defective augmentation by glucose. Diabetologia 38:772–778

Ostenson CG (2001) The Goto-Kakizaki rat. In: Sima AAF, Shafrir E (eds) Animal models of diabetes: a primer. Harwood Academic, Amsterdam, pp 197–211

Östenson CG, Khan A, Abdel-Halim SM, Guenifi A, Suzuki K, Goto Y, Efendic S (1993a) Abnormal insulin secretion and glucose metabolism in pancreatic islets from the spontaneously diabetic GK rat. Diabetologia 36:3–8

Östenson CG, Abdel-Halim SM, Rasschaert J, Malaisse-Lagae F, Meuris S, Sener A, Efendic S, Malaisse WJ (1993b) Deficient activity of FAD-linked glycerophosphate dehydrogenase in islets of GK rats. Diabetologia 36:722–726

Östenson CG, Sandberg-Nordqvist AC, Chen J, Hallbrink M, Rotin D, Langel U, Efendic S (2002) Overexpression of protein tyrosine phosphatase PTP sigma is linked to impaired

glucose-induced insulin secretion in hereditary diabetic Goto-Kakizaki rats. Biochem Biophys Res Commun 291:945–950

Plachot C, Movassat J, Portha B (2001) Impaired β-cell regeneration after partial pancreatectomy in the adult Goto-Kakizaki rat, a spontaneous model of type 2 diabetes. Histochem Cell Biol 116:131–139

Portha B (2005) Programmed disorders of β-cell development and function as one cause for type 2 diabetes? The GK rat paradigm. Diabetes Metab Res Rev 21:495–504

Portha B, Serradas P, Bailbé D, Suzuki KI, Goto Y, Giroix MH (1991) β-Cell insensitivity to glucose in the GK rat, a spontaneous nonobese model for type II diabetes. Dissociation between reductions in glucose transport and glucose-stimulated insulin secretion. Diabetes 40:486–491

Portha B, Giroix MH, Serradas P, Gangnerau MN, Movassat J, Rajas F, Bailbé D, Plachot C, Mithieux G, Marie JC (2001) β-cell function and viability in the spontaneously diabetic GK rat. Information from the GK/Par colony. Diabetes 50(Suppl 1):89–93

Portha B, Lacraz G, Dolz M, Giroix MH, Homo-Delarche F, Movassat J (2007) Issues surrounding β-cells and their roles in type 2 diabetes. What tell us the GK rat model. Expert Rev Endocrinol Metab 2:785–795

Portha B, Lacraz G, Kergoat M, Homo-Delarche F, Giroix MH, Bailbé D, Gangnerau MN, Dolz M, Tourrel-Cuzin C, Movassat J (2009) The GK rat β-cell: a prototype for the diseased human β-cell in type 2 diabetes? Mol Cell Endocrinol 297:73–85

Rose T, Efendic S, Rupnik M (2007) Ca^{2+} -secretion coupling is impaired in diabetic Goto Kakizaki rats. J Gen Physiol 129:493–508

Salehi A, Henningsson R, Mosén H, Östenson CG, Efendic S, Lundquist I (1999) Dysfunction of the islet lysosomal system conveys impairment of glucose-induced insulin release in the diabetic GK rat. Endocrinology 140:3045–3053

Salehi A, Meidute Abaraviciene S, Jimenez-Feltstrom J, Östenson CG, Efendic S, Lundquist I (2008) Excessive islet NO generation in type 2 diabetic GK rats coincides with abnormal hormone secretion and is counteracted by GLP1. PLoS ONE 3(5):e2165

Seiça R, Martins MJ, Pessa PB, Santos RM, Rosario LM, Suzuki KI, Martins MI (2003) Morphological changes of islet of Langerhans in an animal model of type 2 diabetes. Acta Med Port 16:381–388

Sener A, Ladrière L, Malaisse WJ (2001) Assessment by D-[(3)H]mannoheptulose uptake of β-cell density in isolated pancreatic islets from Goto-Kakizaki rats. Int J Mol Med 8:177–180

Serradas P, Giroix M-H, Saulnier C, Gangnerau MN, Borg LAH, Welsh M, Portha B, Welsh N (1995) Mitochondrial deoxyribonucleic acid content is specifically decreased in adult, but not fetal, pancreatic islets of the Goto-Kakizaki rat, a genetic model of non insulin-dependent diabetes. Endocrinology 136:5623–5631

Serradas P, Gangnerau MN, Giroix MH, Saulnier C, Portha B (1998) Impaired pancreatic β cell function in the fetal GK rat. Impact of diabetic inheritance. J Clin Invest 101:899–904

Serradas P, Goya L, Lacorne M, Gangnerau MN, Ramos S, Alvarez C, Pascual-Leone AM, Portha B (2002) Fetal insulin-like growth factor-2 production is impaired in the GK rat model of type 2 diabetes. Diabetes 51:392–397

Shang W, Yasuda K, Takahashi A, Hamasaki A, Takehiro M, Nabe K, Zhou H, Naito R, Fujiwara H, Shimono D, Ueno H, Ikeda H, Toyoda K, Yamada Y, Kurose T (2002) Effect of high dietary fat on insulin secretion in genetically diabetic Goto-Kakizaki rats. Pancreas 25:393–399

Simmons R (2006) Developmental origins of adult metabolic disease. Endocrinol Metab Clin N Am 35:193–204

Suzuki KI, Goto Y, Toyota T (1992) Spontaneously diabetic GK (Goto-Kakizaki) rats. In: Shafrir E (ed) Lessons from animal diabetes. Smith–Gordon, London, pp 107–116

Suzuki N, Aizawa T, Asanuma N, Sato Y, Komatsu M, Hidaka H, Itoh N, Yamauchi K, Hashizume K (1997) An early insulin intervention accelerates pancreatic β-cell dysfunction

in young Goto-Kakizaki rats, a model of naturally occurring noninsulin-dependent diabetes. Endocrinology 138:1106–1110

Svensson AM, Östenson CG, Sandler S, Efendic S, Jansson L (1994) Inhibition of nitric oxide synthase by NG-nitro-L-arginine causes a preferential decrease in pancreatic islet blood flow in normal rats and spontaneously diabetic GK rats. Endocrinology 135:849–853

Svensson AM, Östenson CG, Jansson L (2000) Age-induced changes in pancreatic islet blood flow: evidence for an impaired regulation in diabetic GK rats. Am J Physiol Endocrinol Metab 279:E1139–E1144

Tsuura Y, Ishida H, Okamoto Y, Kato S, Sakamoto K, Horie M, Ikeda H, Okada Y, Seino Y (1993) Glucose sensitivity of ATP-sensitive K^+ channels is impaired in β-cells of the GK rat. A New Genet Model NIDDM Diabetes 42:1446–1453

Váradi A, Molnár E, Östenson CG, Ashcroft SJ (1996) Isoforms of endoplasmic reticulum Ca^{2+}–ATPase are differentially expressed in normal and diabetic islets of Langerhans. Biochem J 319:521–527

Villar-Palasi C, Farese RV (1994) Impaired skeletal muscle glycogen synthase activation by insulin in the Goto-Kakizaki (G/K) rat. Diabetologia 37:885–888

Wallis RH, Wallace KJ, Collins SC, Mc Ateer M, Argoud K, Bihoreau MT, Kaisaki PJ, Gauguier D (2004) Enhanced insulin secretion and cholesterol metabolism in congenic strains of the spontaneously diabetic (type 2) Goto-Kakizaki rat are controlled by independent genetic loci in rat chromosome 8. Diabetologia 47:1096–1106

Wallis RH, Collins SC, Kaisaki PJ, Argoud K, Wilder SP, Wallace KJ, Ria M, Ktorza A, Rorsman P, Bihoreau MT, Gauguier D (2008) Pathophysiological, genetic and gene expression features of a novel rodent model of the cardio-metabolic syndrome. PLoS ONE 3(8):e2962

Warwar N, Efendic S, Östenson CG, Haber EP, Cerasi E, Nesher R (2006) Dynamics of glucose-induced localization of PKC isoenzymes in pancreatic β-cells. Diabetes-related changes in the GK rat. Diabetes 55:590–599

Weng HB, Gu Q, Liu M, Cheng NN, Li D, Gao X (2008) Increased secretion and expression of amylin in spontaneously diabetic Goto-Kakizaki rats treated with rhGLP1(7–36). Acta Pharmacol Sin 29:573–579

Yasuda K, Okamoto Y, Nunoi K, Adachi T, Shihara N, Tamon A, Suzuki N, Mukai E, Fujimoto S, Oku A, Tsuda K, Seino Y (2002) Normalization of cytoplasmic calcium response in pancreatic β-cells of spontaneously diabetic GK rat by the treatment with T-1095, a specific inhibitor of renal Na^+−glucose co-transporters. Horm Metab Res 34:217–221

Zhang W, Khan A, Östenson CG, Berggren PO, Efendic S, Meister B (2002) Down-regulated expression of exocytotic proteins in pancreatic islets of diabetic GK rats. Biochem Biophys Res Commun 291:1038–1044

Zhou YP, Östenson CG, Ling ZC, Grill V (1995) Deficiency of pyruvate dehydrogenase activity in pancreatic islets of diabetic GK rats. Endocrinology 136:3546–3551

β-Cell Function in Obese-Hyperglycemic Mice (*ob/ob* Mice)

27

Per Lindström

Contents

Abstract

This review summarizes key aspects of what has been learned about β-cell physiology from studies in *ob/ob* mice. *Ob/ob* mice lack functional leptin. They are grossly overweight and hyperphagic particularly at young ages and develop severe insulin resistance with hyperglycemia and hyperinsulinemia. *Ob/ob* mice have large pancreatic islets. The β-cells respond adequately to most stimuli, and *ob/ob* mice have been used as a rich source of pancreatic islets with high insulin release capacity. Depending on the genetic background, *ob/ob* mice can be described as a model for a constant prediabetic state or as a model for β-cell events leading to overt type 2 diabetes. The large capacity for islet growth and insulin release makes *ob/ob* mice from the C57Bl/6J or Umeå *ob/ob* strain a good model for studies on how β-cells can cope with prolonged functional stress.

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_15,

Keywords
Mouse • Pancreatic islet • β-cell • Leptin

The *ob*/*ob* Mouse

The *ob*/*ob* syndrome was found in 1949 in an outbred mouse colony at Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine (Ingalls et al. 1950) and was transferred to the already well-characterized C57Bl mice colony that had been established during the 1930s. The discovery that the *ob*/*ob* mouse syndrome is caused by a defective adipocytokine leptin opened a whole new era of metabolic studies and understanding of the endocrine functions of adipose tissue. Obesity is the most obvious characteristic of *ob*/*ob* mice. They are also hyperphagic, hyperinsulinemic, and hyperglycemic and have reduced metabolic rate and a low capacity for thermogenesis (Mayer et al. 1953; Garthwaite et al. 1980). The pancreatic islets are large and contain a high proportion of insulin-producing β-cells. It was soon discovered that *ob*/*ob* mice have a number of other traits except obesity. They are, e.g., infertile and have impaired immune functions.

The *ob*/*ob* syndrome varies considerably depending on the genetic background (Mayer and Silides 1953; Coleman and Hummel 1973). In this presentation *ob*/*ob* mice refer to 6J or Umeå *ob*/*ob* mice unless otherwise stated. On a 6J or Umeå *ob*/*ob* background, hyperglycemia is relatively mild particularly at old age and glycosuria is usually not present in the fasting state. They represent a mouse model for obesity and "diabetes" with moderate hyperglycemia, high insulin release capacity, and marked adiposity (Shafrir et al. 1999). On a KsJ or BTBR background, the mice have a higher food intake (Stoehr et al. 2004) and become overtly diabetic with a reduced life expectancy (Coleman 1978; Ranheim et al. 1997). On a 6J background the mice have a large lipogenic capacity in the liver (Clee et al. 2005), which may render them less susceptible to lipotoxic effects. β-Cells from *ob*/*ob* mice accumulate fat but only a small lipid increase is observed in β-cells from *ob*/*ob* mice on a 6J background (Garris and Garris 2004), which is in keeping with the better-preserved function. The importance of a high insulin release capacity was evident from studies where the *ob* trait was transferred to DBA mice (Chua et al. 2002). Mice with large islets and a high insulin release capacity maintained adiposity, whereas mice with lower serum insulin levels had diminished adiposity and a more severe diabetes (Chua et al. 2002). There are also differences between individual mice from the same colony of 6J and Umeå *ob*/*ob* strains with regard to hyperglycemia and other aspects of a "diabetes-like" condition. This can be used to select subgroups of animals within the same strain for metabolic studies. The fact that genetic background can have such a profound influence on the consequences of leptin deficiency has prompted a series of recent studies in search for factors involved in the development of diabetes. A comparison between BTBR and 6J *ob*/*ob* mouse islets indicates that

the Alzheimer gene *App* may be one of the top candidates for the regulation of insulin release capacity (Tu et al. 2012). Cell cycle regulatory genes are differentially expressed in islets from C57Bl/6J and BTBR mice (Davis et al. 2010), and genes important for cell survival are overexpressed in C57Bl/6J *ob*/*ob* mice islets when compared with diabetes prone BTBR mice (Keller et al. 2008; Singh et al. 2013). Calcium channel blockers increase β-cell survival and insulin sensitivity in BTRB *ob*/*ob* mice (Xu et al. 2012). The adaptive unfolded protein response is better developed in islets from *ob*/*ob* mice compared with db/db mice (Chan et al. 2013), and the capacity for adaptive unfolded protein response is reduced in *ob*/*ob* mouse β-cells if they become autophagy deficient (Quan et al. 2012).

Ob/*ob* mice are indistinguishable from their lean littermates at birth, but within 2 weeks they become heavier and develop hyperinsulinemia. The syndrome becomes much more pronounced after weaning and overt hyperglycemia is observed during the fourth week. The blood glucose rises to reach a peak after 3–5 months when the mice also have a very high food intake and a rapid growth (Westman 1968a; Edvell and Lindström 1995, 1999). After that, blood glucose values decrease and eventually become nearly normal at old age. Serum insulin levels are also very high and peak at a higher age than blood glucose values (Westman 1968a). The animals remain insulin resistant but impaired glucose tolerance and glycosuria after a glucose load are observed mostly in the post-weaning period of rapid growth (Westman 1968a; Herberg et al. 1970; Danielsson et al. 1968; Edvell and Lindström 1998).

Discovery of Leptin

Elegant parabiosis experiments showed that *ob*/*ob* mice lack but are very sensitive to a circulating factor produced by their normal siblings (Coleman 1973, 1978). By extensive positional cloning experiments, this factor could be identified in 1994 by Friedman and co-workers as leptin produced in adipose tissue (Friedman et al. 1991; Zhang et al. 1994). The *ob*/*ob* syndrome can be reversed almost completely even in adult animals by exogenous leptin or transfection with the leptin gene (Larcher et al. 2001; Pelleymounter et al. 1995; Halaas et al. 1995). There are cases with leptin deficiency in obese humans, but this is uncommon; so *ob*/*ob* mice do not present a good model for the etiology of human obesity (Clement 2006). It has not been clarified if hyperglycemia and insulin resistance depend on the adiposity or are a consequence of leptin deficiency. However, the discovery of leptin has widened our understanding of the regulation of food intake, metabolic turnover, and obesity. We also have learned a lot more about the interrelationship between metabolism and other functions such as reproduction and the immune system. Much of what we know about the physiology of leptin has been achieved through studies in *ob*/*ob* mice but also from observations in animal models with leptin receptor defects such as *db*/*db* mice and *fa*/*fa* rats (Chehab et al. 2004; Unger and Orci 2001).

Insulin Resistance and Absence of Leptin

Ob/*ob* mice have severe insulin resistance. Peripheral insulin resistance induces hyperglycemia and worsens the functional load on the β-cells. *Ob*/*ob* mouse β-cells are insulin resistant from an early age (Loreti et al. 1974; Zawalich et al. 2002). Insulin inhibits insulin release, and insulin resistance coupled to reduced PI3K-dependent signaling may result in disinhibition of glucose-induced insulin release (Zawalich et al. 2002). Insulin resistance can therefore be beneficiary for β-cell function.

β-Cells have full-length leptin receptors and leptin inhibits insulin release and insulin biosynthesis in most studies (Kieffer et al. 1996; Emilsson et al. 1997; Melloul et al. 2002). Lack of leptin effects may enhance β-cell function and explain some of the functional differences between *ob*/*ob* mice and normal mice. The main signaling pathways for leptin are the JAK/STAT transduction cascade, the mitogen-activated protein kinase (MAPK) cascade, the phosphoinositide 3-kinase (PI3K), IRS, and the 5′-AMP-activated protein kinase (AMPK) pathways (Sweeney 2002; Frühbeck 2006; Cirillo et al. 2008). The role of these signal mediators in β-cell function has not been entirely clarified but the majority of findings suggest that AMPK (Rutter et al. 2003) and p38 MAPK (Cuenda and Nebreda 2009; Sumara et al. 2009) inhibit glucose-induced insulin release. There are different isoforms of the leptin receptor. The full-length leptin receptor present in pancreatic β-cells is required for the JAK/STAT response, and activation is accompanied also by a rise in suppressor of cytokine signaling (SOCS) (Seufert 2004). A shorter receptor form, which activates PI3K, is predominant in skeletal muscle (Dyck et al. 2006) but PI3K activation is found also in β-cells (Seufert 2004). Leptin signaling pathways may interact with insulin signaling at several points including JAKs, PI3K, and MAPK (Lulu Strat et al. 2005). This interaction between insulin and leptin is complex, but studies in *ob*/*ob* mice clearly indicate that the net effect of leptin is to increase insulin sensitivity (Lulu Strat et al. 2005; Rattarasarn 2006) and that leptin resistance worsens insulin resistance. Absence of leptin can therefore be one of the causes of insulin resistance in *ob*/*ob* mice. Obese individuals are usually both insulin resistant and leptin resistant. However, the total absence of leptin signaling already from the onset of obesity in *ob*/*ob* mice is in sharp contrast to obesity in humans, and the cross talk between the cellular effects of insulin and leptin is obviously absent.

Parasympathetic cholinergic axons are numerous and widespread throughout mouse islets, whereas sympathetic neurons are more abundant in the islet periphery (Rodriguez-Diaz et al. 2011). Leptin is a central mediator of autonomic nervous function and may inhibit islet function through activation of sympathetic neurons (Hinoi et al. 2008; Tentolouris et al. 2008). β-Cells from *ob*/*ob* mice are more sensitive than lean mouse β-cells to the stimulatory effect of acetylcholine and the inhibitory effect of noradrenalin on glucose-induced insulin release (Tassava et al. 1992). This could be because of sympathetic disinhibition due to the lack of leptin. However, there is an age dependence for these effects of neurotransmitters. Islets from young *ob*/*ob* mice have an increased β-cell responsiveness to cholinergic

stimulation already from 10 to 12 days of age (Chen and Romsos 1995). The sensitivity to acetylcholine is reduced at old age, whereas the sensitivity to vagal neuropeptides may be increased (Persson-Sjögren and Lindström 2004; Persson-Sjögren et al. 2006). A reduced cholinergic activity at old age paralleling improved glycemic control is consistent with the finding that M3 receptor knockout in *ob*/*ob* mice reduces the severity of most of the phenotype (Gautam et al. 2008). Much of the effects of leptin are exerted at the level of the central nervous system, and it has been found that hypothalamic endocannabinoid signaling can be very important for the large insulin release capacity of leptin-deficient *ob*/*ob* mice (Li et al. 2013).

Ob/*ob* mouse islets have a rich supply of small vessels but a lower blood flow than lean mouse islets when calculated on the basis of islet size (Carlsson et al. 1996). *Ob*/*ob* mouse islet vessels are also more sensitive to sympathetic inhibition of islet circulation (Rooth and Täljedal 1987). This suggests that they have a reduced capacity to increase blood flow to meet metabolic demands (Carlsson et al. 1996), and this can increase β-cell stress. The increased demand for blood supply of *ob*/*ob* mouse β-cells may be met by vascular dilatation rather than by angiogenesis (Dai et al. 2013). Amyloid deposits surrounding islet cells is observed in most islets from type 2 diabetics (Butler et al. 2003) and may be part of the pathogenesis for β-cell damage. Mice do not normally form islet amyloid deposits, but *ob*/*ob* mice have high serum levels of the islet amyloid polypeptide (IAPP) (Leckström et al. 1999), and the islet content of IAPP increases during *ob*/*ob* syndrome development (Takada et al. 1996). The interaction between leptin and IAPP has not been much studied in *ob*/*ob* mouse islets but leptin inhibits IAPP release in lean mice (Karlsson et al. 1998). Leptin deficiency could therefore increase IAPP content in *ob*/*ob* mice. IAPP inhibits insulin and glucagon release (Ahrén and Sörhede Winzell 2008), and it has been suggested that IAPP also induces insulin resistance (Nyholm et al. 1998).

Pancreatic Islets

The islet volume is up to ten times higher in *ob*/*ob* mice than in normal mice (Bleisch et al. 1952; Gepts et al. 1960), and insulin-producing β-cells are by far the most numerous (Westman 1968a, b; Gepts et al. 1960; Baetens et al. 1978). The architecture and size distribution have been reevaluated in a comparative study of islets from different species (Kim et al. 2009). It was found that the distribution of islet sizes closely overlaps between species. Markedly large islets are found in *ob*/*ob* mice but also in humans and monkeys and in pregnant mice.

Both stimulatory and inhibitory effects of leptin have been found with regard to β-cell proliferation and survival (Marroquí et al. 2012). The islet hyperplasia is probably not caused by a primary abnormality in the islets due to leptin deficiency although this can contribute; the size and form of islets found in *ob*/*ob* mice are probably characteristic of islets able to adapt to increasing demands and not a feature specific for leptin deficiency. The growth may be triggered by hyperglycemia but also by other blood borne factors and nerve stimulation and is evident from

the fourth week (Edvell and Lindström 1999). Partial pancreatectomy in *ob*/*ob* mice in a phase of rapid growth and severe hyperglycemia results in a huge expansion of islet area and islet number (Chen et al. 1989). The islet growth normally continues for more than 6 months and is paralleled by reduced insulin content per islet volume during conditions of free access to food (Tomita et al. 1992). The large islets with many insulin-producing β-cells are in contrast to the decreased β-cell mass found in diabetes (Wajchenberg 2007).

Intranuclear rodlets containing the cytoskeletal protein class III β-tubulin were first described in neurons (Milman et al. 2010). These rodlets are also found in β-cells from humans and normal mice but are in very low numbers in β-cells from *ob*/*ob* mice (Woulfe and Munoz 2000). The functional significance of intranuclear rodlets is not known. Advanced glycation end products are formed intracellularly in β-cells as a consequence of long-standing hyperglycemia and may be involved in β-cell damage leading to insufficient insulin release and overt type 2 diabetes (Han et al. 2013). The receptor for glycation products was upregulated in *ob*/*ob* mouse β-cells when compared with controls, but levels were lower than in *db*/*db* mouse β-cells (Han et al. 2013).

The cellular mechanisms for glucose-induced insulin release is not the subject of this article but islets isolated from *ob*/*ob* mice respond adequately to stimulators and inhibitors of insulin release in most experimental conditions (Hahn et al. 1974; Hellman et al. 1974), and they have been used in several hundred papers as a rich source of β-cells in studies of islet function. After an overnight fast the blood glucose is nearly normalized and *ob*/*ob* mouse islets release larger quantities of insulin after fasting when compared with normal mouse islets (Lavine et al. 1977). However, transplantation of coisogenic (+/+) islets to *ob*/*ob* mice lowered blood glucose values to nearly normal for 1 month (Barker et al. 1977).

The persistent hyperglycemia can therefore be a sign of insufficient β-cell function despite the high capacity to secrete insulin, and the *ob*/*ob* mouse can perhaps be described to be in a constant prediabetic state. The threshold for glucose-induced insulin release occurs at a lower glucose concentration than in lean mouse islets (Lavine et al. 1977; Chen et al. 1993). The mechanisms for this may in part be similar to the glucose hypersensitivity observed after prolonged exposure to elevated glucose in islets from normoglycemic animals and involve both metabolic and ionic events (Ling and Pipeleers 1996; Khaldi et al. 2004).

Oscillatory Insulin Release

Serum insulin shows diurnal oscillations, and it is thought that the effect of insulin on target organs is improved when insulin is delivered in a pulsatile manner. We know little about the periodicity of serum insulin in *ob*/*ob* mice, but serum insulin levels vary considerably in the same mouse also when sampled under tightly controlled conditions (Lindström unpublished). The oscillations can be triggered by several mechanisms including variations in cytosolic calcium and metabolic oscillations (Heart and Smith 2007). Variation in cAMP levels is also a likely

candidate as evidenced from studies in *ob*/*ob* mouse β-cells (Grapengiesser et al. 1991) and β-cell lines (Dyachok et al. 2006). *Ob*/*ob* mouse islets have a reduced capacity to accumulate cAMP (Black et al. 1986, 1988a), but they are more sensitive to a rise in cAMP for stimulation of insulin release (Black et al. 1986). The β-cells have an increased Na/K-ATPase activity (Elmi 2001) and may be more sensitive to voltage-dependent events (Fournier et al. 1990) perhaps due to a reduced activation of K_{ATP} channels (Seufert 2004). However, the function of voltage-dependent Ca^{2+} channels is impaired (Black et al. 1988b), and there is a disturbed pattern of cytoplasmic calcium changes after glucose stimulation (Ravier et al. 2002). *Ob*/*ob* mouse β-cells also do not show the same type of cell-specific Ca^{2+} responses from individual cells that are found in lean mouse islets (Gustavsson et al. 2006). There is an excessive firing of cytoplasmic Ca^{2+} transients when *ob*/*ob* mouse β-cells are stimulated with glucagon (Ahmed and Grapengiesser 2001). This effect could be a direct consequence of leptin deficiency because it was reduced when leptin was also added. Ryanodine receptors in the endoplasmic reticulum may be involved in β-cell calcium regulation and stimulation of insulin release, but the precise role is controversial (Islam 2002; Bruton et al. 2003). In one study it was reported that β-cells from *ob*/*ob* mice have less ryanodine receptor activation than β-cells from lean mice (Takasawa et al. 1998).

An increased sensitivity to cAMP could also have other effects. Uncoupling protein-2 (UCP-2) was demonstrated in β-cells a decade ago, and it has been suggested that UCP-2 is important as a negative regulator of glucose-induced insulin release and protection against oxidative stress. cAMP was found to reduce the inhibitory effect of a rise in UCP-2 through PKA-mediated inhibition of the K_{ATP} channel (McQuaid et al. 2006). *Ob*/*ob* mouse β-cells have increased activity of UCP-2 (Saleh et al. 2006; Zhang et al. 2001) when compared with lean mice from the same background. Inhibition of UCP-2 improved glucose tolerance (De Souza et al. 2007), but knockdown of UCP-2 expression had no effect on glucose-induced insulin release in *ob*/*ob* mouse islets (Saleh et al. 2006). ACTH receptor activation is coupled to a rise in cAMP (Enyeart 2005). Leptin stimulates both CRF and ACTH secretion (Malendowicz et al. 2007), but *ob*/*ob* mice show signs of increased ACTH activity. Serum ACTH levels are high and islets from *ob*/*ob* mice respond with a larger increase in insulin release after stimulation with ACTH 1-39 (Bailey and Flatt 1987).

β-Cell Mass

One of the features of *ob*/*ob* mice is that they have large pancreatic islets consisting of mostly β-cells, and *ob*/*ob* mice have been used in studies of β-cell proliferation. β-Cell growth is probably directly or indirectly stimulated by hyperglycemia. There is a good correlation between the level of hyperglycemia and islet cell replication in rat (Bonner-Weir et al. 1989) and obese hyperglycaemic mice (Andersson et al. 1989), and the morphology of *ob*/*ob* mice islets reaggregated in vitro depends on the glucose concentration (Norlund et al. 1987). The source of β-cell expansion

is still controversial. It has been suggested that cells recruited from the bone marrow increase the insulin release capacity in *ob*/*ob* mice (Kojima et al. 2004). Duct progenitor cells can also be involved in the expansion of the β-cell mass, but mitotic figures have been demonstrated in β-cells from *ob*/*ob* mice (Edvell and Lindström 1999; Gepts et al. 1960), and cells within existing islets can be important for expansion of the total islet mass (Bock et al. 2003). *Ob*/*ob* mice have a growth-promoting environment for β-cells depending on (extra) pancreatic factors (Tyrberg et al. 2001; Flier et al. 2001) perhaps including insulin (Lee and Nielsen 2009). Oncogenes stimulate *ob*/*ob* mice β-cell replication as a sign that they can be manipulated extrinsically (Welsh et al. 1988). Blood-borne factors probably include NPY (Cho and Kim 2004) and GLP-1 (Edvell and Lindström 1999; Stoffers et al. 2000; Blandino-Rosano et al. 2008) which both stimulate *ob*/*ob* mouse β-cell replication. Interestingly, NPY inhibits insulin release and *ob*/*ob* mouse islets have reduced expression of NPY receptors (Imai et al. 2007). Obesity probably also induces an indirect neuronal signal emanating from the liver which is important for stimulation of islet growth in *ob*/*ob* mice (Imai et al. 2008). Cytokines and growth hormone may be important mitogens for β-cells (Black et al. 1988b; Lindberg et al. 2005). Intracellular signaling for GH receptors includes JAK/STAT activation, and this is inhibited by SOCS that inhibit cytokine signaling (Black et al. 1988b; Gysemans et al. 2008). Inhibition of cytokine signaling by SOCS may prevent β-cell death induced by several cytokines such as IL-1β, TNFα, and IFNγ (Gysemans et al. 2008). Leptin activates both JAK/STAT and SOCS, and it is possible that the net effect of leptin deficiency is to stimulate β-cell growth through a lowering of SOCS. Low-grade inflammation may be important for increased adiposity and for the pathogenesis of type 2 diabetes (Hill et al. 2009; Donath et al. 2008). Leptin stimulates the immune system and is involved in macrophage activation and release of cytokines (Lam and Lu 2007). This could be part of the explanation why leptin deficiency may prevent β-cell death. Few studies have specifically addressed the effect of cytokines in *ob*/*ob* mouse islets, but they respond normally to cytokine activators and inhibitors (Prieto et al. 1992; Zaitseva et al. 2006; Peterson et al. 2008).

Glucotoxicity and Lipotoxicity

Glucotoxicity caused by long-standing hyperglycemia may be one factor inducing β-cell death in the development of type 2 diabetes. The toxicity may be caused by induction of reactive oxygen species and by inducing endoplasmic reticulum (ER) stress (Wajchenberg 2007). ER stress is probably an important cause of β-cell dysfunction in diabetes (Eizirik et al. 2008). ER stress can be an important cause of leptin resistance (Ozcan et al. 2009), but this may not be relevant to *ob*/*ob* mice since they lack leptin. However, *ob*/*ob* mice show clear signs of hepatocyte ER stress (Marí et al. 2006; Yang et al. 2007; Sreejayan et al. 2008), and it is likely that also the β-cells have ER stress because of the increased demands for protein synthesis.

Ob/*ob* mice are living proof that prolonged hyperglycemia is not necessarily deleterious. It is possible that the insulin resistance and leptin absence protects the

β-cells from the damage that constant glucose stimulation would otherwise cause. However, only few differences from lean mice have been reported with regard to β-cell metabolic signaling and enzyme activities. The mitochondrial enzyme FAD-linked glycerophosphate dehydrogenase (m-GDH) is thought to play a key role in the glucose-sensing mechanism of the insulin-producing β-cell. It catalyzes a rate-limiting step of the glycerol phosphate shuttle, but there was no difference between islets in enzyme activity between normal and *ob*/*ob* mice (Sener et al. 1993). Perhaps *ob*/*ob* mouse β-cells are protected because they have an increased glucose cycling through glucose-6-phosphatase (Khan et al. 1995). They also have lower levels of the glucose transporter (GLUT2) (Jetton et al. 2001). A reduced glucokinase activity could lessen β-cell stress. There are conflicting data as to whether glucokinase is lower (Jetton et al. 2001) or higher (Hinoi et al. 2008) than in lean mouse islets, but glucokinase activation increases the insulin release capacity (Park et al. 2013).

Elevated serum levels of free fatty acids in the presence of hyperglycemia and aberrant lipoprotein profiles could cause lipotoxic damage to β-cells. *Ob*/*ob* mouse islets show signs of a reduced fatty acid oxidation in the presence of high glucose (Berne 1975) which could lead to toxic effects of lipids. However, β-cells from *ob*/*ob* mice lack lipoprotein lipase (Nyrén et al. 2012) and show low levels of hormone-sensitive lipase (Khan et al. 2001). Leptin treatment restores hormone levels to those found in normal islets (Nyrén et al. 2012; Khan et al. 2001). The lack of intracellular lipase may be important for the balance between glucose and lipid metabolism in *ob*/*ob* mouse islets making them less vulnerable to diabetic insult. *Ob*/*ob* mice have low serum VLDL levels and high HDL levels (Camus et al. 1988), and this can also be protective. It is likely that the large capacity to accumulate fat in adipose tissue protects *ob*/*ob* mice against β-cell lipotoxicity (Flowers et al. 2007).

Incretins

The incretins GLP-1 and GIP are released in response to food ingestion and play an important role in stimulating insulin release when blood glucose levels are elevated. The half-life in circulation is short because of enzymatic digestion through dipeptidyl peptidase-4 (DPP-4). GLP-1 and GLP-1 analogues stimulate β-cell proliferation (Edvell and Lindström 1999; Stoffers et al. 2000; Blandino-Rosano et al. 2008) and glucose-induced insulin release in *ob*/*ob* mice (Cullinan et al. 1994; Young et al. 1999; Rolin et al. 2002), and inhibition of DPP-4 improves β-cell function (Moritoh et al. 2008). On the other hand, chemical ablation of the GIP receptors causes normalization of hyperglycemia, serum insulin, insulin sensitivity, glucose tolerance, and islet hypertrophy in *ob*/*ob* mice (Gault et al. 2005; Irwin et al. 2007). This indicates that different incretins can have both beneficiary and adverse effects in obesity-related hyperglycemia and insulin resistance. Glucagon levels are high in *ob*/*ob* mice (Dubuc et al. 1977). It was early hypothesized that elevated glucagon secretion contributes to the altered metabolism of *ob*/*ob* mice (Mayer 1960) and immunoneutralization of endogenous glucagon improves

metabolic control (Sorensen et al. 2006). There is a correlation between serum glucagon levels and hepatic glucose output in type 2 diabetic patients (Gastaldelli et al. 2000), and reduction of serum glucagon may be a target for diabetes treatment.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily. PPAR-γ and PPAR-α exert profound effects on lipid handling. PPAR-γ directs lipid toward adipose tissue and PPAR-α activation predominantly stimulates lipid oxidation. PPAR agonists have been used in the treatment of type 2 diabetes to reduce insulin resistance and improve β-cell function.

The adaptation of *ob*/*ob* mouse islets to insulin resistance and hyperglycemia is dependent on intact PPAR-γ_2 signaling (Medina-Gomez et al. 2009; Vivas et al. 2011), and treatment with both PPAR-γ agonists (Diani et al. 2004) and PPAR-α agonists (Lalloyer et al. 2006) improved glucose-stimulated insulin release in *ob*/*ob* mice. This is another indication that *ob*/*ob* mouse β-cells are normally under functional stress.

Conclusions

Ob/*ob* mouse islets are large and contain a high proportion of insulin-producing β-cells. They respond adequately to most stimulators and inhibitors of insulin release and have been used as a rich source of β-cells for in vitro studies of islet function. *Ob*/*ob* mouse β-cells show insulin resistance and other signs of leptin deficiency. The lack of leptin must always be taken into account when using *ob*/*ob* mice as a model. Nevertheless, *ob*/*ob* mice represent an excellent model for studies on how β-cells can adapt to increased demand and maintain a high insulin release capacity during prolonged functional stress.

Cross-References

- ▶ (Dys)Regulation of Insulin Secretion by Macronutrients
- ▶ Apoptosis in Pancreatic β-Islet Cells in Type 1 and Type 2 Diabetes
- ▶ Islet Structure and Function in the GK Rat
- ▶ The β-Cell in Human Type 2 Diabetes

References

Ahmed M, Grapengiesser E (2001) Pancreatic β-cells from obese-hyperglycemic mice are characterized by excessive firing of cytoplasmic Ca^{2+} transients. Endocrine 15:73–78

Ahrén B, Sörhede Winzell M (2008) Disturbed α-cell function in mice with β-cell specific overexpression of human islet amyloid polypeptide. Exp Diabetes Res 2008:304–313

Andersson A, Korsgren O, Naeser P (1989) DNA replication in transplanted and endogenous pancreatic islets of obese-hyperglycemic mice at different stages of the syndrome. Metabolism 38:974–978

Baetens D, Stefan Y, Ravazzola M, Malaisse-Lagae F, Coleman DL, Orci L (1978) Alteration of islet cell populations in spontaneously diabetic mice. Diabetes 27:1–7

Bailey CJ, Flatt PR (1987) Insulin releasing effects of adrenocorticotropin (ACTH 1-39) and ACTH fragments (1-24 and 18-39) in lean and genetically obese hyperglycaemic (*ob*/*ob*) mice. Int J Obes 11:175–181

Barker CF, Frangipane LG, Silvers WK (1977) Islet transplantation in genetically determined diabetes. Ann Surg 186:401–410

Berne C (1975) The metabolism of lipids in mouse pancreatic islets. The oxidation of fatty acids and ketone bodies. Biochem J 152:661–666

Black MA, Heick HM, Begin-Heick N (1986) Abnormal regulation of insulin secretion in the genetically obese (*ob*/*ob*) mouse. Biochem J 238:863–869

Black MA, Heick HM, Begin-Heick N (1988a) Abnormal regulation of cAMP accumulation in pancreatic islets of obese mice. Am J Physiol 255:E833–E838

Black MA, Fournier LA, Heick HM, Begin-Heick N (1988b) Different insulin secretory responses to calcium-channel blockers in islets of lean and obese (*ob*/*ob*) mice. Biochem J 249:401–407

Blandino-Rosano M, Perez-Arana G, Mellado-Gil JM, Segundo C, Aguilar- Diosdado M (2008) Anti-proliferative effect of pro-inflammatory cytokines in cultured β cells is associated with extracellular signal-regulated kinase 1/2 pathway inhibition: protective role of glucagon-like peptide -1. J Mol Endocrinol 41:35–44

Bleisch VR, Mayer J, Dickie MM (1952) Familiar diabetes mellitus in mice associated with insulin resistance, obesity, and hyperplasia of the islands of Langerhans. Am J Pathol 28:369–385

Bock T, Pakkenberg B, Buschard K (2003) Increased islet volume but unchanged islet number in *ob*/*ob* mice. Diabetes 52:1716–1722

Bonner-Weir S, Deery D, Leahy JL, Weir GC (1989) Compensatory growth of pancreatic β-cells in adult rats after short-term glucose infusion. Diabetes 38:49–53

Bruton JD, Lemmens R, Shi CL, Persson-Sjögren S, Westerblad H, Ahmed M, Pyne NJ, Frame M, Furman BL, Islam MS (2003) Ryanodine receptors of pancreatic β cells mediate a distinct context-dependent signal for insulin secretion. FASEB J 17:301–303

Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC (2003) β-Cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 52:102–110

Camus MC, Aubert R, Bourgeois F, Herzog J, Alexiu A, Lemonnier D (1988) Serum lipoprotein and apolipoprotein profiles of the genetically obese *ob*/*ob* mouse. Biochim Biophys Acta 961:53–64

Carlsson PO, Andersson A, Jansson L (1996) Pancreatic islet blood flow in normal and obese-hyperglycemic (*ob*/*ob*) mice. Am J Physiol 271:E990–E995

Chan JY, Luzuriaga J, Bensellam M, Biden TJ, Laybutt DR (2013) Failure of the adaptive unfolded protein response in islets of obese mice is linked with abnormalities in β-cell gene expression and progression to diabetes. Diabetes 62:1557–1568

Chehab FF, Qiu J, Ogus S (2004) The use of animal models to dissect the biology of leptin. Recent Prog Horm Res 59:245–266

Chen NG, Romsos DR (1995) Enhanced sensitivity of pancreatic islets from preobese 2-week-old *ob*/*ob* mice to neurohormonal stimulation of insulin secretion. Endocrinology 13:505–511

Chen L, Komiya I, Inman L, McCorkle K, Alam T, Unger RH (1989) Molecular and cellular responses of islets during perturbations of glucose homeostasis determined by in situ hybridization histochemistry. Proc Natl Acad Sci USA 86:1367–1371

Chen NG, Tassava TM, Romsos DR (1993) Threshold for glucose-stimulated insulin secretion in pancreatic islets of genetically obese (*ob*/*ob*) mice is abnormally low. J Nutr 123:1567–1574

Cho YR, Kim CW (2004) Neuropeptide Y promotes β-cell replication via extracellular signal-regulated kinase activation. Biochem Biophys Res Commun 314:773–780

Chua S Jr, Liu SM, Li Q, Yang L, Thassanapaff VT, Fisher P (2002) Differential β cell responses to hyperglycaemia and insulin resistance in two novel congenic strains of diabetes (FVB- Lepr (db)) and obese (DBA- Lep (ob)) mice. Diabetologia 45:976–990

Cirillo D, Rachiglio AM, la Montagna R, Giordano A, Normanno N (2008) Leptin signaling in breast cancer: an overview. J Cell Biochem 10:956–964
Clee SM, Nadler ST, Attie AD (2005) Genetic and genomic studies of the BTBR *ob*/*ob* mouse model of type 2 diabetes. Am J Ther 12:491–498
Clement K (2006) Genetics of human obesity. C R Biol 329:608–622
Coleman DL (1973) Effects of parabiosis of obese with diabetes and normal mice. Diabetologia 9:294–298
Coleman DL (1978) Obese and diabetes: two mutant genes causing diabetes obesity syndromes in mice. Diabetologia 14:141–148
Coleman DL, Hummel KP (1973) The influence of genetic background on the expression of the obese (ob) gene in the mouse. Diabetologia 9:287–293
Cuenda A, Nebreda AR (2009) p38 delta and PKD1: kinase switches for insulin secretion. Cell 136:209–210
Cullinan CA, Brady EJ, Saperstein R, Leibowitz MD (1994) Glucose-dependent alterations of intracellular free calcium by glucagon-like peptide-1(7-36amide) in individual *ob*/*ob* mouse β-cells. Cell Calcium 15:391–400
Dai C, Brissova M, Reinert RB, Nyman L, Liu EH, Thompson C, Shostak A, Shiota M, Takahashi T, Powers AC (2013) Pancreatic islet vasculature adapts to insulin resistance through dilation and not angiogenesis. Diabetes 62:4144–4153 [Epub ahead of print]
Danielsson Å, Hellman B, Täljedal I-B (1968) Glucose tolerance in the period preceding the appearance of the manifest obese-hyperglycemic syndrome in mice. Acta Physiol Scand 72:81–84
Davis DB, Lavine JA, Suhonen JI, Krautkramer KA, Rabaglia ME, Sperger JM, Fernandez LA, Yandell BS, Keller MP, Wang IM, Schadt EE, Attie AD (2010) FoxM1 is up-regulated by obesity and stimulates β-cell proliferation. Mol Endocrinol 24:1822–1834
De Souza CT, Araújo EP, Stoppiglia LF, Pauli JR, Ropelle E, Rocco SA, Marin RM, Franchini KG, Carvalheira JB, Saad MJ, Boschero AC, Carneiro EM, Velloso LA (2007) Inhibition of UCP2 expression reverses diet-induced diabetes mellitus by effects on both insulin secretion and action. FASEB J 21:1153–1163
Diani AR, Sawada G, Wyse B, Murray FT, Khan M (2004) Pioglitazone preserves pancreatic islet structure and insulin secretory function in three murine models of type 2 diabetes. Am J Physiol 286:E116–E122
Donath MY, Schumann DM, Faulenbach M, Ellingsgaard H, Perren A, Ehses JA (2008) Islet inflammation in type 2 diabetes: from metabolic stress to therapy. Diabetes Care 31(Suppl 2):S161–S164
Dubuc PU, Mobley PW, Mahler RJ, Ensinck JW (1977) Immunoreactive glucagon levels in obese-hyperglycemic (*ob*/*ob*) mice. Diabetes 26:841–846
Dyachok O, Isakov Y, Sågetorp J, Tengholm A (2006) Oscillations of cyclic AMP in hormone-stimulated insulin-secreting β-cells. Nature 439:349–352
Dyck DJ, Heigenhauser GJ, Bruce CR (2006) The role of adipokines as regulators of skeletal muscle fatty acid metabolism and insulin sensitivity. Acta Physiol 186:5–16
Edvell A, Lindström P (1995) Development of insulin secretory function in young obese hyperglycemic mice (Umeå *ob*/*ob*). Metabolism 44:906–913
Edvell A, Lindström P (1998) Vagotomy in young obese hyperglycemic mice: effects on syndrome development and islet proliferation. Am J Physiol 274:E1034–E1039
Edvell A, Lindström P (1999) Initiation of increased pancreatic growth in young normoglycemic mice (Umeå +/?). Endocrinology 140:778–783
Eizirik DL, Cardozo AK, Cnop M (2008) The role for endoplasmic reticulum stress in diabetes mellitus. Endocr Rev 29:42–61
Elmi A (2001) Increased number of Na^+/K^+ ATPase enzyme units in *Ob*/*Ob* mouse pancreatic islets. Pancreas 23:113–115
Emilsson V, Liu YL, Cawthorne MA, Morton NM, Davenport M (1997) Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. Diabetes 46:313–316

Enyeart JJ (2005) Biochemical and ionic signaling mechanisms for ACTH-stimulated cortisol production. Vitam Horm 70:265–279

Flier SN, Kulkarni RN, Kahn CR (2001) Evidence for a circulating islet cell growth factor in insulin-resistant states. Proc Natl Acad Sci USA 98:7475–7480

Flowers JB, Rabaglia ME, Schueler KL, Flowers MT, Lan H, Keller MP, Ntambi JM, Attie AD (2007) Loss of stearoyl-CoA desaturase-1 improves insulin sensitivity in lean mice but worsens diabetes in leptin-deficient obese mice. Diabetes 5:1228–1239

Fournier LA, Heick HM, Begin-Heick N (1990) The influence of K^+-induced membrane depolarization on insulin secretion in islets of lean and obese (*ob*/*ob*) mice. Biochem Cell Biol 68:243–248

Friedman JM, Leibel RL, Siegel DS, Walsh J, Bahary N (1991) Molecular mapping of the mouse ob mutation. Genomics 11:1054–1062

Frühbeck G (2006) Intracellular signalling pathways activated by leptin. Biochem J 393:7–20

Garris DR, Garris BL (2004) Cytochemical analysis of pancreatic islet hypercytolipidemia following diabetes (db/db) and obese (*ob*/*ob*) mutation expression: influence of genomic background. Pathobiology 71:231–240

Garthwaite TL, Martinson DR, Tseng LF, Hagen TC, Menahan LA (1980) A longitudinal hormonal profile of the genetically obese mouse. Endocrinology 107:671–676

Gastaldelli A, Baldi S, Pettiti M, Toschi E, Camastra S, Natali A, Landau BR, Ferrannini E (2000) Influence of obesity and type 2 diabetes on gluconeogenesis and glucose output in humans: a quantitative study. Diabetes 49:1367–1373

Gault VA, Irwin N, Green BD, McCluskey JT, Greer B, Bailey CJ, Harriott P, O'harte FP, Flatt PR (2005) Chemical ablation of gastric inhibitory polypeptide receptor action by daily (Pro3)GIP administration improves glucose tolerance and ameliorates insulin resistance and abnormalities of islet structure in obesity-related diabetes. Diabetes 54:2436–2446

Gautam D, Jeon J, Li JH, Han SJ, Hamdan FF, Cui Y, Lu H, Deng C, Gavrilova O, Wess J (2008) Metabolic roles of the M3 muscarinic acetylcholine receptor studied with M3 receptor mutant mice: a review. J Recept Signal Transduct Res 28:93–108

Gepts W, Christophe J, Mayer J (1960) Pancreatic islets in mice with the obese hyperglycemic syndrome: lack of effect of carbutamide. Diabetes 9:63–69

Grapengiesser E, Gylfe E, Hellman B (1991) Cyclic AMP as a determinant for glucose induction of fast Ca^{2+} oscillations in isolated pancreatic β-cells. J Biol Chem 266:12207–12210

Gustavsson N, Larsson-Nyren G, Lindström P (2006) Cell specificity of the cytoplasmic Ca^{2+} response to tolbutamide is impaired in β-cells from hyperglycemic mice. J Endocrinol 190:461–470

Gysemans C, Callewaert H, Overbergh L, Mathieu C (2008) Cytokine signalling in the β-cell: a dual role for INFγ. Biochem Soc Trans 36:328–333

Hahn HJ, Hellman B, Lernmark Å, Sehlin J, Täljedal I-B (1974) The pancreatic β-cell recognition of insulin secretogogues. Influence of neuraminidase treatment on the release of insulin and the islet content of insulin, sialic acid, and cyclic adenosine 3′:5′- monophosphate. J Biol Chem 249:5275–5284

Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543–546

Han D, Yamamoto Y, Munesue S, Motoyoshi S, Saito H, Win MT, Watanabe T, Tsuneyama K, Yamamoto H (2013) Induction of receptor for advanced glycation end products by insufficient leptin action triggers pancreatic β-cell failure in type 2 diabetes. Genes Cells 18:302–314

Heart E, Smith PJ (2007) Rhythm of the β-cell oscillator is not governed by a single regulator: multiple systems contribute to oscillatory behavior. Am J Physiol 292:E1295–E1300

Hellman B, Idahl L-Å, Lernmark Å, Sehlin J, Täljedal I-B (1974) The pancreatic β-cell recognition of insulin secretagogues. Comparisons of glucose with glyceraldehyde isomers and dihydroxyacetone. Arch Biochem Biophys 162:448–457

Herberg L, Major E, Hennings U, Grüneklee D, Freytag G, Gries FA (1970) Differences in the development of the obese-hyperglycemic syndrome in obob and NZO mice. Diabetologia 6:292–299

Hill MJ, Metcalfe D, McTernan PG (2009) Obesity and diabetes: lipids, 'nowhere to run to'. Clin Sci 116:113–123

Hinoi E, Gao N, Jung DY, Yadav V, Yoshizawa T, Myers MG Jr, Chua SC Jr, Kim JK, Kaestner KH, Karsenty G (2008) The sympathetic tone mediates leptin's inhibition of insulin secretion by modulating osteocalcin bioactivity. J Cell Biol 183:1235–1242

Imai Y, Patel HR, Hawkins EJ, Doliba NM, Matschinsky FM, Ahima RS (2007) Insulin secretion is increased in pancreatic islets of neuropeptide Y-deficient mice. Endocrinology 148:5716–5723

Imai J, Katagiri H, Yamada T, Ishigaki Y, Suzuki T, Kudo H, Uno K, Hasegawa Y, Gao J, Kaneko K, Ishihara H, Niijima A, Nakazato M, Asano T, Minokoshi Y, Oka Y (2008) Regulation of pancreatic β cell mass by neuronal signals from the liver. Science 322:1250–1254

Ingalls AM, Dickie MM, Snell GD (1950) Obese, a new mutation in the house mouse. J Hered 41:317–318

Irwin N, McClean PL, O'Harte FP, Gault VA, Harriott P, Flatt PR (2007) Early administration of the glucose-dependent insulinotropic polypeptide receptor antagonist (Pro3)GIP prevents the development of diabetes and related metabolic abnormalities associated with genetically inherited obesity in *ob*/*ob* mice. Diabetologia 50:1532–1540

Islam MS (2002) The ryanodine receptor calcium channel of β-cells: molecular regulation and physiological significance. Diabetes 51:1299–1309

Jetton TL, Liang Y, Cincotta AH (2001) Systemic treatment with sympatholytic dopamine agonists improves aberrant β-cell hyperplasia and GLUT2, glucokinase, and insulin immunoreactive levels in *ob*/*ob* mice. Metabolism 50:1377–1384

Karlsson E, Stridsberg M, Sandler S (1998) Leptin regulation of islet amyloid polypeptide secretion from mouse pancreatic islets. Biochem Pharmacol 56:1339–1346

Keller MP, Choi Y, Wang P, Davis DB, Rabaglia ME, Oler AT, Stapleton DS, Argmann C, Schueler KL, Edwards S, Steinberg HA, Chaibub Neto E, Kleinhanz R, Turner S, Hellerstein MK, Schadt EE, Yandell BS, Kendziorski C, Attie AD (2008) A gene expression network model of type 2 diabetes links cell cycle regulation in islets with diabetes susceptibility. Genome Res 18:706–716

Khaldi MZ, Guiot Y, Gilon P, Henquin JC, Jonas JC (2004) Increased glucose sensitivity of both triggering and amplifying pathways of insulin secretion in rat islets cultured for 1 wk in high glucose. Am J Physiol 287:E207–E217

Khan A, Hong-Lie C, Landau BR (1995) Glucose-6-phosphatase activity in islets from *ob*/*ob* and lean mice and the effect of dexamethasone. Endocrinology 136:1934–1938

Khan A, Narangoda S, Ahren B, Holm C, Sundler F, Efendic S (2001) Long-term leptin treatment of *ob*/*ob* mice improves glucose-induced insulin secretion. Int J Obes Relat Metab Disord 25:816–821

Kieffer TJ, Heller RS, Habener JF (1996) Leptin receptors expressed on pancreatic β-cells. Biochem Biophys Res Commun 224:522–527

Kim A, Miller K, Jo J, Kilimnik G, Wojcik P, Hara M (2009) Islet architecture: a comparative study. Islets 1:129–136

Kojima H, Fujimiya M, Matsumura K, Nakahara T, Hara M, Chan L (2004) Extrapancreatic insulin-producing cells in multiple organs in diabetes. Proc Natl Acad Sci USA 101:2458–2463

Lalloyer F, Vandewalle B, Percevault F, Torpier G, Kerr-Conte J, Oosterveer M, Paumelle R, Fruchart JC, Kuipers F, Pattou F, Fiévet C, Staels B (2006) Peroxisome proliferator-activated receptor α improves pancreatic adaptation to insulin resistance in obese mice and reduces lipotoxicity in human islets. Diabetes 55:1605–1613

Lam QJ, Lu L (2007) Role of leptin in immunity. Cell Mol Immunol 4:1–13

Larcher F, Del Rio M, Serrano F, Segovia JC, Ramirez A, Meana A, Page A, Abad JL, Gonzalez MA, Bueren J, Bernad A, Jorcano JL (2001) A cutaneous gene therapy approach to human

leptin deficiencies: correction of the murine *ob*/*ob* phenotype using leptin targeted keratinocyte grafts. FASEB J 15:1529–1538
Lavine RL, Voyles N, Perrino PV, Recant L (1977) Functional abnormalities of islets of Langerhans of obese hyperglycemic mouse. Am J Physiol 233:E86–E90
Leckström A, Lundquist I, Ma Z, Westermark P (1999) Islet amyloid polypeptide and insulin relationship in a longitudinal study of the genetically obese (*ob*/*ob*) mouse. Pancreas 18:266–273
Lee YC, Nielsen JH (2009) Regulation of β cell replication. Mol Cell Endocrinol 297:18–27
Li Z, Schmidt SF, Friedman JM (2013) Developmental role for endocannabinoid signaling in regulating glucose metabolism and growth. Diabetes 62:2359–2367
Lindberg K, Rønn SG, Tornehave D, Richter H, Hansen JA, Rømer J, Jackerott M, Billestrup N (2005) Regulation of pancreatic β-cell mass and proliferation by SOCS-3. J Mol Endocrinol 35:231–243
Ling Z, Pipeleers DG (1996) Prolonged exposure of human β cells to elevated glucose levels results in sustained cellular activation leading to a loss of glucose regulation. J Clin Invest 98:2805–2812
Loreti L, Dunbar JC, Chen S, Foà PP (1974) The autoregulation of insulin secretion in the isolated pancreas islets of lean (obob) and obese-hyperglycemic (obob) mice. Diabetologia 10:309–315
Lulu Strat A, Kokta TA, Dodson MV, Gertler A, Wu Z, Hill RA (2005) Early signaling interactions between the insulin and leptin pathways in bovine myogenic cells. Biochim Biophys Acta 1744:164–175
Malendowicz LK, Rucinski M, Belloni AS, Ziolkowska A, Nussdorfer GG (2007) Leptin and the regulation of the hypothalamic-pituitary-adrenal axis. Int Rev Cytol 263:63–102
Marí M, Caballero F, Colell A, Morales A, Caballeria J, Fernandez A, Enrich C, Fernandez-Checa JC, García-Ruiz C (2006) Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis. Cell Metab 4:185–198
Marroquí L, Gonzalez A, Ñeco P, Caballero-Garrido E, Vieira E, Ripoll C, Nadal A, Quesada I (2012) Role of leptin in the pancreatic β-cell: effects and signaling pathways. J Mol Endocrinol 49:R9–R17
Mayer J (1960) The obese hyperglycaemic syndrome of mice as an example of "metabolic" obesity. Am J Clin Nutr 8:712–718
Mayer J, Silides N (1953) A quantitative method of determination of the diabetogenic activity of growth hormone preparations. Endocrinology 52:54–56
Mayer J, Russel E, Bates MV, Dickie MM (1953) Metabolic, nutritional and endocrine studies of the hereditary obesity-diabetes syndrome of mice and mechanisms of its development. Metabolism 2:9–21
McQuaid TS, Saleh MC, Joseph JW, Gyulkhandanyan A, Manning-Fox JE, MacLellan JD, Wheeler MB, Chan CB (2006) cAMP-mediated signaling normalizes glucose stimulated insulin secretion in uncoupling protein-2 overexpressing β-cells. J Endocrinol 190:669–680
Medina-Gomez G, Yetukuri L, Velagapudi V, Campbell M, Blount M, Jimenez-Linan M, Ros M, Oresic M, Vidal-Puig A (2009) Adaptation and failure of pancreatic β cells in murine models with different degrees of metabolic syndrome. Dis Model Mech 2:582–592
Melloul D, Marshak S, Cerasi E (2002) Regulation of insulin gene transcription. Diabetologia 45:309–326
Milman P, Fu A, Screaton RA, Woulfe JM (2010) Depletion of intranuclear rodlets in mouse models of diabetes. Endocr Pathol 21:230–235
Moritoh Y, Takeuchi K, Asakawa T, Kataoka O, Odaka H (2008) Chronic administration of alogliptin, a novel, potent, and highly selective dipeptidyl peptidase-4 inhibitor, improves glycemic control and β-cell function in obese diabetic *ob*/*ob* mice. Eur J Pharmacol 588:325–332
Norlund R, Norlund L, Täljedal I-B (1987) Morphogenetic effects of glucose on mouse islet-cell re-aggregation in culture. Med Biol 65:209–216

Nyholm B, Fineman MS, Koda JE, Schmitz O (1998) Plasma amylin immunoreactivity and insulin resistance in insulin resistant relatives of patients with noninsulin-dependent diabetes mellitus. Horm Metab Res 30:206–212

Nyrén R, Chang CL, Lindström P, Barmina A, Vorrsjö E, Ali Y, Juntti-Berggren L, Bensadoun A, Young SG, Olivecrona T, Olivecrona G (2012) Localization of lipoprotein lipase and GPIHBP1 in mouse pancreas: effects of diet and leptin deficiency. BMC Physiol 12:14

Ozcan L, Ergin AS, Lu A, Chung J, Sarkar S, Nie D, Myers MG Jr, Ozcan U (2009) Endoplasmic reticulum stress plays a central role in development of leptin resistance. Cell Metab 9:35–51

Park K, Lee BM, Kim YH, Han T, Yi W, Lee DH, Choi HH, Chong W, Lee CH (2013) Discovery of a novel phenylethyl benzamide glucokinase activator for the treatment of type 2 diabetes mellitus. Bioorg Med Chem Lett 23:537–542

Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F (1995) Effects of the obese gene product on body weight regulation in *ob*/*ob* mice. Science 269:540–543

Persson-Sjögren S, Lindström P (2004) Effects of cholinergic m-receptor agonists on insulin release in islets from obese and lean mice of different ages: the importance of bicarbonate. Pancreas 29:90–99

Persson-Sjögren S, Forsgren S, Lindström P (2006) Vasoactive intestinal polypeptide and pituitary adenylate cyclase activating polypeptide: effects on insulin release in isolated mouse islets in relation to metabolic status and age. Neuropeptides 40:283–290

Peterson SJ, Drummond G, Kim DH, Li M, Kruger AL, Ikehara S, Abraham NG (2008) L-4F treatment reduces adiposity, increases adiponectin levels, and improves insulin sensitivity in obese mice. J Lipid Res 49:1658–1669

Prieto J, Kaaya EE, Juntti-Berggren L, Berggren PO, Sandler S, Biberfeld P, Patarroyo M (1992) Induction of intercellular adhesion molecule-1 (CD54) on isolated mouse pancreatic β cells by inflammatory cytokines. Clin Immunol Immunopathol 65:247–253

Quan W, Hur KY, Lim Y, Oh SH, Lee JC, Kim KH, Kim GH, Kim SW, Kim HL, Lee MK, Kim KW, Kim J, Komatsu M, Lee MS (2012) Autophagy deficiency in β cells leads to compromised unfolded protein response and progression from obesity to diabetes in mice. Diabetologia 55:392–403

Ranheim T, Dumke C, Schueler KL, Cartee GD, Attie AD (1997) Interaction between BTBR and c57Bl/6J genomes produces an insulin resistance syndrome in [BTBNR x C57Bl/6J] F1 mice. Arterioscler Thromb Vasc Biol 17:3286–3293

Rattarasarn C (2006) Physiological and pathophysiological regulation of regional adipose tissue in the development of insulin resistance and type 2 diabetes. Acta Physiol 186:87–101

Ravier MA, Sehlin J, Henquin JC (2002) Disorganization of cytoplasmic Ca^{2+} oscillations and pulsatile insulin secretion in islets from *ob*/*ob* mice. Diabetologia 45:1154–1163

Rodriguez-Diaz R, Abdulreda MH, Formoso AL, Gans I, Ricordi C, Berggren PO, Caicedo A (2011) Innervation patterns of autonomic axons in the human endocrine pancreas. Cell Metab 14:45–54

Rolin B, Larsen MO, Gotfredsen CF, Deacon CF, Carr RD, Wilken M, Knudsen LB (2002) The long-acting GLP-1 derivative NN2211 ameliorates glycemia and increases β-cell mass in diabetic mice. Am J Physiol 283:E745–E752

Rooth P, Täljedal I-B (1987) Vital microscopy of islet blood flow: catecholamine effects in normal and *ob*/*ob* mice. Am J Physiol 252:E130–E135

Rutter GA, Da Silva Xavier G, Leclerc I (2003) Roles of 5′-AMP-activated protein kinase (AMPK) in mammalian glucose homoeostasis. Biochem J 375:1–16

Saleh MC, Wheeler MB, Chan CB (2006) Endogenous islet uncoupling protein-2 expression and loss of glucose homeostasis in *ob*/*ob* mice. Endocrinology 190:659–667

Sener A, Anak O, Leclercq-Meyer V, Herberg L, Malaisse WJ (1993) FAD glycerophosphate dehydrogenase activity in pancreatic islets and liver of *ob*/*ob* mice. Biochem Mol Biol Int 30:397–402

Seufert J (2004) Leptin effects on pancreatic β-cell gene expression and function. Diabetes 53(Suppl 1):S152–S158

Shafrir E, Ziv E, Mosthaf L (1999) Nutritionally induced insulin resistance and receptor defect leading to β-cell failure in animal models. Ann NY Acad Sci 892:223–246

Singh H, Farouk M, Bose BB, Singh P (2013) Novel genes underlying β cell survival in metabolic stress. Bioinformation 9:37–41

Sorensen H, Brand CL, Neschen S, Holst JJ, Fosgerau K, Nishimura E, Shulman GI (2006) Immunoneutralization of endogenous glucagon reduces hepatic glucose output and improves long-term glycemic control in diabetic *ob*/*ob* mice. Diabetes 55:2843–2848

Sreejayan N, Dong F, Kandadi MR, Yang X, Ren J (2008) Chromium alleviates glucose intolerance, insulin resistance, and hepatic ER stress in obese mice. Obesity 16:1331–1337

Stoehr JP, Byers JE, Clee SM, Lan H, Boronenkov OIV, Schueler KL, Yandell BS, Attie AD (2004) Identification of major quantitative trait loci controlling body weight variation in *ob*/*ob* mice. Diabetes 53:245–249

Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Bonner-Weir S, Habener JF, Egan JM (2000) Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. Diabetes 49:741–747

Sumara G, Formentini I, Collins S, Sumara I, Windak R, Bodenmiller B, Ramracheya R, Caille D, Jiang H, Platt KA, Meda P, Aebersold R, Rorsman P, Ricci R (2009) Regulation of PKD by the MAPK p38delta in insulin secretion and glucose homeostasis. Cell 136:235–248

Sweeney G (2002) Leptin signaling. Cell Signal 14:655–663

Takada K, Kanatsuka A, Tokuyama Y, Yagui K, Nishimura M, Saito Y, Makino H (1996) Islet amyloid polypeptide/amylin contents in pancreas change with increasing age in genetically obese and diabetic mice. Diabetes Res Clin Pract 33:153–158

Takasawa S, Akiyama T, Nata K, Kuroki M, Tohgo A, Noguchi N, Kobayashi S, Kato I, Katada T, Okamoto H (1998) Cyclic ADP-ribose and inositol 1,4,5-trisphosphate as alternate second messengers for intracellular Ca^{2+} mobilization in normal and diabetic β-cells. J Biol Chem 273:2497–2500

Tassava TM, Okuda T, Romsos DR (1992) Insulin secretion from *ob*/*ob* mouse pancreatic islets: effects of neurotransmitters. Am J Physiol 262:E338–E343

Tentolouris N, Argyrakopoulou G, Katsilambros N (2008) Perturbed autonomic nervous system function in metabolic syndrome. Neuromol Med 10:169–178

Tomita T, Doull V, Pollock HG, Krizsan D (1992) Pancreatic islets of obese hyperglycemic mice (*ob*/*ob*). Pancreas 7:367–375

Tu Z, Keller MP, Zhang C, Rabaglia ME, Greenawalt DM, Yang X, Wang IM, Dai H, Bruss MD, Lum PY, Zhou YP, Kemp DM, Kendziorski C, Yandell BS, Attie AD, Schadt EE, Zhu J (2012) Integrative analysis of a cross-loci regulation network identifies App as a gene regulating insulin secretion from pancreatic islets. PLoS Genet 8:e1003107

Tyrberg B, Ustinov J, Otonkoski T, Andersson A (2001) Stimulated endocrine cell proliferation and differentiation in transplanted human pancreatic islets: effects of the ob gene and compensatory growth of the implantation organ. Diabetes 50:301–307

Unger RH, Orci L (2001) Diseases of liporegulation: new perspective on obesity and related disorders. FASEB J 15:312–321

Vivas Y, Martínez-García C, Izquierdo A, Garcia-Garcia F, Callejas S, Velasco I, Campbell M, Ros M, Dopazo A, Dopazo J, Vidal-Puig A, Medina-Gomez G (2011) Early peroxisome proliferator-activated receptor gamma regulated genes involved in expansion of pancreatic β cell mass. BMC Med Genomics 4:86

Wajchenberg BL (2007) β-cell failure in diabetes and preservation by clinical treatment. Endocr Rev 28:187–218

Welsh M, Welsh N, Nilsson T, Arkhammar P, Pepinsky RB, Steiner DF, Berggren PO (1988) Stimulation of pancreatic islet β-cell replication by oncogenes. Proc Natl Acad Sci USA 85:116–120

Westman S (1968a) Development of the obese-hyperglycemic syndrome in mice. Diabetologia 4:141–149

Westman S (1968b) The endocrine pancreas of old obese-hyperglycemic mice. Acta Med Upsal 73:81–89

Woulfe J, Munoz D (2000) Tubulin immunoreactive neuronal intranuclear inclusions in the human brain. Neuropathol Appl Neurobiol 26:161–171

Xu G, Chen J, Jing G, Shalev A (2012) Preventing β-cell loss and diabetes with calcium channel blockers. Diabetes 61:848–856

Yang L, Jhaveri R, Huang J, Qi Y, Diehl AM (2007) Endoplasmic reticulum stress, hepatocyte CD1d and NKT cell abnormalities in murine fatty livers. Lab Invest 87:927–937

Young AA, Gedulin BR, Bhavsar S, Bodkin N, Jodka C, Hansen B, Denaro M (1999) Glucose-lowering and insulin-sensitizing actions of exendin-4: studies in obese diabetic (*ob*/*ob*, db/db) mice, diabetic fatty Zucker rats, and diabetic rhesus monkeys (Macaca mulatta). Diabetes 48:1026–1034

Zaitseva II, Sharoyko V, Størling J, Efendic S, Guerin C, Mandrup-Poulsen T, Nicotera P, Berggren PO, Zaitsev SV (2006) RX871024 reduces NO production but does not protect against pancreatic β-cell death induced by proinflammatory cytokines. Biochem Biophys Res Commun 347:1121–1128

Zawalich WS, Tesz GJ, Zawalich KC (2002) Inhibitors of phosphatidylinositol 3-kinase amplify insulin release from islets of lean but not obese mice. J Endocrinol 174:247–258

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432

Zhang CY, Baffy G, Perret P, Krauss S, Peroni O, Grujic D, Hagen T, Vidal-Puig AJ, Boss O, Kim YB, Zheng XX, Wheeler MB, Shulman GI, Chan CB, Lowell BB (2001) Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, β cell dysfunction, and type 2 diabetes. Cell 105:745–755

Role of Reproductive Hormones in Islet Adaptation to Metabolic Stress

28

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Contents

Abstract

There is an interaction between reproduction and energy stores. Both the production and secretion of insulin by pancreatic islet β-cells must adapt to the metabolic demands of various environmental stresses related to reproduction and energy status. These adaptations must occur in a sex-specific manner.

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_44,

It is therefore conceivable that reproductive hormones play a role in β-cell adaptation to environmental stresses. This review explores the roles of estrogen, androgen, progesterone, and lactogens in pancreatic β-cell mass and function under conditions of metabolic stress such as pregnancy, obesity, and diabetes.

Keywords
Islets • β-cell • Metabolic stress • Reproductive hormones • Estrogen • Androgen • Progesterone • Lactogens • Proliferation • β-cell survival • Insulin secretion

Introduction

There is an interaction between reproduction and energy metabolism under conditions of disrupted energy homeostasis such as obesity and cachexia, with both conditions negatively impacting fertility (Mauvais-Jarvis 2011). The pancreatic β-cell of the islets of Langerhans is critical for producing insulin, the main hypoglycemic and anabolic hormone. Insulin is essential for promotion and maintenance of cellular energy stores. It acts by stimulating the storage of glucose in the form of glycogen and triglycerides. Production and secretion of insulin by the β-cells must adapt to changes in metabolic demand associated with various environmental stresses, including those related to reproduction and energy status. It is therefore conceivable that reproductive hormones play sex-specific roles in β-cell adaptation to these environmental stresses. This review focuses on the roles of male and female reproductive hormones including estrogen, androgen, progesterone, prolactin, and placental lactogen as they relate to changes in pancreatic β-cell mass and function in conditions of metabolic stresses such as pregnancy, obesity, and diabetes.

Estrogens

In rodent models, treatment with 17 β-estradiol, the main circulating estrogen in females (E2), protects pancreatic β-cells against various diabetic injuries. These injuries include oxidative stress, amyloid polypeptide toxicity, lipotoxicity, and apoptosis (Tiano and Mauvais-Jarvis 2012a). Three ERs – ERα, ERβ, and the G protein-coupled ER (GPER) – have been identified in both rodent and human β-cells. Unlike the classical nuclear ER that acts as a ligand-activated transcription factor in breast and uterine cells, β-cell ERs reside mainly in extranuclear locations. They exert their effects via cytosolic interactions with kinases such as Src, ERK, and AMPK or via transcription factors of the STAT family (Tiano and Mauvais-Jarvis 2012a, b; Tiano et al. 2011; Wong et al. 2010). Activation of ERα enhances glucose-stimulated insulin biosynthesis (Wong et al. 2010; Alonso-Magdalena et al. 2008) through a pathway involving Src and ERK, and stimulation of nuclear translocation and binding to the insulin promoter of NeuroD1, an insulinotropic transcription factor (Wong et al. 2010). This action may assist islets in adapting to the increased

metabolic demands of pregnancy by enhancing insulin biosynthesis. Activation of ERα reduces islet excess de novo synthesis of fatty acids and lipogenesis as well as accumulation of toxic lipid intermediates (Tiano et al. 2011; Tiano and Mauvais-Jarvis 2012b, c). This anti-lipogenic action involves at least two pathways. First, an extranuclear ERα activates and promotes the nuclear translocation of STAT3. This leads to inhibition of the master regulator of lipogenesis, the liver X receptor LXRβ, as well as its transcriptional targets, the sterol regulatory element-binding protein 1c (SREBP1c) and the carbohydrate response element-binding protein (ChREBP). Suppression of LXRβ and SREBP1c mRNA may be mediated by a pool of ERα associated with the plasma membrane that activates Src and leads to STAT3 activation (Tiano and Mauvais-Jarvis 2012b). In β-cells, chronic LXR activation leads to excess lipogenesis, which, in turn, is associated with lipotoxicity and apoptosis (Choe et al. 2007). Thus, ERα suppression of LXR mRNAs in β-cells may account for the inhibition of lipogenesis and prevention of islet lipotoxicity. In the second pathway, activation of ERα induces AMP kinase to suppress SREBP-1c gene and protein expression (Tiano and Mauvais-Jarvis 2012b). Together, ERα extranuclear actions in β-cells via STAT3 and AMPK lead to decreased expression and activity of the master effector of fatty acid (FA) synthesis under conditions of glucose surplus – fatty acid synthase (FAS). This converts malonyl-CoA into saturated long-chain FA that can then undergo β-oxidation or esterification to MAG, DAG, and TG (Tiano et al. 2011). Activation of ERα also promotes β-cell survival from most proapoptotic stimuli associated with diabetes (Le May et al. 2006; Liu et al. 2009, 2013; Liu and Mauvais-Jarvis 2009). These anti-apoptotic mechanisms involve a combination of rapid actions that are independent of nuclear events and that potentially lead to alteration in protein phosphorylation (Liu et al. 2009, 2013) as well as a more classical genomic mechanism that induces an anti-inflammatory cascade via expression of the liver receptor homolog-1(LRH-1), NR5A5 (Baquie et al. 2011). Activation of ERβ seems to preferentially enhance glucose-stimulated insulin secretion (Soriano et al. 2009, 2012) via a membrane pathway that leads to activation of the ANF receptor and closure of K_{ATP} channels (Soriano et al. 2009). Activation of GPER, however, protects β-cells from lipid accumulation (Tiano et al. 2011; Tiano and Mauvais-Jarvis 2012b), thereby promoting their survival (Liu et al. 2009; Balhuizen et al. 2010; Kumar et al. 2011). Activation of GPER also enhances glucose-stimulated insulin secretion (Balhuizen et al. 2010; Sharma and Prossnitz 2011) via activation of the epidermal growth factor receptor and ERK (Sharma and Prossnitz 2011), although it has no effect on insulin biosynthesis (Wong et al. 2010). However, it has been proposed that GPER induces expression of ERα36, a short isoform of the classical long isoform of ERα, ERα66 (Kang et al. 2010). Both ERα66 and ERα36 are expressed in β-cells (Tiano et al. 2011). Thus, it is unclear whether GPER-mediated effects in β-cells are due to intrinsic GPER actions or if GPER is interacting with ERα36 at the membrane level. Importantly, ERα, ERβ, and GPER are expressed in human β-cells, and the beneficial effects of ER ligands on β-cell survival, function, and nutrient homeostasis that are described above are all observed in human islets (Tiano et al. 2011; Tiano and Mauvais-Jarvis 2012b; Liu et al. 2009; Kumar et al. 2011; Contreras et al. 2002).

Perhaps the most translational prospect of E2 therapy for β-cell protection involves pancreatic islet transplantation (PIT). Fertile women with T1D exhibit E2 deficiency relative to healthy women (Salonia et al. 2006). Therefore, women with T1D undergoing islet transplantation may have lost part of their endogenous E2-related islet protection and could benefit from short-term E2 supplementation. To explore this hypothesis, we used a T1D model with xenotransplantation of a marginal dose of human islets in nude mice rendered insulin deficient by streptozotocin. In this model, a transient 4-week E2 treatment protected functional β-cell mass and enhanced islet revascularization and engraftment (Liu et al. 2013). E2 effects were retained in the presence of immunosuppression and persisted after discontinuation of E2 treatment. E2 treatment produced acutely decreased hypoxic damage and oxidative stress of the islet graft and suppressed graft β-cell apoptosis. Interestingly, E2 also acutely suppressed hyperglucagonemia without altering insulin secretion. These results suggest that transient E2 treatment in women could provide an immediate therapeutic alternative to improve PIT and also achieve insulin independence with fewer islets. This therapeutic approach could be developed long before other surrogate islet β-cell sources or β-cell regeneration therapy can be developed and therefore warrants further investigation.

From a therapeutic point of view, the risk of hormone-dependent cancer precludes use of general estrogen therapy as a chronic treatment for β-cell failure in diabetes. To preferentially target E2 to β-cells without the undesirable effect of general estrogen therapy, we created novel fusion peptides that combine glucagon-like peptide-1 (GLP-1) and E2 in a single molecule (Finan et al. 2012). By combining the pharmacologic properties of GLP-1 and E2, we postulated synergistic actions on β-cell function and survival resulting from the combined insulinotropic and anti-apoptotic activities on pancreatic β-cells that express ER and GLP-1R. Two conjugates were synthesized with E2 stably linked to GLP-1 to avoid E2 release in the circulation and to maximize E2 delivery at target cells: a GLP-1 agonist stably linked to E2 (aGLP1-E2) and an inactive GLP-1 stably linked to E2 (iGLP1-E2). The second conjugate binds GLP-1R normally, but is pharmacologically incapable of activating GLP-1R signaling and used to direct E2 to β-cells. Tiano et al. tested the efficiency of GLP1-E2 conjugates in preventing insulin-deficient diabetes in a model of β-cell destruction induced by multiple low-dose injections of streptozotocin (STZ). They observed that the iGLP1-E2 conjugate prevented STZ-induced insulin-deficient diabetes, thereby demonstrating that, in vivo, the inactive GLP-1 was able to bind the GLP-1R and to direct E2 to β-cells for protection. Most importantly, the aGLP1-E2 conjugate was more potent than either the GLP-1 agonist or the iGLP1-E2 individually in preventing STZ-induced diabetes. All conjugates were devoid of E2 gynecological effects compared to general E2 therapy (Tiano et al. 2012). These observations provide proof of concept that combining GLP-1 and E2 in a single molecule results in synergies for protection of β-cell function without the side effects associated with general estrogen therapy.

E2 might also promote islet β-cell proliferation under specific physiological and experimental conditions. An effect of estrogen on islet regeneration was initially suggested by Houssay et al. who observed that subtotal pancreatectomy followed by implantation of an estrogen pellet in the remaining pancreas induced regeneration of surrounding islets (Houssay et al. 1954). Further, the stimulatory effect of estrogen on islet and β-cell regeneration was also observed in the alloxan-induced diabetic rat model (Goodman and Hazelwood 1974) and in rat pancreatic islets damaged by streptozotocin (Yamabe et al. 2010). E2 also increases cultured rat islet cell proliferation (Sorenson et al. 1993). However, in these studies, estrogen was used at pharmacological concentrations, so the relevance of these observations to physiology is unclear. Nonetheless, in one study, physiological doses of estrogen have been reported to increase β-cell proliferation and restore the decrease in β-cell mass observed in ovariectomized rodents with subtotal pancreatectomy. This effect was associated with an increase in expression of IRS-2 and Pdx1 proteins via activation of the cAMP response element-binding protein (Choi et al. 2005). Thus, in classical models of β-cell regeneration or at high doses, E2 can induce β-cell proliferation. Regardless, in most of our studies, E2 – used at doses leading to physiological serum concentrations – has never induced significant β-cell proliferation in either male or female rodent models of diabetes with β-cell apoptosis induced by streptozotocin or lipotoxicity (Tiano et al. 2011; Le May et al. 2006; Liu et al. 2013).

Interestingly, GPER has recently been implicated in β-cell proliferation. Pregnancy is associated with an expansion of functional β-cell mass as a means to adapt to increased metabolic demand. In rodents, GPER expression is markedly upregulated during pregnancy. In addition, expansion of β-cell mass during pregnancy was associated with decreased expression of the islet microRNA, miR-338-3p. In rodents, downregulation of this small noconding RNA promoted β-cell proliferation and protected β-cells against apoptosis. In contrast, miR-338-3p upregulation triggered β-cell apoptosis and was associated with a decreased β-cell mass (Jacovetti et al. 2012). In liver cancer cells miR-338-3p expression was downregulated, and restoration of its expression produced a suppression of the invasive potential of cancer cells (Huang et al. 2011). In isolated rat islets, exposure to E2 or the GPER agonist G1 decreased miR-338-3p to levels observed in gestation, a level that was associated with increased β-cell proliferation. These E2 effects depend on cAMP and protein kinase A. However, under these conditions there is no proliferation of human β-cells (Liu et al. 2013). Nonetheless, E2 exposure reduces the level of miR-338-3p in human islet cells (Jacovetti et al. 2012). However, neither E2 nor silencing of miR-338-3p elicited replication of human β-cells in culture. Thus, the impressive effect of E2, GPER, and miR-338-3p observed in rodent β-cell proliferation is not observed in human β-cells. Finally, E2 was reported to promote the proliferation and inhibit the differentiation of adult human islet-derived precursor cells via ERα (Ren et al. 2010). Thus, in classical rodent models of β-cell regeneration and at pharmacological doses, E2 can induce β-cell proliferation. Still, further studies are needed to determine the validity of these findings in human β-cells.

Androgens

Although the role of the major male androgen, testosterone, in β-cell biology is poorly understood, aging men with testosterone deficiency exhibit increased T2D risk (Mauvais-Jarvis 2011; Zitzmann 2009). In addition, men who are on androgen depletion therapy for prostate cancer are also at high risk of T2D (Keating et al. 2012). Although the impact of testosterone deficiency on development of visceral obesity and insulin resistance (IR) in men is established (Mauvais-Jarvis 2011; Zitzmann 2009; Basaria et al. 2006; Khaw and Barrett-Connor 1992; Pitteloud et al. 2005; Zitzmann et al. 2006), the role of testosterone deficiency in β-cell dysfunction remains unknown. Nonetheless, low testosterone levels have been implicated in the pathogenesis of T2D (Haffner et al. 1996; Oh et al. 2002), raising the possibility that testosterone deficiency may predispose to β-cell failure. Early studies reported that in male mice in which β-cell destruction was induced by streptozotocin, testosterone accelerates hyperglycemic decompensation via a pathway involving the AR (Maclaren et al. 1980; Paik et al. 1982). By contrast, it was also reported that testosterone protects early apoptotic damage induced by streptozotocin in male rat pancreas via an AR-dependent mechanism (Morimoto et al. 2005; Palomar-Morales et al. 2010). In the latter study, however, the effect of testosterone on diabetes incidence was not reported. We have generated a β-cell-specific AR knockout mouse to examine the direct role of AR in male β-cell physiology (βARKO$^{-/y}$) (Navarro and Mauvais-Jarvis 2013). Male βARKO$^{-/y}$ mice exhibit decreased glucose-stimulated insulin secretion (GSIS) leading to glucose intolerance. The decreased GSIS is reproduced in cultured male βARKO$^{-/y}$ islets and in human islets treated with flutamide, an AR antagonist. This suggests that AR is a physiological regulator of male β-cell function, a finding that has important implications for prevention of T2D in aging men. A previous report suggested that testosterone stimulates islet insulin mRNA and content in culture and in vivo (Morimoto et al. 2001), but we found no evidence of AR involvement in insulin synthesis.

Women with hyperandrogenemia display β-cell dysfunction. Women with functional hyperandrogenism have significantly higher basal insulin secretory rates and attenuated secretory responses to meals (O'Meara et al. 1993). Women with polycystic ovary syndrome (PCOS) have been reported to show inadequate acute insulin release to the degree of insulin resistance (Dunaif and Finegood 1996) or an exaggerated early insulin response to glucose. These are not accounted for by insulin resistance and are closely associated with hyperandrogenicity (Holte et al. 1994). In these PCOS women, there is a robust relationship between β-cell function and bioavailable testosterone, raising the possibility that excess testosterone in women leads to insulin hypersecretion (Goodarzi et al. 2005). Thus, women with hyperandrogenism display β-cell hyperfunction which may predispose to secondary failure. Consistent with this hypothesis, in female mice, testosterone accelerates hyperglycemic decompensation in experimental models of insulin-dependent diabetes in which β-cell destruction is induced by oxidative stress or inflammation (Maclaren et al. 1980; Liu et al. 2010). In addition,

hyperandrogenemia in women with PCOS is accompanied by systemic oxidative stress (Gonzalez et al. 2006), and we showed that excess testosterone in female mice induces systemic oxidative stress (Liu et al. 2010). Further, in the presence of a prior β-cell injury induced by streptozotocin, female mice exposed to excess testosterone are predisposed to β-cell failure via an AR-dependent mechanism (Liu et al. 2010). We also reported that female mice exposed to chronic androgen excess exhibit an islet failure to compensate for high-fat-feeding-induced insulin resistance that leads to T2D (Navarro et al. 2011). Androgen-excess-induced insulin resistance and hyperglycemia is eliminated in female βARKO$^{-/-}$ mice. Thus, excess AR activation in β-cells (and other tissues) may predispose to the β-cell dysfunction observed in women with androgen excess.

Progesterone

The presence of progesterone receptors in the human endocrine pancreas suggests a direct role of progesterone on pancreatic islet function (Doglioni et al. 1990). In vivo progesterone treatment of intact male and female mice stimulates islet α- and β-cell proliferation (Nieuwenhuizen et al. 1999). However, this effect is not observed in gonadectomized mice, suggesting that progesterone requires intact gonadal function to induce islet cell proliferation. Indeed, this effect of progesterone is not observed in cultured rat islet cells (Sorenson et al. 1993). In contrast, female mice deficient in the progesterone receptor (PR) have enhanced glucose tolerance related to improved β-cell function (Picard et al. 2002). The improved β-cell function in these female mice is attributed to increased β-cell mass with enhanced proliferation. The increased β-cell proliferation is not associated with differences in islet expression levels of the cell cycle regulators p21, p27, cyclin D1, cyclin B1, or cyclin E. In contrast, the protein levels of the tumor suppressor p53 were markedly decreased in PR-deficient islets, which may enhance islet proliferation. Progesterone did not affect miR-338-3p levels in cultured INS cells (Jacovetti et al. 2012). In MIN6 β-cells, progesterone was reported to enhance basal- and glucose-stimulated insulin secretion, in part by increasing glucokinase activity and amplifying cAMP levels (Shao et al. 2004).

Lactogens

Lactogens – prolactin (PRL) and placental lactogen (PL) – mediate their biological responses through a common prolactin receptor (PRLR) (Goffin et al. 1999). In situ hybridization analysis has revealed the presence of both the short and long forms of the PRLR mRNA in the endocrine pancreas (Moldrup et al. 1993; Ouhtit et al. 1994). This was confirmed by immunohistochemical staining of PRLR in islet β-cells (Brelje et al. 2002). Lactogens play a role in regulation of normal islet development. Male and female PRLR-deficient mice exhibit reduced islet mass and density that is observed as early as 3 weeks of age (Freemark et al. 2002).

There is a blunted insulin secretory response to glucose in male PRLR-deficient mice and in isolated cultured islets from PRLR-deficient mice of both sexes.

Lactogens are especially important during pregnancy, when islets are exposed to metabolic stress and need to adapt to the increased metabolic demands of the fetus (Newberna and Freemark 2011). Physiological changes associated with pregnancy include β-cell proliferation, lowering of the glucose threshold for insulin release, increased glucose-stimulated insulin secretion, and islet β-cell coupling (Terra et al. 2011). This suggests that prolactin may increase β-cell sensitivity to glucose. Indeed, during pregnancy, increased islet glucokinase and GLUT2 glucose transporters are associated with prolactin-induced augmentation of glucose-stimulated insulin secretion, as well as increases in β-cell proliferation, increased glucose metabolism, gap-junctional coupling among β-cells, and c-AMP signaling (Sorenson and Brelje 1997). There is evidence to suggest that PRL and placental lactogen are instrumental in inducing these changes and promoting β-cell expansion and insulin production. The increase in serum prolactin and placental lactogen levels parallels increases in β-cell mass (Hughes and Huang 2011; Vetere and Wagner 2012), and expression of PRLR in pancreatic β-cells increases during pregnancy (Hughes and Huang 2011). Further, the onset of placental lactogen secretion during pregnancy occurs at the same time that the earliest changes in β-cell division and insulin secretion are detected (Huang et al. 2009). In vivo and in vitro rodent studies comparing the effect of lactogenic hormones with those observed in pregnancy on islets revealed that lactogens induce the same changes in islets as those observed during pregnancy. This led to the hypothesis that β-cell PRLR is central to the mechanisms islets use to adapt to pregnancy (Sorenson and Brelje 2009). During pregnancy, female PRLR-deficient mice exhibit decreased islet mass and lower rates of β-cell proliferation leading to impaired glucose tolerance (Huang et al. 2009). PRL-induced increases in insulin secretion and β-cell proliferation are also mediated by the JAK2/STAT5 pathway (Brelje et al. 2002; Fujinaka et al. 2007). Increases in proliferation require PRLR signaling through Jak2, Akt, menin/p18, and p21 (Hughes and Huang 2011). Transgenic-specific overexpression of PL β-cells in mice produces a marked increase in islet mass due to augmented islet size and number. These mice are also resistant to STZ-induced β-cell death, suggesting that PL may promote β-cell survival during pregnancy (Vasavada et al. 2000). PRL has been reported to improve islet graft function, and pretreatment of human islets for transplantation with PRL promotes islet viability and survival (Yamamoto et al. 2008; Terra et al. 2011). PRL-treated transplanted islets have increased revascularization and improved function (Johansson et al. 2009). Lactogen-mediated β-cell protection is also mediated by JAK2/STAT5 signaling pathway (Fujinaka et al. 2007; Kondegowda et al. 2012) and involves the upregulation of the anti-apoptotic mediator Bcl-X_L (Fujinaka et al. 2007). PRL upregulates expression of cell cycle regulators (D-type cyclins, CDK4) as well as genes involved in cell proliferation (members of the MAPK signaling pathway) (Bordin et al. 2004). The enhanced compensatory glucose-stimulated insulin secretion during pregnancy also involves the IRS/PI3K and SHC/ERK pathway (Amaral et al. 2004).

In humans, prolactin and placental lactogen play central roles in insulin production (Lombardo et al. 2011). Although limited, studies on human islets indicate that lactogen treatment increases insulin secretion and islet cell proliferation and survival (Yamamoto et al. 2008; Terra et al. 2011; Lombardo et al. 2011). Thus, it is quite likely that in humans as well as in rodents, lactogenic hormones are at least partly responsible for islet adaptation to the metabolic stress of pregnancy (Sorenson and Brelje 2009). The molecular pathway involved in lactogen transduction in human islets involves JAK2/STAT5, IRS-1 and IRS-2, PI3 kinase, MAPKs, as well as the signal transduction mechanisms activated by PL in β-cells in pregnancy (Lombardo et al. 2011). Evidence suggests that during pregnancy, lactogens induce proliferation via serotonin release. PL induces a marked rise of serotonin production in islets in a subpopulation of β-cells (Schraenen et al. 2010). Inhibition of serotonin synthesis blocks β-cell expansion and induces glucose intolerance in pregnant mice without affecting insulin sensitivity (Kim et al. 2010).

Thus, lactogens promote β-cell survival and increase insulin secretion and islet cell proliferation. Further studies are needed to determine how these findings can be translated to therapeutic avenue to protect β-cells at the onset of T2D.

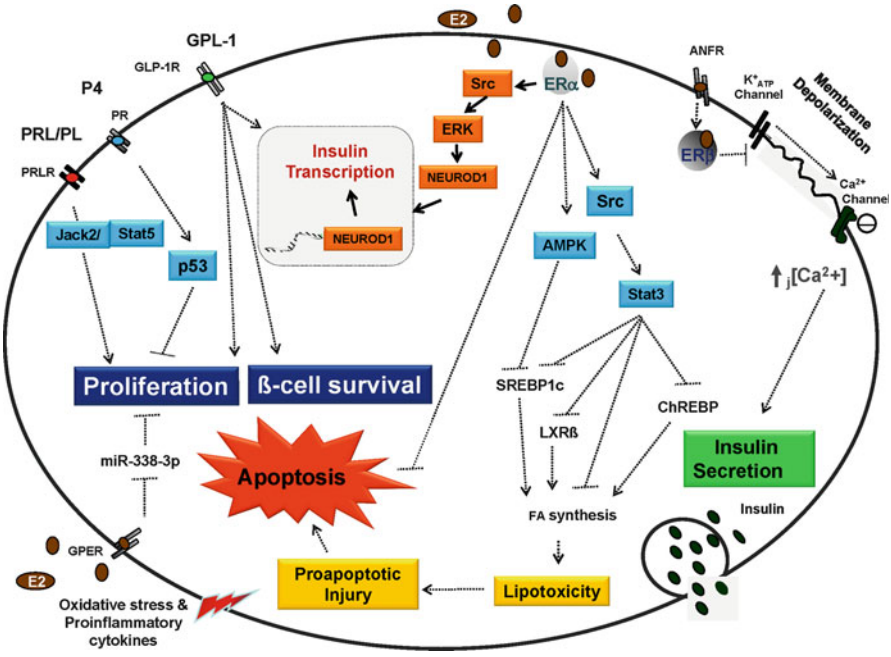

Fig. 1 Summary of reproductive hormone effects in the islet adaptation to metabolic stress. In females, reproductive hormones are important to the β-cell adaptation to the metabolic stress of pregnancy. Thus, placental lactogen (*PL*) and prolactin (*PRL*) promote β-cell expansion to adapt to the increase in insulin demand. This synergizes with 17β-estradiol (*E2*) that increases insulin production and secretion and promotes β-cell survival. Progesterone (*P4*) could act to limit β-cell expansion in vivo. In males, physiological levels of testosterone enhance β-cell function

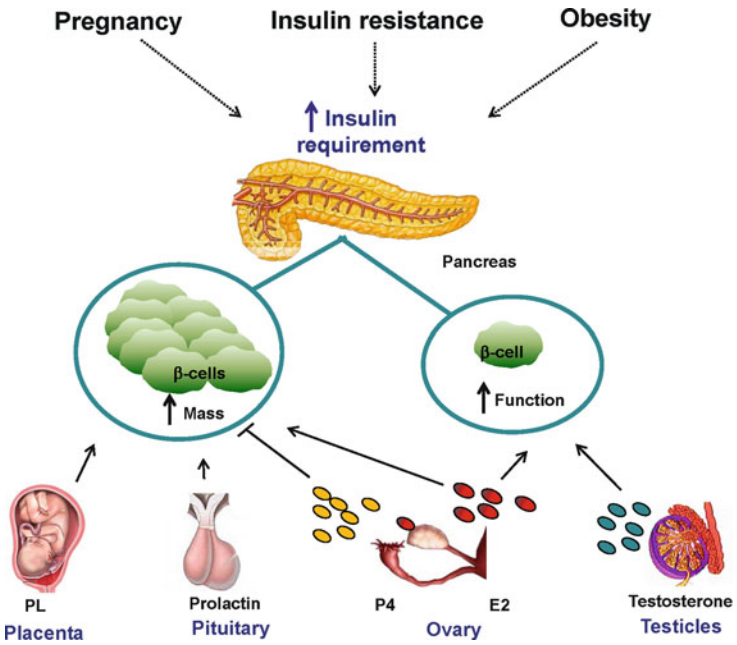

Fig. 2 Molecular pathways used by female reproductive hormones in the islet adaptation to metabolic stress. Prolactin (*PRL*) and placental lactogen (*PL*) promote β-cell proliferation via the lactogen-prolactin receptor (*PRLR*) and the Janus kinase 2/signal transducer and activator of transcription 5 (*JAK2*/*STAT5*) pathway. E2 signals in β-cells via three different receptors, ERα, ERβ, and the G protein coupled ER (*GPER*). ERα enhances glucose-stimulated insulin biosynthesis by enhancing NeuroD1 nuclear translocation to the insulin promoter. ERα suppresses lipogenesis through (1) a Src-signal transducer and activator of transcription 3 (*STAT3*)-dependent pathway inhibiting liver X receptor β (*LXRβ*) mRNA expression and eventually sterol regulatory element-binding protein 1c (*SREBP1c*) and carbohydrate response element-binding protein (*ChREBP*) expression and (2) an 5′ adenosine monophosphate-activated protein kinase (*AMPK*)-dependent pathway directly inhibiting SREBP1c expression. Altogether this prevents lipotoxicity. Activation of ERβ enhances glucose-stimulated insulin secretion (*GSIS*) via activation of the atrial natriuretic peptide receptor (*ANFR*) and closure of K_{ATP} channels. GPER stimulates β-cell mass during pregnancy via a decreased expression of the islet microRNA, miR-338-3p. Finally, progesterone (*P4*) suppresses β-cell proliferation by inducing the expression of tumor suppressor p53

Conclusion

Pancreatic β-cells located in islets of Langerhans have the ability to adapt to the increased insulin requirements by increasing their function, mass, or both. During pregnancy, reproductive hormones play a critical role in this adaptive process by increasing β-cell function, growth, and survival via β-cell receptors. These effects and their mechanisms are summarized in Figs. 1 and 2. A better understanding of the mechanisms of action of reproductive hormones in β-cells promises to yield therapeutic avenues to protect functional β-cell mass in diabetes.

Acknowledgments This work was supported by grants from NIH RO1 DK074970, the Juvenile Diabetes Research Foundation (1-2006-837) and the March of Dimes (6-FY7-312).

Cross-References

- ▶ Glucose-Induced Apoptosis in Pancreatic Islets
- ▶ Prevention of β-Cell Destruction in Autoimmune Diabetes: Current Approaches and Future Prospects
- ▶ The β-Cell in Human Type 2 Diabetes
- ▶ β-Cell Function in Obese-Hyperglycemic Mice

References

Alonso-Magdalena P, Ropero AB, Carrera MP, Cederroth CR, Baquie M et al (2008) Pancreatic insulin content regulation by the estrogen receptor ER α. PLoS One 3:e2069

Amaral ME, Cunha DA, Anhe GF, Ueno M, Carneiro EM et al (2004) Participation of prolactin receptors and phosphatidylinositol 3-kinase and MAP kinase pathways in the increase in pancreatic islet mass and sensitivity to glucose during pregnancy. J Endocrinol 183:469–476

Balhuizen A, Kumar R, Amisten S, Lundquist I, Salehi A (2010) Activation of G protein-coupled receptor 30 modulates hormone secretion and counteracts cytokine-induced apoptosis in pancreatic islets of female mice. Mol Cell Endocrinol 320:16–24

Baquie M, St-Onge L, Kerr-Conte J, Cobo-Vuilleumier N, Lorenzo PI et al (2011) The liver receptor homolog-1 (LRH-1) is expressed in human islets and protects β-cells against stress-induced apoptosis. Hum Mol Genet 20:2823–2833

Basaria S, Muller DC, Carducci MA, Egan J, Dobs AS (2006) Hyperglycemia and insulin resistance in men with prostate carcinoma who receive androgen-deprivation therapy. Cancer 106:581–588

Bordin S, Amaral ME, Anhe GF, Delghingaro-Augusto V, Cunha DA et al (2004) Prolactin-modulated gene expression profiles in pancreatic islets from adult female rats. Mol Cell Endocrinol 220:41–50

Brelje TC, Svensson AM, Stout LE, Bhagroo NV, Sorenson RL (2002) An immunohistochemical approach to monitor the prolactin-induced activation of the JAK2/STAT5 pathway in pancreatic islets of Langerhans. J Histochem Cytochem 50:365–383

Choe SS, Choi AH, Lee JW, Kim KH, Chung JJ et al (2007) Chronic activation of liver X receptor induces β-cell apoptosis through hyperactivation of lipogenesis: liver X receptor-mediated lipotoxicity in pancreatic β-cells. Diabetes 56:1534–1543

Choi SB, Jang JS, Park S (2005) Estrogen and exercise may enhance β-cell function and mass via insulin receptor substrate 2 induction in ovariectomized diabetic rats. Endocrinology 146:4786–4794

Contreras JL, Smyth CA, Bilbao G, Young CJ, Thompson JA et al (2002) 17β-Estradiol protects isolated human pancreatic islets against proinflammatory cytokine-induced cell death: molecular mechanisms and islet functionality. Transplantation 74:1252–1259

Doglioni C, Gambacorta M, Zamboni G, Coggi G, Viale G (1990) Immunocytochemical localization of progesterone receptors in endocrine cells of the human pancreas. Am J Pathol 137:999–1005

Dunaif A, Finegood DT (1996) β-cell dysfunction independent of obesity and glucose intolerance in the polycystic ovary syndrome. J Clin Endocrinol Metab 81:942–947

Finan B, Yang B, Ottaway N, Stemmer K, Muller TD et al (2012) Targeted estrogen delivery reverses the metabolic syndrome. Nat Med 18:1847–1856

Freemark M, Avril I, Fleenor D, Driscoll P, Petro A et al (2002) Targeted deletion of the PRL receptor: effects on islet development, insulin production, and glucose tolerance. Endocrinology 143:1378–1385

Fujinaka Y, Takane K, Yamashita H, Vasavada RC (2007) Lactogens promote β cell survival through JAK2/STAT5 activation and Bcl-XL upregulation. J Biol Chem 282:30707–30717

Goffin V, Binart N, Clement-Lacroix P, Bouchard B, Bole-Feysot C et al (1999) From the molecular biology of prolactin and its receptor to the lessons learned from knockout mice models. Genet Anal 15:189–201

Gonzalez F, Rote NS, Minium J, Kirwan JP (2006) Increased activation of nuclear factor kappaB triggers inflammation and insulin resistance in polycystic ovary syndrome. J Clin Endocrinol Metab 91:1508–1512

Goodarzi MO, Erickson S, Port SC, Jennrich RI, Korenman SG (2005) β-Cell function: a key pathological determinant in polycystic ovary syndrome. J Clin Endocrinol Metab 90:310–315

Goodman MN, Hazelwood RL (1974) Short-term effects of oestradiol benzoate in normal, hypophysectomized and alloxan-diabetic male rats. J Endocrinol 62:439–449

Haffner SM, Laakso M, Miettinen H, Mykkanen L, Karhapaa P et al (1996) Low levels of sex hormone-binding globulin and testosterone are associated with smaller, denser low density lipoprotein in normoglycemic men. J Clin Endocrinol Metab 81:3697–3701

Holte J, Bergh T, Berne C, Berglund L, Lithell H (1994) Enhanced early insulin response to glucose in relation to insulin resistance in women with polycystic ovary syndrome and normal glucose tolerance. J Clin Endocrinol Metab 78:1052–1058

Houssay BA, Foglia VG, Rodriguez RR (1954) Production or prevention of some types of experimental diabetes by oestrogens or corticosteroids. Acta Endocrinol (Cph) 17:146–164

Huang C, Snider F, Cross J (2009) Prolactin receptor is required for normal glucose homeostasis and modulation of β-cell mass during pregnancy. Endocrinology 150:1618–1626

Huang X, Chen J, Wang Q, Chen X, Wen W et al (2011) miR-338-3p suppresses invasion of liver cancer cell by targeting smoothened. J Pathol 225:463–472

Hughes E, Huang C (2011) Participation of Akt, menin, and p21 in pregnancy-induced β-cell proliferation. Endocrinology 152:847–855

Jacovetti C, Abderrahmani A, Parnaud G, Jonas JC, Peyot ML et al (2012) MicroRNAs contribute to compensatory β cell expansion during pregnancy and obesity. J Clin Invest 122:3541–3551

Johansson M, Olerud J, Jansson L, Carlsson PO (2009) Prolactin treatment improves engraftment and function of transplanted pancreatic islets. Endocrinology 150:1646–1653

Kang L, Zhang X, Xie Y, Tu Y, Wang D et al (2010) Involvement of estrogen receptor variant ER-α36, not GPR30, in nongenomic estrogen signaling. Mol Endocrinol 24:709–721

Keating NL, O'Malley A, Freedland SJ, Smith MR (2012) Diabetes and cardiovascular disease during androgen deprivation therapy: observational study of veterans with prostate cancer. J Natl Cancer Inst 104(19):1518–1523

Khaw KT, Barrett-Connor E (1992) Lower endogenous androgens predict central adiposity in men. Ann Epidemiol 2:675–682

Kim H, Toyofuku Y, Lynn FC, Chak E, Uchida T et al (2010) Serotonin regulates pancreatic β cell mass during pregnancy. Nat Med 16:804–U106

Kondegowda N, Mozar A, Chin C, Otero A, Garcia-Ocaña A et al (2012) Lactogens protect rodent and human β cells against glucolipotoxicity-induced cell death through Janus kinase-2 (JAK2)/signal transducer and activator of transcription-5 (STAT5) signalling. Diabetologia 55:1721–1732

Kumar R, Balhuizen A, Amisten S, Lundquist I, Salehi A (2011) Insulinotropic and antidiabetic effects of 17β-estradiol and the GPR30 agonist G-1 on human pancreatic islets. Endocrinology 152:2568–2579

Le May C, Chu K, Hu M, Ortega CS, Simpson ER et al (2006) Estrogens protect pancreatic β-cells from apoptosis and prevent insulin-deficient diabetes mellitus in mice. Proc Natl Acad Sci U S A 103:9232–9237

Liu S, Mauvais-Jarvis F (2009) Rapid, nongenomic estrogen actions protect pancreatic islet survival. Islets 1:273–275

Liu S, Le May C, Wong WP, Ward RD, Clegg DJ et al (2009) Importance of extranuclear estrogen receptor-α and membrane G protein-coupled estrogen receptor in pancreatic islet survival. Diabetes 58:2292–2302

Liu S, Navarro G, Mauvais-Jarvis F (2010) Androgen excess produces systemic oxidative stress and predisposes to β-cell failure in female mice. PLoS One 5:e11302

Liu S, Kilic G, Meyers MS, Navarro G, Wang Y et al (2013) Oestrogens improve human pancreatic islet transplantation in a mouse model of insulin deficient diabetes. Diabetologia 56:370–381

Lombardo MF, De Angelis F, Bova L, Bartolini B, Bertuzzi F et al (2011) Human placental lactogen (hPL-A) activates signaling pathways linked to cell survival and improves insulin secretion in human pancreatic islets. Islets 3:250–258

Maclaren NK, Neufeld M, McLaughlin JV, Taylor G (1980) Androgen sensitization of streptozotocin-induced diabetes in mice. Diabetes 29:710–716

Mauvais-Jarvis F (2011) Estrogen and androgen receptors: regulators of fuel homeostasis and emerging targets for diabetes and obesity. Trends Endocrinol Metab 22:24–33

Moldrup A, Petersen ED, Nielsen JH (1993) Effects of sex and pregnancy hormones on growth hormone and prolactin receptor gene expression in insulin-producing cells. Endocrinology 133:1165–1172

Morimoto S, Cerbón MA, Alvarez-Alvarez A, Romero-Navarro G, Díaz-Sánchez V (2001) Insulin gene expression pattern in rat pancreas during the estrous cycle. Life Sci 68(26):2979–2985

Morimoto S, Mendoza-Rodriguez CA, Hiriart M, Larrieta ME, Vital P et al (2005) Protective effect of testosterone on early apoptotic damage induced by streptozotocin in rat pancreas. J Endocrinol 187:217–224

Navarro G, Mauvais-Jarvis F (2013) The role of the Androgen Receptor in β-cell function in male mice. Diabetes 62(Suppl 1):A571

Navarro G, Suhuan Liu P, De Gendt K, Verhoeven G, Mauvais-Jarvis F (2011) Importance of the β – cell Androgen Receptor in type 2 diabetes. Endocr Rev 32:OR22–OR23

Newberna N, Freemark M (2011) Placental hormones and the control of maternal metabolism and fetal growth. Curr Opin Endocrinol Diabetes Obes 18:409–416

Nieuwenhuizen AG, Schuiling GA, Liem SM, Moes H, Koiter TR et al (1999) Progesterone stimulates pancreatic cell proliferation in vivo. Eur J Endocrinol 140:256–263

O'Meara NM, Blackman JD, Ehrmann DA, Barnes RB, Jaspan JB et al (1993) Defects in β-cell function in functional ovarian hyperandrogenism. J Clin Endocrinol Metab 76:1241–1247

Oh JY, Barrett-Connor E, Wedick NM, Wingard DL (2002) Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. Diabetes Care 25:55–60

Ouhtit A, Kelly PA, Morel G (1994) Visualization of gene expression of short and long forms of prolactin receptor in rat digestive tissues. Am J Physiol 266:G807–G815

Paik SG, Michelis MA, Kim YT, Shin S (1982) Induction of insulin-dependent diabetes by streptozotocin. Inhibition by estrogens and potentiation by androgens. Diabetes 31:724–729

Palomar-Morales M, Morimoto S, Mendoza-Rodriguez CA, Cerbon MA (2010) The protective effect of testosterone on streptozotocin-induced apoptosis in β cells is sex specific. Pancreas 39:193–200

Picard F, Wanatabe M, Schoonjans K, Lydon J, O'Malley BW et al (2002) Progesterone receptor knockout mice have an improved glucose homeostasis secondary to β-cell proliferation. Proc Natl Acad Sci U S A 99:15644–15648

Pitteloud N, Mootha VK, Dwyer AA, Hardin M, Lee H et al (2005) Relationship between testosterone levels, insulin sensitivity, and mitochondrial function in men. Diabetes Care 28:1636–1642

Ren Z, Zou C, Ji H, Zhang YA (2010) Oestrogen regulates proliferation and differentiation of human islet-derived precursor cells through oestrogen receptor α. Cell Biol Int 34:523–530

Salonia A, Lanzi R, Scavini M, Pontillo M, Gatti E et al (2006) Sexual function and endocrine profile in fertile women with type 1 diabetes. Diabetes Care 29:312–316

Schraenen A, Lemaire K, de Faudeur G, Hendrickx N, Granvik M et al (2010) Placental lactogens induce serotonin biosynthesis in a subset of mouse β cells during pregnancy. Diabetologia 53:2589–2599

Shao J, Qiao L, Friedman JE (2004) Prolactin, progesterone, and dexamethasone coordinately and adversely regulate glucokinase and cAMP/PDE cascades in MIN6 β-cells. Am J Physiol Endocrinol Metab 286:E304–E310

Sharma G, Prossnitz ER (2011) Mechanisms of estradiol-induced insulin secretion by the G protein-coupled estrogen receptor GPR30/GPER in pancreatic β-cells. Endocrinology 152:3030–3039

Sorenson RL, Brelje TC (1997) Adaptation of islets of langerhans to pregnancy: β-cell growth, enhanced insulin secretion and the role of lactogenic hormones. Horm Metab Res 29:301–307

Sorenson RL, Brelje TC (2009) Prolactin receptors are critical to the adaptation of islets to pregnancy. Endocrinology 150:1566–1569

Sorenson RL, Brelje TC, Roth C (1993) Effects of steroid and lactogenic hormones on islets of Langerhans: a new hypothesis for the role of pregnancy steroids in the adaptation of islets to pregnancy. Endocrinology 133:2227–2234

Soriano S, Ropero AB, Alonso-Magdalena P, Ripoll C, Quesada I et al (2009) Rapid regulation of K_{ATP} channel activity by 17β-estradiol in pancreatic β-cells involves the estrogen receptor β and the atrial natriuretic peptide receptor. Mol Endocrinol 23:1973–1982

Soriano S, Alonso-Magdalena P, Garcia-Arevalo M, Novials A, Muhammed SJ et al (2012) Rapid insulinotropic action of low doses of bisphenol-A on mouse and human islets of Langerhans: role of estrogen receptor β. PLoS One 7:e31109

Terra LF, Garay-Malpartida MH, Wailemann RA, Sogayar MC, Labriola L (2011) Recombinant human prolactin promotes human β cell survival via inhibition of extrinsic and intrinsic apoptosis pathways. Diabetologia 54:1388–1397

Tiano JP, Mauvais-Jarvis F (2012a) Importance of oestrogen receptors to preserve functional β-cell mass in diabetes. Nat Rev Endocrinol 8:342–351

Tiano JP, Mauvais-Jarvis F (2012b) Molecular mechanisms of estrogen receptors' suppression of lipogenesis in pancreatic β-cells. Endocrinology 153:2997–3005

Tiano J, Mauvais-Jarvis F (2012c) Selective estrogen receptor modulation in pancreatic β-cells and the prevention of type 2 diabetes. Islets 4:173–176

Tiano JP, Delghingaro-Augusto V, Le May C, Liu S, Kaw MK et al (2011) Estrogen receptor activation reduces lipid synthesis in pancreatic islets and prevents β cell failure in rodent models of type 2 diabetes. J Clin Invest 121:3331–3342

Tiano J, Finan B, DiMarchi R, Mauvais-Jarvis F (2012) A Glucagon-like peptide-1-estrogen fusion peptide shows enhanced efficacy in preventing insulin-deficient diabetes in mice. Endocr Rev 33:OR21–OR26

Vasavada RC, Garcia-Ocana A, Zawalich WS, Sorenson RL, Dann P et al (2000) Targeted expression of placental lactogen in the β cells of transgenic mice results in β cell proliferation, islet mass augmentation, and hypoglycemia. J Biol Chem 275:15399–15406

Vetere A, Wagner BK (2012) Chemical methods to induce β-cell proliferation. Int J Endocrinol 2012:925143

Wong WP, Tiano JP, Liu S, Hewitt SC, Le May C et al (2010) Extranuclear estrogen receptor-α stimulates NeuroD1 binding to the insulin promoter and favors insulin synthesis. Proc Natl Acad Sci USA 107:13057–13062

Yamabe N, Kang KS, Zhu BT (2010) Beneficial effect of 17β-estradiol on hyperglycemia and islet β-cell functions in a streptozotocin-induced diabetic rat model. Toxicol Appl Pharmacol 249:76–85

Yamamoto T, Ricordi C, Mita A, Miki A, Sakuma Y et al (2008) β-Cell specific cytoprotection by prolactin on human islets. Transplant Proc 40:382–383

Zitzmann M (2009) Testosterone deficiency, insulin resistance and the metabolic syndrome. Nat Rev Endocrinol 5:673–681

Zitzmann M, Faber S, Nieschlag E (2006) Association of specific symptoms and metabolic risks with serum testosterone in older men. J Clin Endocrinol Metab 91:4335–4343

The β-Cell in Human Type 2 Diabetes

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Lorella Marselli, Mara Suleiman, Farooq Syed, Franco Filipponi, Ugo Boggi, Piero Marchetti, and Marco Bugliani

Contents

Abstract

β-cell dysfunction is central to the onset and progression of type 2 diabetes. Reduced islet number and/or diminished β-cell mass/volume in the pancreas of type 2 diabetic subjects have been reported by many authors, mainly due to increased apoptosis not compensated for by adequate regeneration. In addition, ultrastructural analysis has shown reduced insulin granules and morphological changes in several β-cell organelles, including mitochondria and endoplasmic

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_16,

reticulum. Several quantitative and qualitative defects of β-cell function have been described in human type 2 diabetes using isolated islets, including alterations in early phase and glucose-stimulated insulin release. These survival and functional changes are accompanied by modifications of islet gene and protein expression. The impact of genotype in affecting β-cell function and survival has been addressed in a few studies, and a number of gene variants have been associated with β-cell dysfunction. Among acquired factors, the role of glucotoxicity and lipotoxicity could be of particular importance, due to the potential deleterious impact of elevated levels of glucose and/or free fatty acids in the natural history of β-cell damage. More recently, it has been proposed that inflammation might also play a role in the dysfunction of the β-cell in type 2 diabetes. Encouraging, although preliminary, data show that some of these defects might be directly counteracted, at least in part, by appropriate in vitro pharmacological intervention.

Keywords
β-cell volume • β-cell mass • Insulin secretion • Apoptosis • Regeneration • Mitochondria • Endoplasmic reticulum • Gene polymorphisms • Gene expression • Protein expression • Glucotoxicity • Lipotoxicity • Inflammation

Introduction

β-cell dysfunction is central to the development and progression of type 2 diabetes (American Diabetes Association 2008; Stumvoll et al. 2005; Kahn 2003). Reduced β-cell functional mass in diabetes and other categories of glucose intolerance has been described in patients, and decreased islet and/or β-cell volume in the pancreas of type 2 diabetic patients has been consistently observed (Marchetti et al. 2008; Wajchenberg 2007; Meier 2008). These findings are in agreement with the results obtained with healthy humans who underwent hemipancreatectomy for the purpose of organ donation, and 43 % of cases developed impaired fasting glucose, impaired glucose tolerance, or diabetes on 3–10 years of follow-up (Kumar et al. 2008). In addition, studies in patients and the use of isolated islets have shown both quantitative and qualitative defects of glucose-stimulated insulin secretion in type 2 diabetes (Porte 1991; Ferrannini and Mari 2004; Kahn et al. 2008). The importance of β-cell function (in the absence of obvious reduction of β-cell mass) is supported by the MODY2 type of diabetes, due to mutations of the enzyme glucokinase, leading to decreased glycolytic flux in the β-cell (Vaxillaire and Froguel 2008). In this chapter, we describe the mass and functional defects of β-cells in type 2 diabetes and discuss the accompanying molecular alterations. Then, the role of a few genetic and acquired factors affecting the β-cell is briefly discussed, followed by the description of the beneficial effects that some compounds directly have on the diabetic β-cell.

β-Cell Mass Defects

Early work reported that total islet number was approximately 30 % lower in pancreatic histology samples from type 2 diabetic subjects as compared to those from nondiabetic individuals (Saito et al. 1978). The reduction in total islet volume in diabetic vs. nondiabetic pancreata (1.01 ± 0.12 vs. 1.60 ± 0.16 cm^3) was confirmed (Westermark and Wilander 1978) and resulted even more marked when corrected for the presence of amyloid (Westermark and Wilander 1978). Successively, it was found that β-cell volume was 30–40 % reduced in type 2 diabetic islets (Saito et al. 1979). In the following years, although a few authors were not able to find differences in β-cell amount in diabetic versus nondiabetic pancreas specimens (Stefan et al. 1982; Rahier et al. 1983), several studies have consistently shown that β-cell mass is reduced in type 2 diabetes (Clark et al. 1988; Sakuraba et al. 2002; Yoon et al. 2003; Butler et al. 2003; Rahier et al. 2008). Clark and colleagues studied the pancreas of 15 type 2 diabetic and ten control subjects and observed 24 % β-cell area reduction in the diabetic samples (Clark et al. 1988). More recently, it has been reported that islet β-cell volume density and total β-cell mass were significantly lower (~30 %) in pancreatic specimens from Japanese type 2 diabetic patients in comparison with those obtained from nondiabetic individuals (Sakuraba et al. 2002). Accordingly, when pancreas samples following surgical removal were studied (Yoon et al. 2003), it was found that in the nondiabetic cases β-cell volume was 1.94 ± 0.7 %, whereas specimens from type 2 diabetic patients contained a lower β-cell volume (1.37 ± 1.0 %). In addition, in the diabetic samples, no correlation was found between β-cell volume and diabetes duration (Yoon et al. 2003). Pancreatic autoptic samples from type 2 diabetic patients, subjects with impaired fasting glycemia (IFG), and nondiabetic individuals (the groups were subdivided into lean or obese according to BMI) have been studied lately (Butler et al. 2003). In normoglycemic cases, obesity was associated with 50 % higher β-cell volume, as compared to nonobese individuals. However, obese subjects with IFG or diabetes had 40–60 % reduction in β-cell volume in comparison to BMI-matched, nondiabetic cases. This was due to β-cell number decrease, rather than changes in islet size. In the nonobese group, diabetes was associated with 41 % reduction in the volume of the β-cells. A detailed study has been published very recently (Rahier et al. 2008). The authors analyzed autoptic samples from 57 type 2 diabetic and 52 nondiabetic European subjects and confirmed that β-cell mass was lower (around 30 %) in the former (Fig. 1). However, there was marked intersubject variability and large overlap between the two groups (Fig.1). No difference was found between diabetic patients treated with oral agents and insulin, whereas β-cell mass increased with BMI values and decreased with duration of diabetes (Rahier et al. 2008). Finally, a reduced number of β-cells in islets from type 2 diabetic subjects has been demonstrated by electron microscopy as well, which also showed that volume density of mature insulin granules was lower in type 2 diabetic than in nondiabetic β-cells (Marchetti et al. 2004).

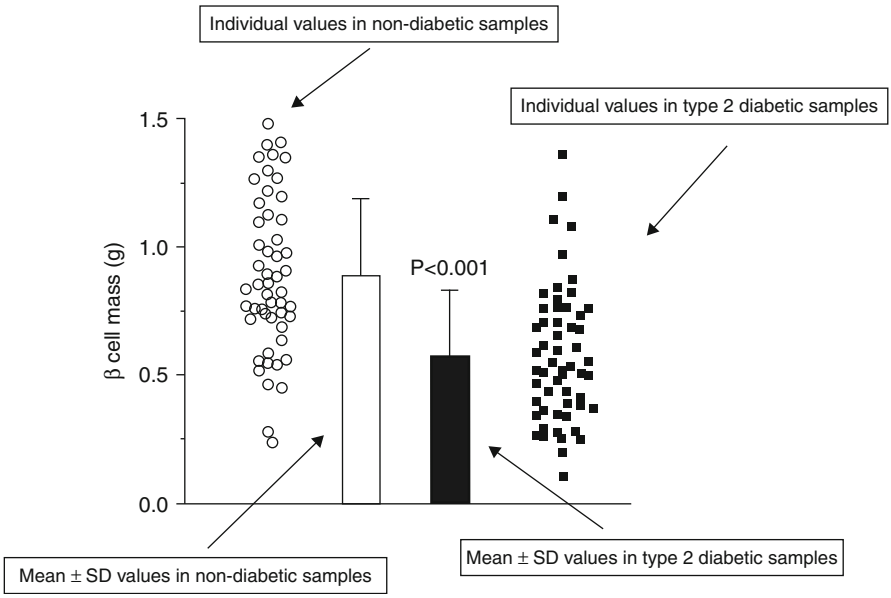

Fig. 1 β-cell mass is reduced in type 2 diabetic patients, as compared to nondiabetic controls, although there is a marked intersubject variability and clear overlap between the two groups (Adapted from Rahier et al. (2008))

It is generally assumed that β-cell loss in type 2 diabetes is mainly due to increased β-cell apoptosis (Butler et al. 2003; Marchetti et al. 2007). As a matter of fact, in autoptic samples, apoptosis was shown to be three- and tenfold higher in obese and lean type 2 diabetic samples, respectively, than in BMI-matched, normoglycemic individuals (Butler et al. 2003), and increased β-cell apoptosis in diabetic islets has been reported following electron microscopy analysis (Marchetti et al. 2007). In addition, by assessing cytoplasmic histone-associated DNA fragments, it has been observed that there is a twofold higher amount of islet cell death with isolated diabetic islets, as compared to nondiabetic islets (Marchetti et al. 2004) (Fig.2). This was accompanied by a significant increase in the activity of caspase-3 and caspase-8, key molecules in the apoptotic pathway (Marchetti et al. 2004) (Fig.2). Several factors can contribute to cause β-cell apoptosis (see below), and intracellular organelles, including the endoplasmic reticulum, are likely to be actively involved (Marchetti et al. 2007). On the other hand, the enhanced β-cell death rate does not seem to be adequately compensated for by regenerative phenomena in diabetic islets. In autoptic specimens, it has been reported that the relative rate of new islet formation, estimated by fraction of duct cells positive for insulin, and the frequency of β-cell replication, assessed by Ki67 staining, were substantially similar in type 2 diabetic and control pancreata (Butler et al. 2003).

Therefore, current evidence shows a reduced β-cell amount in human type 2 diabetes, possibly due to increased apoptosis without adequate regeneration.

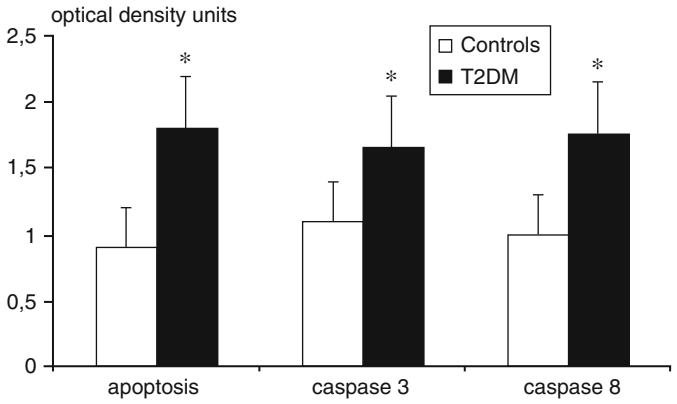

Fig. 2 Isolated type 2 diabetic (*T2DM*) islets show increased apoptosis and enhanced caspase-3 and caspase-8 activities, as compared to nondiabetic controls. Death was measured by ELISA methods evaluating cytoplasmic histone-associated DNA fragments, and caspase activity was determined using a colorimetric assay. *$p < 0.05$ vs. controls (Adapted from Marchetti et al. (2004))

However, the loss of β-cell appears to be 30 % on average, which is unlikely to lead to overt diabetes, unless a defect in β-cell function is present as well.

β-Cell Functional Defects

Several functional properties of the pancreatic β-cells in type 2 diabetes have been directly evaluated ex vivo following islet isolation from the human pancreas. Earlier work showed that the release of insulin evoked by glucose was lower in type 2 diabetic than in nondiabetic islets (Fernandez-Alvarez et al. 1994). However, the secretory response to the combination of l-leucine and l-glutamine appeared less severely altered (Fernandez-Alvarez et al. 1994). In a detailed study by Deng and colleagues, islets isolated from eight diabetic and nine normal donors were evaluated by in vitro islet perifusion experiments (Deng et al. 2004). Basal insulin secretion was similar for both normal and diabetic islets. However, the islets from diabetic donors released less total insulin in response to glucose and also exhibited an elevated threshold for insulin secretion triggering. In addition, it was observed that in comparison with normal islets, an equivalent amount of type 2diabetic islets did not fully reverse the hyperglycemic condition when transplanted into diabetic mice (Deng et al. 2004). In another study, when insulin secretion was measured in response to glucose, arginine, and glibenclamide in isolated nondiabetic and type 2 diabetic islets, again no significant difference as for insulin release in response to 3.3 mmol/l glucose was observed (Del Guerra et al. 2005). However, when challenged with 16.7 mmol/l glucose, diabetic islets secreted significantly less insulin than did nondiabetic cells. Insulin secretion during arginine and glibenclamide stimulation was also lower from diabetic islets than from control islets; however, type 2 diabetic islets released a significantly higher amount of insulin in response to

arginine and glibenclamide than in response to glucose. In addition, when perifusion experiments were performed, glucose stimulation did not elicit any apparent increase in the early insulin secretion phase from diabetic islets, which however promptly released insulin when challenged with arginine or sulfonylurea (Del Guerra et al. 2005). Consistent with the observation that β-cell insulin secretion defects in type 2 diabetes β-cells are more selective for glucose-induced stimulation, it has been observed that in type 2 diabetic islets glucose oxidation is reduced, as compared to nondiabetic islets (Fernandez-Alvarez et al. 1994; Del Guerra et al. 2005). This has led to the speculation that mitochondria might be involved in causing β-cell dysfunction in type 2 diabetes. In this regard, the morphology and the function of mitochondria in human type 2 diabetic β-cells have been studied (Anello et al. 2005). By electron microscopy, mitochondria in type 2 diabetes β-cells appeared round shaped, hypertrophic, and with higher density volume when compared to control β-cells. When adenine nucleotide content was measured, it was found that islets from diabetic subjects were not able to increase their ATP content in the presence of acute glucose stimulation (Fig.3). As a consequence, the ATP/ADP ratio was approximately 40 % lower in diabetic than in control islets, which could contribute to the blunted or absent glucose-stimulated insulin release in the former (Anello et al. 2005) (Fig.3).

In summary, insulin secretion defects in human type 2 diabetic islets have been described by several authors, and data show more marked changes in insulin release in response to glucose, as compared to other fuel and nonfuel stimuli. This suggests that type 2 diabetic β-cells may have alterations in some steps of glucose metabolism, including those at the mitochondria level, leading to reduced ATP production.

Molecular Changes

Changes at the gene and protein expression levels have been reported in type 2 diabetic pancreatic islets by several authors. Using oligonucleotide microarrays of pancreatic islets isolated from humans with type 2 diabetes vs. normal glucose tolerant controls, Gunton et al. found that 370 genes were differently expressed in the two groups (243 upregulated and 137 downregulated) (Gunton et al. 2005). Quantitative RT-PCR studies were performed on selected genes, which confirmed changes in the expression of genes known to be important in β-cell function, including major decreases in the expression of HNF4 α, insulin receptor, IRS2, Akt2, and several glucose-metabolic-pathway genes. There was also a 90 % decrease in the expression of the transcription factor ARNT/HIF1β (hydrocarbon nuclear receptor translocator/hypoxia-inducible factor 1β) (Gunton et al. 2005). Successively, several genes encoding for the following proteins were found to be downregulated in type 2 diabetic islets by real-time RT-PCR: insulin, glucose transporter 1, glucose transporter 2, glucokinase, and molecules involved in insulin granules exocytosis (Del Guerra et al. 2005; Ostenson et al. 2006). Conversely, several genes implicated in differentiation and proliferation pathways have been reported to be increased in diabetic islets, including PDX-1, Foxo-1, Pax-4,

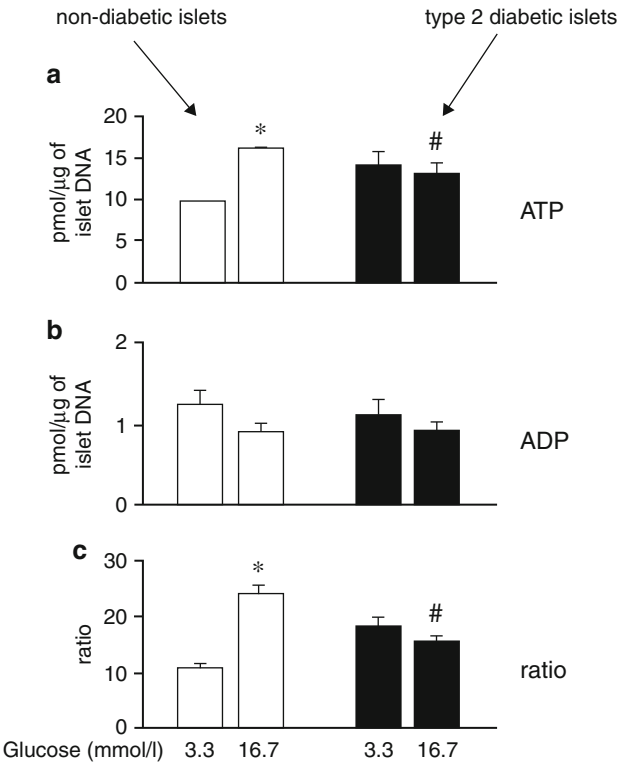

Fig. 3 ATP production and ATP/ADP ratio increase in nondiabetic but not in type 2 diabetic islets following exposure to 3.3–16.7 mmol/l glucose concentration.*: significantly higher vs.3.3 mmol/l glucose; #: significantly lower vs. nondiabetic islets at16.7 mmol/l glucose (Adapted from Anello et al. (2005))

andTCF7L2 (Del Guerra et al. 2005; Brun et al. 2008; Lyssenko et al. 2007). Furthermore, changes at the level of the expression of genes involved in regulating cell redox balance have been shown (Marchetti et al. 2004). As a matter of fact, mRNA expression of NADPH oxidase has been found to be increased and that of manganese and copper/zinc superoxide dismutases to be decreased in diabetic islets, together with enhanced expression of catalase and GSH peroxidase (Marchetti et al. 2004). In a recent paper, the expression of several genes associated with the function of the endoplasmic reticulum (in particular, those encoding for immunoglobulin heavy chain-binding protein, BiP, and X-box binding protein 1, XBP-1) has been described to be induced by exposure to high glucose in type 2 diabetic islets but not in control islets (Marchetti et al. 2007). When β-cell-enriched preparations obtained by the laser capture microdissection technique were studied (Marselli et al. 2007), transcript to some analysis preliminarily performed on four type 2 diabetic and four samples showed that in diabetic samples, there were 1,532 upregulated and 528 downregulated genes (Marselli et al. 2007).

Some information is also available as for protein expression in type 2 diabetic islets. The amount of insulin has been reported to be decreased 30–40 % in diabetic islet cells (Marchetti et al. 2004; Ostenson et al. 2006). The expression of AMP-activated kinase, IRS-2, PDX-1 (this latter at odds with gene expression data), and proteins involved in exocytosis was also found to be decreased in type 2 diabetic islets in comparison to nondiabetic samples (Marchetti et al. 2004; Ostenson et al. 2006). Preliminary data on type 2 diabetic islet protein profiling have been reported recently (Nyblom et al. 2007). The results showed that although considerable variability existed within the individuals, 31 differentially expressed peaks were detected, and the intensities of some of them were significantly correlated with ex vivo islet insulin release (Nyblom et al. 2007).

Whereas many defects at the gene and protein expression level have been described in islet cells from type 2 diabetic subjects, at present it is not possible to distinguish between primary β-cell molecular changes (leading to diabetes) and those occurring as a consequence of the unfavorable microenvironment associated with the diabetic conditions (see below). Since prospective studies in this regard are not feasible for obvious reasons, it would be of interest to compare the molecular properties of β-cells from individuals at different stages of disease.

The Role of Genetic and Acquired Factors

Type 2 diabetes is a polygenic disease, and in the past few years, link age studies, candidate-gene approaches, and genome-wide association studies have identified several gene variants which associate with this form of diabetes (Jafar-Mohammadi and McCarthy 2008; Owen and McCarthy 2007; Groop and Lyssenko 2008; Parikh and Groop 2004; Vaxillaire and Froguel 2008; Hattersley and Pearson 2006). The majority of these genes are involved in β-cell function and survival, and for some of them the description is available as for their direct effects on some β-cell features in humans. The common Gly(972) → Arg amino acid polymorphism of insulin receptor substrate 1, Arg(972) IRS-1, has been found to be associated with functional and morphological alterations of isolated human islets, including increased susceptibility to apoptosis, diminished glucose-stimulated insulin secretion, and lower amount of insulin granules (Marchetti et al. 2002; Federici et al. 2001). Similarly, the E23K variant of KCNJ11 gene, encoding the pancreatic β-cell adenosine 5′-triphosphate-sensitive potassium channel subunit Kir6.2 and associated with an increased risk of secondary failure to sulfonylurea in patients with type 2 diabetes (Sesti et al. 2006), has been shown to be associated with impairment of glibenclamide-induced insulin release following 24-h exposure to high glucose concentration. However, those studies were performed on islets isolated from nondiabetic subjects. More recently, genetic variants in the gene encoding for transcription factor-7-like 2 (TCF7L2) have been associated with type 2 diabetes and impaired β-cell function (Cauchi and Froguel 2008). It has been shown that the CT/TT genotypes of SNP rs7903146 strongly predicted future diabetes in independent cohorts of patients and that TCF7L2 expression in human islets was increased

fivefold in type 2 diabetes, particularly in carriers of the TT genotype (Lyssenko et al. 2007). In this study, overexpression of TCF7L2 in human islets reduced glucose-stimulated insulin secretion. However, in another report, depleting TCF7L2 by siRNA resulted in decreased glucose-stimulated insulin release, increased β-cell apoptosis, and decreased β-cell proliferation in human islets (Shu et al. 2008). In contrast, overexpression of TCF7L2 protected islets from glucose and cytokine-induced apoptosis and impaired function (Shu et al. 2008). It cannot be excluded that in the presence of diabetes, phenotypic changes occurring independent of the genotype may render the overall picture less clear.

Several acquired factors can affect β-cell survival and function (Stumvoll et al. 2005; Kahn 2003; Marchetti et al. 2008; Wajchenberg 2007; Meier 2008). In particular, the effects of glucotoxicity and lipotoxicity (terms used to indicate the deleterious effects induced on tissues and cells by prolonged exposure to increased glucose or free fatty acid concentrations) have been studied with isolated islets. Both conditions can lead to increased apoptosis, reduced glucose-stimulated insulin release, and molecular changes (Marchetti et al. 2008). Unfortunately, very little information is available on gluco- and/or lipotoxicity on human type 2 diabetic islets. In a recently published study (Marchetti et al. 2007), several features of β-cell endoplasmic reticulum were investigated in islets from nondiabetic and type 2 diabetic subjects. Whereas signs of endoplasmic reticulum stress were found in diabetic β-cells, it was also reported that when the islets were cultured for 24 h in 11.1 mmol/l glucose, there was the induction of immunoglobulin heavy chain-binding protein (BiP) and X-box binding protein 1(XBP-1) in the type 2 diabetic islets (Marchetti et al. 2007) (Fig.4). Obviously, more work is needed on these issues.

The mechanisms mediating the deleterious effects of acquired factors are being actively investigated, with increased oxidative stress probably playing an important role (Poitout and Robertson 2002). As a matter of fact, when the presence of 8-hydroxy-2′-deoxyguanosine (a marker of oxidative stress-induced DNA damage) and 4-hydroxy-2-nonenal modified proteins (a marker of lipid peroxidation products) was determined by immunostaining in islets of type 2 diabetic patients, both markers significantly increased as compared with nondiabetic individuals (Sakuraba et al. 2002). In addition, reduced staining of Cu/Zn superoxide dismutase was observed in the diabetic islet cells (Sakuraba et al. 2002). Similar findings were reported in a study performed with isolated type 2 diabetic islets (Marchetti et al. 2004), which showed increased content of nitrotyrosine and 8-hydroxy-2′-deoxyguanosine and reduced expression of Cu/Zn- and Mn superoxide dismutase. All this may contribute to produce a proinflammatory soil, which has been proposed to lead to β-cell damage in type 2 diabetes (Böni-Schnetzler et al. 2008; Ehses et al. 2008). Pancreatic islets may respond to metabolic stress by producing inflammatory factors, such as IL-1, and macrophage infiltration has been found in human type 2 diabetic islets. It is however possible that some of these pathways may be activated in subgroups of patients (Welsh et al. 2005).

Dealing with all the information continuously and rapidly coming from genetic studies is not an easy task, but the assessment of the relationships between β-cell

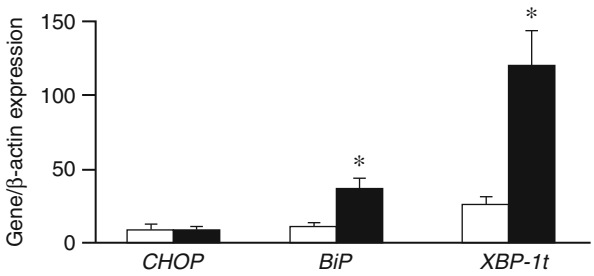

Fig. 4 When isolated type 2 diabetic islets were exposed for 24 h at increased glucose concentration (see text for details), a significant induction of genes involved in endoplasmic reticulum stress (BiP and XBP-1t) was observed, as measured by quantitative RT-PCR. The expression of another gene (CHOP) did not change (Adapted from Marchetti et al. (2007))

genotype and phenotype is crucial to understand why the β-cell fails in type 2 diabetes and in which way it is affected by acquired factors.

Reversal of β-Cell Damage in Type 2 Diabetes

The possibility that pancreatic β-cell damage induced by acquired factors can be prevented has been demonstrated in isolated nondiabetic islets exposed to different metabolic perturbations (Marchetti et al. 2008). More importantly, a few studies have shown that β-cell dysfunction in type 2 diabetes may be reversible. Exposure of isolated type 2 diabetic islets to antioxidants has led to improved glucose-stimulated insulin secretion and normalized expression of a few ROS scavenging enzymes (Del Guerra et al. 2005; Lupi et al. 2007). As mentioned above, a study showed that isolated type 2 diabetic islets were characterized by reduced insulin content, decreased amount of mature insulin granules, impaired glucose-induced insulin secretion, reduced insulin mRNA expression, and increased apoptosis with enhanced caspase-3 and caspase-8 activities (Marchetti et al. 2004). These alterations were associated with increased oxidative stress, as shown by higher nitrotyrosine concentrations, increased expression of protein kinase C-β2 and NADH oxidase, and changes in mRNA expression of Mn superoxide dismutase, Cu/Zn superoxide dismutase, catalase, and glutathione peroxidase (Marchetti et al. 2004). When these islets were incubated for 24 h in the presence of therapeutic concentration of metformin, insulin content and the number of mature insulin granules increased (Fig.5), and glucose-induced insulin release improved, with induction of insulin mRNA expression. Moreover, apoptosis was reduced, with concomitant decrease of caspase-3 and -8 activities. These changes were accompanied by reduction or normalization of markers of oxidative stress (Marchetti et al. 2004). Recently, the role of incretins (GLP-1, glucose-dependent insulinotropic polypeptide [GIP], and some of their analogs) in the therapy of diabetes has received much attention, mainly because of the beneficial actions of

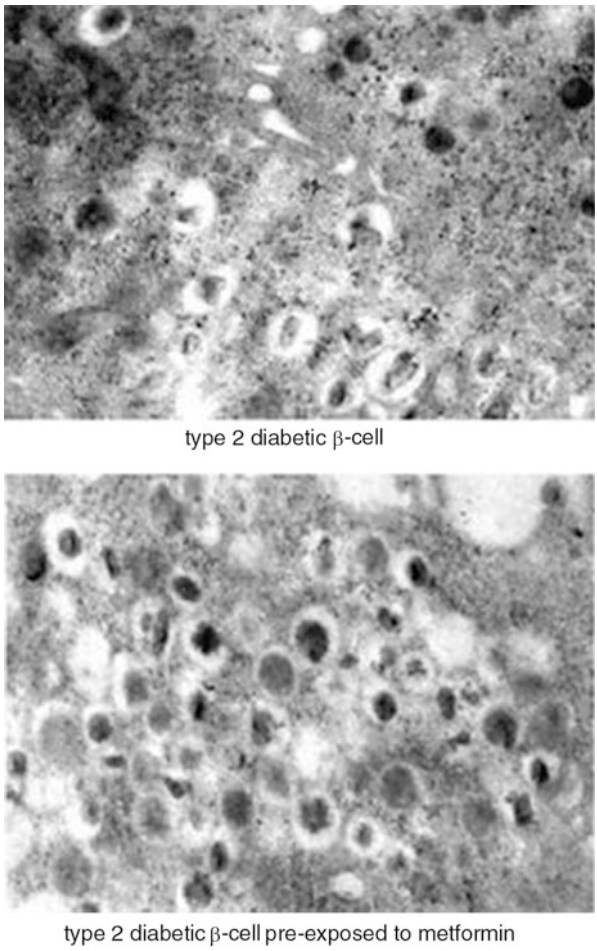

Fig. 5 The amount of insulin granules in type 2 diabetic β-cells increases following preexposure for 24 h with therapeutic concentration of metformin. Electron microscopy evaluation, magnification × 160,000 (Reproduced with modifications from Marchetti et al. (2004)

these molecules (GLP-1 in particular) on the β-cell (Drucker and Nauck 2006). In a recent study (Lupi et al. 2008), pancreatic islets were prepared from the pancreas of nondiabetic and type 2 diabetic donors and then incubated in the presence of 5.5 mmol/l glucose, with or without the addition of exendin-4 (a long-acting GLP-1 mimetic). Insulin secretion from the type 2 diabetic islets improved after incubation with exendin-4, which also induced a significantly higher expression of insulin, glucose transporter 2, glucokinase, and some β-cell regeneration and differentiation factors, including pancreas duodenum homeobox-1(Pdx-1).

Therefore, acting directly at the β-cell level to prevent damage or restore functional and survival competence is feasible in vitro. Strategies need to be developed to deliver the appropriate treatment to the β-cell in vivo, to be combined with therapies aiming to limit the negative impact on the islets of acquired conditions such as glucotoxicity and lipotoxicity (see above).

Table 1 Main defects of pancreatic β-cells in human type 2 diabetes

β-cell mass
Increased apoptosis
Not sufficient proliferation
Not sufficient neogenesis
β -cell function
Reduced glucose-stimulated insulin secretion
Blunted or absent early phase insulin secretion
Increased proinsulin/insulin ratio
Altered pulsatility of insulin release
Molecular features
Altered expression of genes involved in β-cell function and survival
Altered expression of proteins involved in β-cell function and survival
Increased production of reactive oxygen and nitrogen species

Conclusions

Pancreatic β-cells in type 2 diabetes have several defects (Table 1). Decreased β-cell mass is due to increased apoptosis not compensated for by adequate β-cell regeneration. Insulin secretion defects are more marked in response to glucose, suggesting that handling of this fuel by the β-cell is defective somewhere along the road leading to ATP production. These alterations are accompanied by several molecular defects, possibly due, at least in part, to genetic variations and acquired factors, which still need to be set in a more comprehensive picture. The observation that β-cell defects may be reversible supports the concept that β-cell dysfunction in human type 2 diabetes could not be relentless.

Acknowledgments Supported in part by the Italian Ministry of University and Research (PRIN2007–2008).

Cross-References

► (Dys)Regulation of Insulin Secretion by Macronutrients
► Apoptosis in Pancreatic β-Islet Cells in Type 1 and Type 2 Diabetes
► Islet Structure and Function in the GK Rat
► Microscopic Anatomy of the Human Islet of Langerhans

References

American Diabetes Association (2008) Diagnosis and classification of diabetes mellitus. Diabetes Care 31(Suppl 1):S55–S60

Anello M, Lupi R, Spampinato D, Piro S, Masini M, Boggi U, Del Prato S, Rabuazzo AM, Purrello F, Marchetti P (2005) Functional and morphological alterations of mitochondria in pancreatic β cells from type 2 diabetic patients. Diabetologia 48:282–289

Böni-Schnetzler M, Thorne J, Parnaud G, Marselli L, Ehses JA, Kerr-Conte J, Pattou F, Halban PA, Weir GC, Donath MY (2008) Increased interleukin IL-1β messenger ribonucleic acid expression in β-cells of individuals with type 2 diabetes and regulation of IL-1β inhuman islets by glucose and autostimulation. J Clin Endocrinol Metab 93:4065–4074

Brun T, Hu He KH, Lupi R, Boehm B, Wojtusciszyn A, Sauter N, Donath M, Marchetti P, Maedler K, Gauthier BR (2008) The diabetes-linked transcription factor Pax4 is expressed in human pancreatic islets and is activated by mitogens and GLP-1. Hum Mol Genet 17:478–489

Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC (2003) β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 52:102–110

Cauchi S, Froguel P (2008) TCF7L2 genetic defect and type 2 diabetes. Curr Diabetes Rep 8:149–155

Clark A, Wells CA, Buley ID et al (1988) Islet amyloid, increased α-cells, reduced β-cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. Diabetes Res 9:151–159

Del Guerra S, Lupi R, Marselli L, Masini M, Bugliani M, Sbrana S, Torri S, Pollera M, Boggi U, Mosca F, Del Prato S, Marchetti P (2005) Functional and molecular defects of pancreatic islets inhuman type 2 diabetes. Diabetes 54:727–735

Deng S, Vatamaniuk M, Huang X, Doliba N, Lian MM, Frank A et al (2004) Structural and functional abnormalities in the islets isolated from type 2 diabetic subjects. Diabetes 53:624–632

Drucker DJ, Nauck MA (2006) The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet 368:1696–1705

Ehses JA, Boni-Schnetzler M, Faulenbach M, Donath MY (2008) Macrophages, cytokines and β cell death in type 2 diabetes. Biochem Soc Trans 36:340–342

Federici M, Hribal ML, Ranalli M, Marselli L, Porzio O, Lauro D, Borboni P, Lauro R, Marchetti P, Melino G, Sesti G (2001) The common Arg972 polymorphism in insulin receptorsubstrate-1 causes apoptosis of human pancreatic islets. FASEB J 15:22–24

Fernandez-Alvarez J, Conget I, Rasschaert J, Sener A, Gomis R, Malaisse WJ (1994) Enzymatic, metabolic and secretory patterns in human islets of type 2 (non-insulin-dependent) diabetic patients. Diabetologia 37:177–181

Ferrannini E, Mari A (2004) β-cell function and its relation to insulin action in humans: a critical appraisal. Diabetologia 47:943–956

Groop L, Lyssenko V (2008) Genes and type 2 diabetes mellitus. Curr Diabetes Rep 8:192–197

Gunton JE, Kulkarni RN, Yim S, Okada T, Hawthorne WJ, Tseng YH, Roberson RS, Ricordi C, O'Connell PJ, Gonzalez FJ, Kahn CR (2005) Loss of ARNT/HIF1β mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. Cell 122:337–349

Hattersley AT, Pearson ER (2006) Minireview: pharmacogenetics and beyond: the interaction of therapeutic response, β-cell physiology, and genetics in diabetes. Endocrinology 147:2657–2663

Jafar-Mohammadi B, McCarthy MI (2008) Genetics of type 2 diabetes mellitus and obesity-a review. Ann Med 40:2–10

Kahn SE (2003) The relative contribution of insulin resistance and β-cell dysfunction to the pathophysiology of type 2 diabetes. Diabetologia 46:3–19

Kahn SE, Carr DB, Faulenbach MV, Utzschneider KM (2008) An examination of β-cell function measures and their potential use for estimating β-cell mass. Diabetes Obes Metab 10(Suppl 4):63–76

Kumar AF, Gruessner RW, Seaquist ER (2008) Risk of glucose intolerance and diabetes in hemipancreatectomized donors selected for normal preoperative glucose metabolism. Diabetes Care 31:1639–1643

Lupi R, Del Guerra S, Mancarella R, Novelli M, Valgimigli L, Pedulli GF, Paolini M, Soleti A, Filipponi F, Mosca F, Boggi U, Del Prato S, Masiello P, Marchetti P (2007) Insulin secretion defects of human type 2 diabetic islets are corrected in vitro by a new reactive oxygen species scavenger. Diabetes Metab 33:340–345

Lupi R, Mancarella R, Del Guerra S (2008) Effects of exendin-4 on islets Bugliani M, Del PratoS, Boggi U, Mosca F, Filipponi F, Marchetti P. from type 2 diabetes patients. Diabetes Obes Metab 10:515–519

Lyssenko V, Lupi R, Marchetti P, Del Guerra S, Orho-Melander M, Almgren P, Sjogren M, Ling C, Eriksson KF, Lethagen UL, Mancarella R, Berglund G, Tuomi T, Nilsson P, Del Prato S, Groop L (2007) Mechanisms by which common variants in the TCF7L2 gene increase risk of type2 diabetes. J Clin Invest 117:2155–2163

Marchetti P, Lupi R, Federici M, Marselli L, Masini M, Boggi U, Del Guerra S, Patane G, Piro S, Anello M, Bergamini E, Purrello F, Lauro R, Mosca F, Sesti G, Del Prato S (2002) Insulin secretory function is impaired in isolated human islets carrying the Gly(972) → Arg IRS-1polymorphism. Diabetes 51:1419–1424

Marchetti P, Del Guerra S, Marselli L, Lupi R, Masini M, Pollera M (2004) Pancreatic islets from type 2 diabetic patients have functional defects and increased apoptosis that are ameliorated by metformin. J Clin Endocrinol Metab 89:5535–5541

Marchetti P, Bugliani M, Lupi R, Marselli L, Masini M, Boggi U, Filipponi F, Weir GC, Eizirik DL, Cnop M (2007) The endoplasmic reticulum in pancreatic β cells of type 2 diabetes patients. Diabetologia 50:2486–2494

Marchetti P, Dotta F, Lauro D, Purrello F (2008) An overview of pancreatic β-cell defects inhuman type 2 diabetes: implications for treatment. Regul Pept 146:4–11

Marselli L, Sgroi DC, Thorne J, Dahiya S, Torri S, Omer A, Del Prato S, Towia L, Out HH, Sharma A, Bonner-Weir S, Marchetti P, Weir GC (2007) Evidence of inflammatory markers in β-cells of type 2 diabetic subjects. Diabetologia 50(Suppl 1):S178

Meier JJ (2008) β cell mass in diabetes: a realistic therapeutic target? Diabetologia 51:703–713

Nyblom HK, Bugliani M, Marchetti P, Bergsten P (2007) Islet protein expression from type 2 diabetic donors correlating with impaired secretory response. Diabetologia 50(Suppl 1):S178

Ostenson CG, Gaisano H, Sheu L, Tibell A, Bartfai T (2006) Impaired gene and protein expression of exocytotic soluble N-ethylmaleimide attachment protein receptor complex proteins in pancreatic islets of type 2 diabetic patients. Diabetes 55:435–440

Owen KR, McCarthy MI (2007) Genetics of type 2 diabetes. Curr Opin Genet Dev 17:239–244

Parikh H, Groop L (2004) Candidate genes for type 2 diabetes. Rev Endocr Metab Disord 5:151–176

Poitout V, Robertson RP (2002) Minireview: secondary β-cell failure in type 2 diabetes – a convergence of glucotoxicity and lipotoxicity. Endocrinology 143:339–342

Porte D Jr (1991) Banting lecture 1990. β-cells in type II diabetes mellitus. Diabetes 40:166–180

Rahier J, Goebbels RM, Henquin JC (1983) Cellular composition of the human diabetic pancreas. Diabetologia 24:366–371

Rahier J, Guiot Y, Goebbels RM, Sempoux C, Henquin JC (2008) Pancreatic β-cell mass in European subjects with type 2 diabetes. Diabetes Obes Metab 10(Suppl 4):32–42

Saito K, Takahashi T, Yaginuma N, Iwama N (1978) Islet morphometry in the diabetic pancreas of man. Tohoku J Exp Med 125:185–197

Saito K, Yaginuma N, Takahashi T (1979) Differential volumetry of A, B and D cells in the pancreatic islets of diabetic and non diabetic subject. Tohoku J Exp Med 129:273–283

Sakuraba H, Mizukami H, Yagihashi N, Wada R, Hanyu C, Yagihashi S (2002) Reduced β cell mass and expression of oxidative stress related DNA damage in the islets of Japanese type 2diabetic patients. Diabetologia 45:85–96

Sesti G, Laratta E, Cardellini M, Andreozzi F, Del Guerra S, Irace C, Gnasso A, Grupillo M, Lauro R, Hribal ML, Perticone F, Marchetti P (2006) The E23K variant of KCNJ11 encoding the pancreatic β-cell K_{ATP} channel subunits Kir6.2 is associated with an increased risk of secondary failure to sulfonylurea in patients with type 2 diabetes. J Clin Endocrinol Metab 91:2334–2339

Shu L, Sauter NS, Schulthess FT, Matveyenko AV, Oberholzer J, Maedler K (2008) Transcription factor 7-like 2 regulates β-cell survival and function in human pancreatic islets. Diabetes 57:645–653

Stefan Y, Orci L, Malaisse-Lagae F et al (1982) Quantitation of endocrine cell content in the pancreas of nondiabetic and diabetic humans. Diabetes 31:694–700

Stumvoll M, Goldstein BJ, van Haeften TW (2005) Type 2 diabetes: principles of pathogenesis and therapy. Lancet 365:1333–13

Vaxillaire M, Froguel P (2008) Monogenic diabetes in the young, pharmacogenetics and relevance to multifactorial forms of type 2 diabetes. Endocr Rev 29:254–264

Wajchenberg BL (2007) β-cell failure in diabetes and preservation by clinical treatment. Endocr Rev 28:187–218

Welsh N, Cnop M, Kharroubi I, Bugliani M, Lupi R, Marchetti P, Eizirik DL (2005) Is there a role for locally produced interleukin-1 in the deleterious effects of high glucose or the type 2 diabetes milieu to human pancreatic islets? Diabetes 54:3238–3244

Westermark P, Wilander E (1978) The influence of amyloid deposits on the islet volume in maturity onset diabetes mellitus. Diabetologia 15:417–421

Yoon KH, Ko SH, Cho JH et al (2003) Selective β-cell loss and α-cell expansion in patients with type 2 diabetes in Korea. J Clin Endocrinol Metab 88:2300–2308

Pancreatic β Cells in Metabolic Syndrome 30

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Contents

Abstract

Obesity is considered a major public health problem worldwide. Metabolic syndrome is a cluster of signs that increases the risk of developing cardiovascular disease and type 2 diabetes mellitus (T2DM). The main characteristics of metabolic syndrome are central obesity, dyslipidemia, hypertension, hyperinsulinemia, and insulin resistance. It is clear that the progression of

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_27,

metabolic syndrome to T2DM depends on the environment and the genetic traits of individuals.

Pancreatic β cells are fundamental for nutrient homeostasis. They are the unique cells in the organisms that produce and secrete insulin. The actions of insulin are anabolic, stimulating glucose entry to adipose tissue and skeletal muscle, and promoting nutrient storage.

However, insulin receptors are present in every mammalian cell, and not all the physiological effects of this hormone are completely understood. Nutrients, other hormones, and neurotransmitters regulate insulin secretion, and the main ones will be discussed in this chapter. We will summarize how metabolic changes modify β-cell physiology and the actions of insulin in metabolic syndrome, eventually leading to the development of T2DM.

Keywords

β-cell exhaustion • Insulin resistance • Obesity • Ion channels • Cytokines • β-cell dysfunction • Insulin hypersecretion • Hyperinsulinemia

Glossary

ACh	Acetylcholine
acyl-CoA	Acyl coenzyme A
ADP	Adenosine diphosphate
AMPK	5′ adenosine monophosphate-activated protein kinase
AR	Adrenoreceptor
ATP	Adenosine triphosphate
BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
CAP	Cbl-associated protein
Cbl	Casitas B-lineage lymphoma proto-oncogene
Cytokine R	R Cytokine receptor
DAG	Diacylglycerol
DPP-4	Enzyme dipeptidylpeptidase-4
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
GK	Glucokinase
GLP-1	Glucagon-like peptide-1
GLUT2	Glucose transporters type 2
GSIS	Glucose-stimulated insulin secretion
IGF1R	Insulin-like growth factor 1 receptor
IKK	Kinase of IKB (inhibitor of KB)
IL-6	Interleukin-6
IR	Insulin receptor
IRS	Insulin receptor substrate

JAKs	Kinases of the Janus family
JNK	c-Jun N-terminal kinase
K_{ATP}	ATP-sensitive potassium channel
MAPK	Mitogen-activated protein kinase
MODY	Maturity onset diabetes of the young
MS	Metabolic syndrome
mTOR	Mammalian target of rapamycin
NEFAs	Nonesterified fatty acids
NGF	Nerve growth factor
PDK	Phosphoinositide-dependent kinase
PHHI	Persistent hypoglycemic hyperinsulinemia of the infancy
PI3K	Phosphoinositol-3 kinase
PKA	Protein kinase A
PKB/Akt	Protein kinase B
PKC	Protein kinase C
PPAR gamma	Peroxisome-proliferation-activated receptor gamma
PTPs	Protein tyrosine phosphatases
Ras	Rat sarcoma protein family
RBP4	Retinol binding protein-4
ROS	Reactive oxygen species
SNARE	Soluble NSF attachment protein receptor
SOCS	Suppressor of cytokine signaling
SREBP	Sterol regulatory element-binding protein
T2DM	Type 2 diabetes mellitus
TLR4	Toll-like receptor 4
TNFa	Tumor necrosis factor α
TNFR	Tumor necrosis factor receptor
TrkA	Tyrosine kinase receptor A
TRP	Transient receptor channels
WAT	White adipose tissue

Introduction

Insulin secretion is fundamental for nutrient homeostasis. Insulin is an anabolic hormone that regulates the storage of carbohydrates, lipids, and proteins in the liver, muscle, and adipose tissue but also glucose uptake in the last two. Although insulin receptor is ubiquitously expressed in mammalian cells, the physiological effects of this hormone are not completely understood in every tissue. The autoimmune destruction of pancreatic β cells leads to a chronic insulin deprivation, a pathologic condition called type 1 diabetes mellitus. On the other hand, type 2 diabetes mellitus (T2DM) results from a combination of insulin resistance and an impaired insulin secretion, which is not enough to maintain euglycemia.

The metabolic syndrome (MS) is a cluster of signs including central obesity, dyslipidemia, hypertension, hyperinsulinemia, and insulin resistance that increase the risk to develop cardiovascular disease and diabetes mellitus (Hunt et al. 2004), reviewed by Larqué et al. (2011). The progression from MS to T2DM depends on individual genetic traits.

According to the World Health Organization (2013), diabetes is one of the leading causes of death worldwide. It is clear that some risk factors like obesity and metabolic syndrome are associated with T2DM; for instance, in North America 60 % of the population exhibits overweight or obesity. In 2010, it has been estimated that 79 million people around the world presented a prediabetic diagnosis. It is mandatory to understand how obesity and MS contribute to β-cell exhaustion and finally to diabetes. Here, we review the β-cell physiology and the physiopathological changes in MS.

Insulin Secretion

Pancreatic β-cells may be considered fuel sensors. They are continuously monitoring and responding to changes in the concentration of circulating nutrients and thus regulating their homeostasis. Insulin secretion is stimulated by several secretagogues like metabolized nutrients, neurotransmitters, hormones, and drugs that bind to membrane receptors (Fig. 1).

Glucose-Stimulated Insulin Secretion (GSIS)

The best characterized mechanism of insulin secretion is the stimulated by glucose (Fig. 1) (see also chapter "► (Dys)Regulation of Insulin Secretion by Macronutrients"). When its level rises, glucose enters the β cell mainly via glucose transporters: GLUT2 in rodents (SLC2A2) or GLUT1 in humans (SLC2A1). This is followed by glucose phosphorylation catalyzed by glucokinase (GK) (Prentki and Matschinsky 1987; Prentki et al. 2013). The glycolytic flux is regulated by a combination of the glucose flux and GK activity. Studies of GK mutations in animals, as well as in some human conditions, have led to similar conclusions. Mutations that increase GK activity promote persistent hypoglycemic hyperinsulinemia of the infancy (PHHI). A reduction in the GK catalytic activity has been documented in patients with maturity onset diabetes of the youth (MODY) and type 2 diabetes (Huypens et al. 2012). Excellent reviews on the importance of glucose metabolism have been published elsewhere, and we will not review them in this chapter (Jensen et al. 2008; Nolan and Prentki 2008).

It is well established that GSIS is dependent on β-cell electrical activity (Drews et al. 2010; Henquin and Meissner 1984) (see also chapter "► Electrophysiology of Islet Cells"). At glucose concentrations below 3 mM, the cell is electrically silent with a resting membrane potential of about −70 mV. Rising external glucose produces a slow depolarization, which is dependent on the sugar concentration.

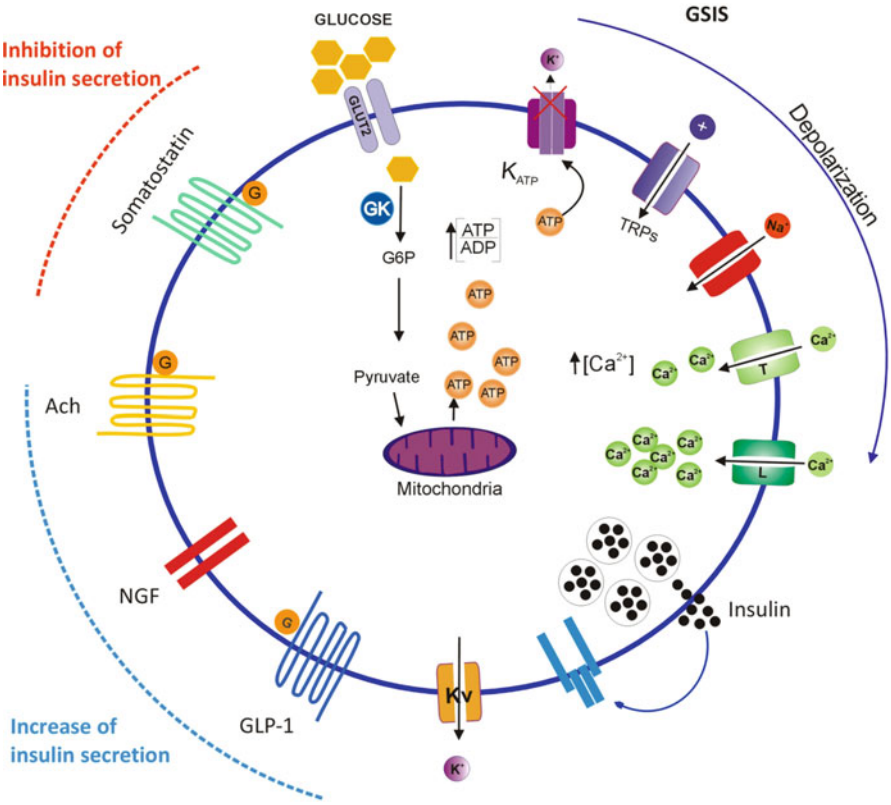

Fig. 1 Insulin secretion in pancreatic β-cells. Glucose-stimulated insulin secretion (GSIS) depends on the activation of different types of ionic channels placed on the cell membrane. Insulin secretion is regulated by other hormones, neurotransmitters, growth factors and incretins

When plasma glucose rises, the rate of metabolism by the cell is stimulated. Therefore, the intracellular ATP/ADP ratio increases, which leads to the closure of K_{ATP} channels and membrane depolarization (see also chapter "► ATP-Sensitive Potassium Channels in Health and Disease"). This step is also dependent on the activity of TRP channels, which are nonselective cationic channels. Membrane depolarization results in the activation of voltage-dependent sodium and low-threshold calcium channels, which accentuate it. Consequently, high-threshold voltage-dependent calcium channels (mainly L-type channels) are activated and action potentials start from a plateau potential. The Ca^{2+} influx through these channels increases the intracellular Ca^{2+} concentration, which triggers insulin exocytosis from secretory granules (see also chapter "► Exocytosis in Islet β-Cells"; Hiriart and Aguilar-Bryan 2008).

Delayed rectifier voltage-dependent K^+ channels and high-conductance calcium- dependent K^+ channels (K_{Ca}) repolarize the membrane to the resting potential (Drews et al. 2010). The process of ATP synthesis, closure of K_{ATP}

channels, and raising of intracellular calcium levels is repeated in subsequent cycles (see also chapter "► Electrical, Calcium, and Metabolic Oscillations in PancreaticIslets").

The membrane potential of β cell begins to oscillate at a suprathreshold glucose concentration. It is known that lasting of both, the depolarized burst phases with superimposed spikes and the silent hyperpolarized inter-burst phases are glucose dependent. As the glucose concentration increases, burst phases are longer and inter-burst phases are shortened until continuous activity is achieved at a glucose concentration above 25 mM (Drews et al. 2010).

It is well established that triggering Ca^{2+} signals is essential for both first and second phases of GSIS. However, glucose also induces insulin secretion by an amplifying pathway, sensitizing the exocytotic machinery to cytosolic calcium concentration changes, independently of K_{ATP} and membrane potential. This pathway is strongly dependent on metabolism and is only mimicked by other metabolizable secretagogues (see also chapter "► Electrical, Calcium, and Metabolic Oscillations in Pancreatic Islets"; Henquin 2011).

It is discussed in the literature that amplifying signals produced by glucose are only involved in the second phase of insulin secretion. However, dynamic studies of insulin secretion in mouse islets with various glucose concentrations point to a role also in the first phase that could account for no less than 50 % of the response. The rate of insulin secretion relies on the intracellular Ca^{2+} signals (see also chapter "► Calcium Signaling in the Islets"), while the magnitude of insulin pulses increases with the glucose concentration, reflecting the participation of the amplifying pathway (Henquin 2011).

Furthermore, the distribution of insulin functional pools must be well controlled to guarantee an optimal physiological response to glucose (Rorsman and Renstrom 2003). The initial exocytotic response (see also chapter "► Exocytosis in Islet β-Cells;") may result from stimulus that increases intracellular Ca^{2+}. However, sustained secretion phase can only be elicited by metabolizable secretagogues as glucose, which also promotes the translocation of granules to the membrane, from a reservoir pool, followed by docking and priming (Ohara-Imaizumi et al. 2007). Proteins dependent on GTP/GDP cycling play an important role in vesicle recruitment in this second phase (Henquin 2000; Straub et al. 2004). The fusion of an exocytotic vesicle with the plasma membrane is mediated by SNARE proteins (Soluble NSF attachment protein receptor).

The SNARE core complex includes the plasma membrane proteins syntaxin and SNAP-23/25 and the vesicular protein VAMP (synaptobrevin). This complex is regulated by the Sec1/Munc18 (SM) family of proteins, which selectively bind with high affinity to their syntaxin isoforms (Jewell et al. 2010). Other proteins, such as synaptotagmin III, V, VII, VIII, and IX, act as Ca^{2+} sensors for exocytosis in β cells. The interaction of various SNARE proteins with the voltage-dependent calcium channels (VDCCs) mediates a tight coupling of Ca^{2+} entry and exocytosis of insulin vesicles (reviewed by MacDonald et al. 2005). In summary, glucose-sensing mechanisms are important for amplifying and maintaining insulin secretion.

Amino Acid-Stimulated Insulin Secretion (AASIS)

The role of amino acids in insulin secretion has long been recognized, but their mechanisms of action are still unclear (see also chapter "► (Dys)Regulation of Insulin Secretion by Macronutrients"). Individually, amino acids, such as alanine, asparagine, glycine, glutamate, phenylalanine, and tryptophan, or mixtures of all amino acids are poor secretagogues, and a relatively small number of them promote or synergistically enhance GSIS from β cells. Leucine is an exception, since its stimulatory actions in perfused rat pancreas are eradicated in the presence of glucose (Zhang and Li 2013).

Amino acids that are cotransported with Na^+ depolarize the membrane, causing Ca^{2+} influx and triggering insulin secretion (Newsholme et al. 2007). Alanine and leucine also increase ATP production, promoting the K_{ATP} channel closure.

In normal humans, amino acids also potentiate the GSIS, which is the basis for the application of combinatory therapies of both secretagogues in order to achieve a maximal insulin secretory responsiveness (Li et al. 2012). Furthermore, an important role for amino acids in insulin secretion comes from recent discoveries related to clinical disorders associated with amino acid-sensitive hypoglycemia in children with congenital hyperinsulinism (Zhang and Li 2013).

Glutamine exerts amplifying effects on GSIS, although the precise mechanism is still unknown. Studies in SUR1-KO mice suggested that its effect is mediated by the cAMP-dependent pathways, analogous to the GLP-1 receptor (Zhang and Li 2013).

During GSIS the intracellular levels of many amino acids change, probably via transamination reactions between some amino acids and the intermediaries of the tricarboxylic acid cycle. For example, the intracellular aspartate is reduced, while alanine increases (Li et al. 2012).

Nonesterified Fatty Acids (NEFAs)

It has been demonstrated that normal blood levels of NEFAs are necessary to maintain GSIS in β cells (see also chapter "► (Dys)Regulation of Insulin Secretion by Macronutrients"; Stein et al. 1997). The effects of NEFAs are time and concentration dependent. A transient elevation in the NEFA levels potentiates GSIS, while a long-term increase impairs this process (reviewed by Graciano et al. 2011; Nolan et al. 2006).

Several mechanisms had been proposed to explain the physiological role of NEFAs in GSIS. During fasting state, circulating NEFAs are oxidized, increasing ATP production, β-cell depolarization, and insulin secretion. On the fed state, β-cell NEFAs, associated to the glucose metabolism, regulate the mitochondrial anaplerotic and cataplerotic pathways and thus insulin secretion. Moreover, when glucose levels rise, NEFAs oxidation decreases, leading to long-chain acyl-CoA accumulation which facilitates the fusion of insulin vesicles to the β-cell membrane. In addition, long-chain fatty acids may be esterified increasing intracellular

levels of complex lipids such as triglycerides, DAG, or phospholipids. GPR40 is a G protein-coupled receptor activated by NEFAs that amplifies insulin exocytosis by DAG, PKC, IP3, increased calcium influx, and reduced outward voltage-gated potassium channel conductance. Finally, β-oxidation of NEFAs generates electrons that activate the respiratory chain and promotes mitochondrial production of ROS through complex I (NADH dehydrogenase) and complex III (cytochrome bc1), which are the major producing sites of superoxide, also enhancing insulin secretion (reviewed by Graciano et al. 2011; Nolan et al. 2006).

It has been demonstrated that long-term high blood levels of NEFAs impair insulin secretion. In MS high intracellular levels of NEFAs increase the mitochondrial transmembrane potential and shift the redox state of complex III components toward reduced values favoring superoxide production. In addition, NEFAs may inhibit electron transport by interacting with components of the respiratory chain and promote superoxide production by transferring electrons to complex I. Furthermore, peroxisome metabolism of long-chain fatty acids leads to the generation of hydrogen peroxide due to the O_2 participation as the electron acceptor (reviewed by Graciano et al. 2011; Nolan et al. 2006).

Pancreatic Hormones

Glucagon

Glucagon is secreted by pancreatic α-cells (see also chapter "▶ Physiological and Pathophysiological Control of Glucagon Secretion by Pancreatic α-Cells"), which comprise nearly 15 % of islet volume (reviewed by Taborsky 2010). In rodents, the α cells are localized in the periphery of the islet together with delta cells, while the β cells are concentrated at the core of the islet (Cabrera et al. 2006). Blood flow direction inside the islet impacts the regulation of the different intraislet hormones. Two blood flow pattern have been proposed in mice: (a) the most accepted in the literature, in which the direction is inner to outer, allowing β-cell hormones to reach and regulate α and delta cells physiology, and (b) top to bottom, in which the blood perfuses from one side to the other (Nyman et al. 2008). The endocrine cells in human islets have a distinct distribution, where cells appear to be randomly distributed within the islet. In human islets, 70 % of β cells are in contact with α and delta cells (Gromada et al. 2007). According to cellular arrangement and blood flow, it has been proposed that glucagon can increase insulin secretion by a paracrine mechanism and insulin and gamma-aminobutyric acid (GABA) secreted by β cells may decrease glucagon secretion (Bagger et al. 2011).

Glucagon receptor (GlucR) is expressed in the α and β cells. GlucRs are proteins with seven transmembrane domains, coupled to G proteins (Ahren 2009), adenylyl cyclase (AC), cAMP production, and protein kinase A (PKA), and finally promote calcium-dependent exocytosis (reviewed by Gromada et al. 2007; Koh et al. 2012).

Somatostatin

Somatostatin is secreted by pancreatic δ-cells and inhibits insulin and glucagon secretion. There are five types of somatostatin receptors (SSTRs), which are tissue specific. SSTRs are G protein-coupled receptors with seven transmembrane domains. Human and rat β-cells express type 1 and 5 SSTRs, while human, mouse, and rat α-cells express type 2 SSTRs (Koh et al. 2012).

The inhibitory effectors of somatostatin signaling are potassium channels, promoting membrane potential hyperpolarization and decreasing calcium channel activity, which mediates exocytosis. Moreover somatostatin may inhibit adenylyl cyclase activity resulting in a reduction of intracellular cAMP levels and a decreased activity of PKA. Furthermore, it has been proposed that in high glucose concentrations, somatostatin may modulate insulin secretion by interacting with cholinergic receptors (Youos 2011).

Autocrine Effects of Insulin

β-cell secretion is also regulated by hormones, neurotransmitters, and other proteins that are secreted by themselves or their neighbors causing autocrine and paracrine effects respectively. The autocrine effects of insulin on β cells through insulin autoreceptors are a matter of controversy. However, it has been shown that autocrine regulation of insulin release plays an important role in apoptosis, proliferation, and gene transcription (see also chapter "▶ IGF-1 and Insulin Receptor Signalling in Insulin-Secreting Cells: From Function to Survival"; reviewed by (Leibiger et al. 2008; Rhodes et al. 2013).

Amylin

Amylin, also known as islet amyloid polypeptide protein (IAPP), is another small protein, member of the calcitonin family peptides that is synthesized and co-secreted with insulin by β cells and delta cells. In the literature, the possible role of amylin in insulin secretion is controversial because some articles affirm that it increases insulin secretion, while others describe no effects or significant inhibition of GSIS by physiological doses of this hormone. Other extrapancreatic effects of amylin described throughout the literature are suppression of glucagon secretion, inhibition of glucose release from the liver, decreasing gastric emptying, and stimulating satiety (reviewed by Gebre-Medhin et al. 2000; Pillay and Govender 2013).

Human IAPP aggregates and forms toxic fibrils in T2DM; it is considered that the latter may cause islet dysfunction and apoptosis of β cells (Lorenzo et al. 1994). Rodents under experimental diabetes express more amylin than insulin; however the structure of amylin in these animals is different, and it does not form fibrils or plaques (Chakraborty et al. 2013).

Amylin receptors are formed by a complex of calcitonin receptor and a receptor-modifying protein (RAMP). The affinity of amylin to the receptor is higher in the complex form. Moreover, different subtypes of receptors are formed by splice variants of the calcitonin receptor and different RAMPs, although these aspects have not been fully characterized in β cells (Abedini and Schmidt 2013).

When large amounts of insulin are secreted, there is also an elevated amylin secretion, which eventually could form aggregates. Important information on the impact of amylin aggregation come from transgenic animals for human IAPP, for example, it has been observed that high carbohydrates or fat diets promote amyloid fibril formation in human-amylin transgenic mice (Pillay and Govender 2013; Westermark et al. 2011).

The complete role of amylin in the pathogenesis of T2DM is not yet resolved, but considering its possible peripheral roles, it is now used as a complementary treatment in T1DM.

Gamma-aminobutyric Acid (GABA)

Pancreatic β cells produce and secrete the neurotransmitter GABA. It could be present in the cytoplasm, contained in synaptic-like microvesicles, or stored in a subpopulation of insulin-containing granules. In addition, β cells express both $GABA_A$ and $GABA_B$ receptors, indicating a probable autocrine role of the neurotransmitter (Reetz et al. 1991). It has been demonstrated that glucose induces GABA secretion in a Ca^{2+}-dependent manner (Smismans et al. 1997). However, some evidence suggests that GABA could also be secreted through a non-vesicular and glucose-independent pathway (Braun et al. 2004). The G-protein-coupled $GABA_B$ receptor has been involved in the inhibition of insulin secretion by decreasing the exocytotic process probably through the modulation of voltage-gated calcium and G-coupled inwardly-rectifying potassium channels. Finally, the activation of the ligand-gated Cl^- channel $GABA_A$ receptor may lead to membrane depolarization thus promoting insulin secretion (Braun et al. 2010; Dong et al. 2006).

Nerve Growth Factor

Nerve growth factor (NGF), initially identified as a soluble factor enhancing growth and differentiation of sympathetic ganglia, exerts many other effects on the physiology of neuronal and nonneuronal tissues (Aloe 2011). Pancreatic β cells secrete NGF (Rosenbaum et al. 1998) and also express both high- (TrkA) and low-affinity (p75) receptors for this neurotrophin (Polak et al. 1993). Some of the effects of this neurotrophin on insulin-secreting cells include morphological changes (i.e., neurite-like processes), improved survival, and increased excitability (Hiriart et al. 2001).

The regulatory role of NGF on ion channels that participate in insulin secretion has been extensively studied by our research group. Today, it is well known that a long-term treatment with NGF increases the synthesis of voltage-sensitive Na^+ channels and consequently insulin secretion (Vidaltamayo et al. 2002). The currents through L-type Ca^{2+} channels are also upregulated by NGF due to an increase in the density of ion channels at the plasma membrane and their activity through a direct modulation (Rosenbaum et al. 2001, 2002). The latter supports a role of NGF as an autocrine modulator of β-cell physiology. Nerve growth factor improves the responsiveness of immature β-cells from neonate rats increasing the translocation of voltage-gated Ca^{2+} channels to the plasma membrane (Navarro-Tableros et al. 2007). Furthermore, it has been suggested that NGF guides the vasculature

and sympathetic innervation of pancreatic islets during development (Cabrera-Vasquez et al. 2009). Moreover, NGF deprivation causes a reduction in insulin levels and secretion, as well as an exacerbated apoptosis, which are more prominent in β cells from hyperglycemic rats (Gezginci-Oktayoglu et al. 2012). Indeed, NGF shapes the endocrine physiopathology from islet architecture to the ultrastructure of single β cells.

Interestingly, β cells increase transcription and secretion of NGF after streptozotocin injury, probably as a protective strategy that counteracts apoptosis (Larrieta et al. 2006). Circulating NGF is also associated with diabetes mellitus and metabolic syndrome. It is known that NGF levels are reduced in patients with diabetic neuropathy and correlated with an impairment of motor nerve conduction velocity (Faradji and Sotelo 1990).

NGF participates in the etiology of metabolic syndrome and neuroendocrine disorders (Chaldakov et al. 2010; Chaldakov et al. 2009). In fact, during early stages of MS, hypersecretion of NGF causes alterations of neurotransmitters levels in the brain and leads to vegetodystonia. In the early stages, the vagal tone increases, which also enhances insulin secretion. Moreover, NGF also causes an increased brain expression of the orexigenic neuropeptide Y, as well as the hyperactivation of the hypothalamic–pituitary–adrenal axis that contributes to obesity. The resulting long-term hormonal imbalance and the exhaustion of compensatory mechanisms finally establish hypo-neurotrophinemia in the generalized MS (Hristova and Aloe 2006).

Incretins: A Crosstalk Between the gut and the Endocrine Pancreas

Some intestinal hormones cause trophic effects in β cells and link insulin secretion to nutrient ingestion. Glucagon-like peptide-1 (GLP-1) and the glucose insulinotropic polypeptide (GIP) are named incretins because they account for the higher insulin secretion after an oral challenge of glucose, compared with an intravenous bolus of glucose. Moreover, both incretins decrease apoptosis and potentiate insulin release and β-cell proliferation (Phillips and Prins 2011).

The way GLP-1 affects insulin release involves its binding to a membrane receptor and PKA activation, which in turn increases β-cell excitability and Ca^{2+} entry, in part through cationic nonselective currents (Togashi et al. 2006). Interestingly, application of the GLP-1 mimetic exendin-4 prevents β-cell loss and augments NGF and p75 expression in islets from streptozotocin-treated rodents (Gezginci-Oktayoglu and Bolkent 2009). GLP-1 could also induce cardio- and neuroprotective effects as well as weight loss, which contribute to the beneficial effects in therapeutics of GLP-1 analogs and also the inhibitors of its degrading enzyme dipeptidylpeptidase-4 (DPP-4) (Phillips and Prins 2011).

It is worth to mention that overexpression of GIP in a transgenic mouse model ameliorates diet-induced obesity while improving glucose tolerance and insulin sensitivity (Kim et al. 2012). Moreover, administration of a GLP-1-derived nonapeptide showed similar effects on mice fed with a high-fat diet, indicating its

potential in the treatment of MS (Tomas et al. 2011). Recently, it has been suggested that GLP-1 could physiologically modulate insulin secretion by activating the pancreatic vagal innervation, because vagotomized mice show less insulin secretion in response to intraportal administration of GLP-1 with respect to a control group of animals (Nishizawa et al. 2013).

Controversial results are reported regarding the mechanisms of incretin deregulation in diabetes mellitus. Apparently, an impairment of β-cell response to incretins is more likely than a reduced secretion of these hormones (Ahren 2012). It has been shown that exenatide, a GLP-1 receptor agonist, decreases the postprandial glucose and promotes insulin secretion with a higher efficacy than sitagliptin (a DPP-4 inhibitor) in patients with T2DM (DeFronzo et al. 2008). Interestingly, patients with MS manifested a higher improvement of their metabolic status when treated with exenatide versus those treated with DPP-4 inhibitors, overcoming the GLP-1 resistance (Fadini et al. 2011).

Neurotransmitters

The pancreas is richly innervated by both parasympathetic and sympathetic branches of the autonomic nervous system. These fibers do not form classic synapses with endocrine cells but have release sites near the islet cells (reviewed by Osundiji and Evans 2013). The overall effect of parasympathetic stimulation is an increase in pancreatic insulin release, whereas the net effect of sympathetic nerve stimulation is to lower the plasma insulin concentration (Rodriguez-Diaz and Caicedo 2013).

Sympathetic nerve stimulation is carried out by adrenaline and noradrenaline. The presence of both α2- and β2-adrenergic receptors (AR) have been reported on pancreatic islet cells (Ullrich and Wollheim 1985), and the selective activation of α2- and β-receptors results in inhibition and stimulation of insulin secretion, respectively.

The predominant physiological effect on insulin secretion of the sympathetic system is inhibitory, due to activation of potassium channels, which hyperpolarizes the membrane; another target is the inhibition of adenylyl cyclases activity, preventing the stimulation of insulin release by cAMP, or to the actions of the G protein β-γ subunits, blocking the interaction of the calcium sensor synaptotagmin with the proteins involved in exocytosis (SNAREs) (Straub and Sharp 2012).

The blockade of α2-adrenoceptors with specific antagonists (i.e., phentolamine) (Ahren and Lundquist 1981), silencing of receptor expression, or blockade of downstream effectors reverses this effect. Mice lacking α2A- or α2C-AR show hyperinsulinemia, reduced blood glucose levels, and improved glucose tolerance (Ruohonen et al. 2012). A recent study also supported the role of the α2A-AR in the regulation of β-cell function and glucose homeostasis. In agreement with the above, overexpression of α2-AR in diabetic rats causes impaired insulin-granule membrane docking and less GSIS in β cells (Rosengren et al. 2010).

On the other hand, acetylcholine (ACh) release by parasympathetic innervation stimulates insulin secretion by activating muscarinic receptors (AChRs), linked to Gq proteins signaling pathways that increase the intracellular calcium concentration and activate different PKC isoforms (Gautam et al. 2006). Studies in KO mice showed that M3 receptor subtype is responsible for regulating insulin release (de Azua et al. 2012). Muscarinic M3 receptor in β cells also triggers PKD1 activation and induces insulin secretion (Sumara et al. 2009).

In vivo studies in M3 AChRs KO mice show impaired glucose tolerance and modest insulin release after glucose administration, while transgenic mice with β-cell M3 AChRs overexpression show the opposite metabolic phenotype (Rodriguez-Diaz et al. 2011).

Insulin Functions and Signaling

Insulin increases glucose uptake in adipose tissue and muscle and also inhibits hepatic glucose production. In addition, it stimulates cell growth and differentiation and promotes storage of nutrients by enhancing lipogenesis, glycogenesis, glycolysis, and protein synthesis and by inhibiting lipolysis, gluconeogenesis, glycogenolysis, and protein breakdown. Insulin resistance results in important deregulation of glucose and lipid metabolism (Saltiel and Kahn 2001).

Normal insulin signaling and effects have been extensively reviewed elsewhere (Fig. 2; Saltiel and Kahn 2001; Cheng et al. 2010; Leavens and Birnbaum 2011; Taniguchi et al. 2006; Wang and Jin 2009; White 2003). Briefly, the pathway includes the insulin receptor (IR), which is a prototype of integral membrane proteins with tyrosine kinase (TK) activity, and is formed by two α-β dimers. Insulin binds to the extracellular domain of the α subunit of the receptor and produces a conformational change that promotes the β subunit TK activity. This induces the IR transphosphorylation and phosphorylation of other IR-recruited proteins. However, the binding properties depend on insulin receptor structure, which may vary due to tissue-specific alternative splicing and alternative posttranslational assembly with IGF-1 receptor (White 2003).

Once IR is activated, several proteins are recruited including insulin receptor substrate (IRS) proteins and scaffold proteins such as SHC, CBL, APS, SH2B, GABs, and DOCKs. It has been suggested that IRS proteins mediate most of the intracellular insulin signaling. These proteins can couple to IR and other activated receptors, such as growth hormone receptor, integrins, and some interleukin receptors, through an NH2-terminal pleckstrin homology (PH) domain and a phosphotyrosine-binding (PTB) domain. In addition, IR and IRSs contain other tyrosine and serine/threonine phosphorylation sites capable of binding to numerous effectors or adapter proteins (White 2003). Their phosphorylation could modify the TK activity and interaction with other proteins, thus impairing downstream signaling. IR serine phosphorylation is observed during hyperinsulinemia, resulting from the activation of PKC or AMPK (Vollenweider 2003).

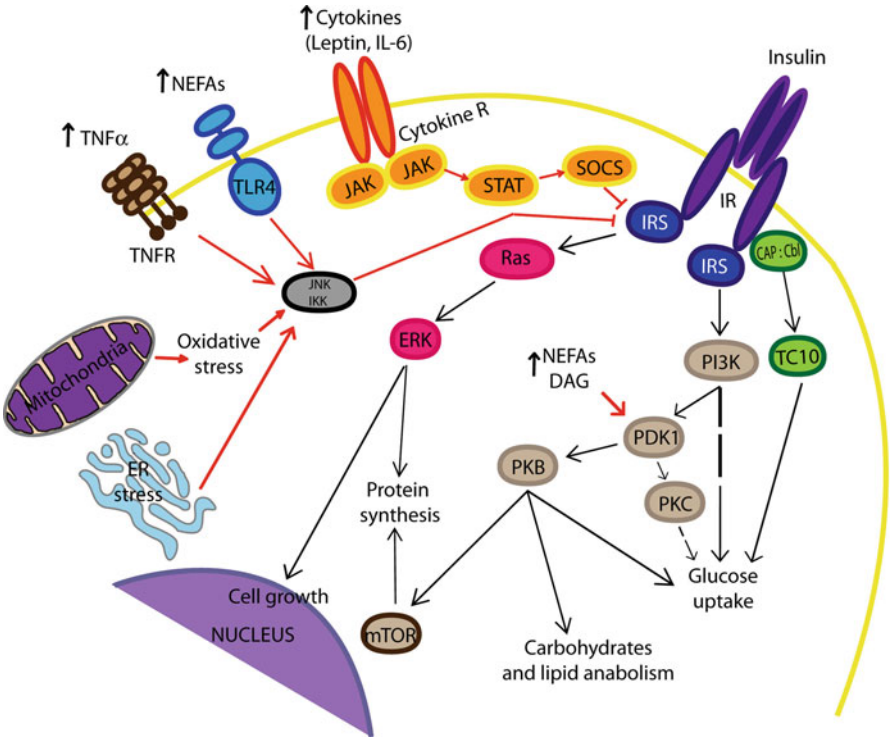

Fig. 2 General insulin signaling pathway and insulin resistance mechanisms. *Black arrows* denote activation processes within insulin signaling pathway. *Red arrows* denote activation processes in other signaling pathways. *Red blocked arrows* represent inhibition processes impairing insulin signaling. *Discontinuous lines* represent activation of intermediate mediators

Phosphorylation of IRS proteins activates three main intracellular downstream signaling pathways. First, the PI3K-PKC-phosphoinositide-dependent kinase (PDK) and PKB/Akt pathway is mainly involved in the metabolic actions of insulin. This cascade modulates glucose-related metabolic enzymes through glycogen synthase kinase 3 (GSK3) and lipid metabolic enzymes, regulating mammalian target of rapamycin (mTOR)-related protein synthesis, phosphorylating the forkhead box O1 (FOXO1), and activating sterol regulatory element-binding protein (SREBP) transcription factors, which regulate metabolism and partially mediate the translocation of GLUT-4-containing vesicles to the cell membrane (Taniguchi et al. 2006). In addition, the Ras-mitogen-activated protein kinase (MAPK) pathway regulates the expression of cell growth- and differentiation-related genes (Taniguchi et al. 2006). Finally, the proto-oncogene Cbl and the Cbl-associated protein (CAP) are released from the IR complex after being activated. They accumulate in the caveolar membrane domains due to an interaction with the protein flotillin. These discrete plasma membrane regions are enriched in lipid-modified signaling proteins,

glycophosphatidylinositol-anchored proteins, glycolipids, sphingolipids, and cholesterol. The Cbl/CAP-activated complex can recruit SH2-containing proteins, such as CRK II and the guanine nucleotide exchange factor C3G. Furthermore, it has been suggested that this complex activates the rho family protein TC10, which partially mediates the translocation of GLUT-4-containing vesicles to the membrane (Vollenweider 2003).

The extent of tyrosine phosphorylation of the IR and downstream proteins reflects a balance between autophosphorylation, in addition to the TK activity of the IR, and their dephosphorylation mediated by protein tyrosine phosphatases (PTPs). The PTB1B is a well studied phosphatase that interacts with the phosphorylated activated IR (Vollenweider 2003; Asante-Appiah and Kennedy 2003), directly dephosphorylating it and finishing the intracellular insulin signaling. Moreover, PTP1B KO mice are resistant to diet-induced obesity, probably due to enhanced hepatic and muscular insulin sensitivity (Asante-Appiah and Kennedy 2003).

The phosphatase SHP-2/PTP1D also binds to ligand-activated receptors with tyrosine kinase activity through their SH2 domains (Vollenweider 2003) and acts as a negative modulator of insulin signaling through dephosphorylation of IR and IRSs (Asante-Appiah and Kennedy 2003).

Metabolic Syndrome and β Cells

The diagnostic criteria for MS are summarized in Table 1, according to the World Health Organization (WHO) and the Adult Treatment Program III (ATPIII) of the National Cholesterol Education Program (NCEP). The presence of at least three factors of Table 1 is required for a positive MS diagnosis. NCEP criteria do not include inflammatory or homeostatic variables. However, they are used because of their clinical feasibility (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2002). Although the pathogenesis of MS is not completely clear, it is known that a genetic predisposition, coupled to a sedentary lifestyle and a high-energy diet, contributes to the development of this syndrome (Rask-Madsen and Kahn 2012).

Insulin resistance is defined as a defect in insulin ability to decrease plasma glucose levels, due in part to an impaired signaling of this hormone in sensitive tissues. The impairment of insulin-dependent biological actions on glucose and lipid metabolism results in fasting and postprandial hyperinsulinemia and increased glucose levels.

Adipose Tissue and Obesity

Obesity is a risk factor to develop insulin resistance, T2DM, and cardiovascular disease. It is well established that hypertrophy and hyperplasia of visceral adipocytes are components of central obesity. Therefore, abdominal circumference,

Table 1 Criteria to diagnose MS according to WHO and NCEP ATP III

Risk factor	WHO	NCEP ATP III
Abdominal girth	Waist to hip ratio > 0.9 in men, > 0.85 in women and/or BMI >30 kg/m^2	> 35 in. (88 cm) in women >40 in. (102 cm) in men
Triglycerides	>1.7mmol/l	>150 mg/dl (1.69 mmol/l) or drug elevated triglycerides
HDL cholesterol	Men < 0.09 mmol/l Women <1.0 mmol/l	Men <40 mg/dl; women <50 mg/dl or drug treatment for reduced HDL-C
Blood pressure	>140/90 mmHg	>130/85 mmHg or drug treatment for elevated tryglicerides
Fasting glucose		>110 mg/dl or drug treatment for elevated glucose
Microalbuminuria	Urinary albumin excretion rate >20 μg/min or Albumin: creatine ratio >30 mg/g	

rather than body mass index (BMI), has become the most important parameter to identify central obesity (Alberti et al. 2009).

Adipose tissue is not only metabolically active and plays an important role in glucose and lipid metabolism, sequestering fat in triglycerides stores, which attenuates the effects of NEFAs, but also secretes hormones called adipokines that could be related to the pathogenesis of various pathological processes including the metabolic syndrome (Hotamisligil 2006).

Interestingly, nearly 25–30 % of morbidly obese humans (BMI > 35 kg/m^2) are insulin sensitive, and the insulin-resistant individuals show a decrease in AMPK activity and increased oxidative stress compared to the insulin-sensitive counterparts (Xu et al. 2012).

The metabolic effects from the abdominal fat are more adverse than those from the fat tissue in other locations, being associated with ectopic fat accumulation in the liver and nonalcoholic fatty liver disease (NAFLD). However, patients with lipodystrophy may also develop NAFLD, independent of obesity, indicating that it is adipose tissue function per se which is important for developing this pathology. Fatty acid fluxes from adipose tissue to the liver increase the availability of NEFAs, particularly in sedentary subjects, which leads to DAG synthesis that contributes to insulin resistance in the liver (Byrne 2012).

Metabolic Effects of Cytokines

Adipose tissue as an endocrine organ is involved in the whole-body energetic balance, by producing and secreting adipokines, such as leptin, adiponectin, resistin, interleukin-6 (IL-6), retinol binding protein-4 (RBP4), NGF, and

brain-derived neurotrophic factor (BDNF). A mixed cytokine excess in MS leads to inflammation and disrupts β-cell functions (see also chapter "▶ Inflammatory Pathways Linked to β Cell Demise in Diabetes").

Leptin is mainly secreted by adipocytes, and its plasma levels correlate with the total body fat mass (Maffei et al. 1995). Leptin inhibits insulin action in white adipose tissue (WAT) and decreases insulin synthesis and secretion in β cells (Denroche et al. 2012). The classical animal models of leptin deficiency exhibit obesity, hyperglycemia, and hyperinsulinemia. The most commonly used are the leptin-deficient *ob*/*ob* (see also chapter "▶ β-Cell Function in Obese-Hyperglycemic Mice") and the leptin receptor-deficient db/db mice, as well as some rat models (fa/fa, Zucker, etc., reviewed by Larqué et al. 2011). Another mouse model with a disrupted signaling domain of leptin receptor in β cells and hypothalamus is characterized by overweight, insulin resistance, impaired β-cell functions, and glucose intolerance (Covey et al. 2006). Recently, it was observed that a variant of this model, deficient in leptin signaling in β cells by a Cre-loxP recombination, shows insulin hypersecretion under basal conditions and a deficient secretion in response to glucose (Tuduri et al. 2013).

Adiponectin is exclusively secreted by adipocytes and its plasma levels show a negative correlation with the risk of obesity, insulin resistance, T2DM, and cardiovascular disease (Harwood 2012). Reports about the direct effects of adiponectin on insulin secretion are variable and inconsistent between animal models and humans and require further clarification (Lee et al. 2011).

Circulating levels of tumor necrosis factor α (TNFα) are also increased in nondiabetic obese and individuals with T2DM (Bays et al. 2004), but the correlation between insulin resistance and plasma TNFa levels is weak in both cases (Hotamisligil et al. 1995). The primary source of TNFa is macrophages from the stromal vascular fraction of adipose tissue; however, differentiated white adipocytes also produce this cytokine.

Little is known about the direct effect of TNFa in the islets. However, plasma levels of insulin and leptin are decreased in $TNFa^{-/-}$ mice (Romanatto et al. 2009) and their islets are pronouncedly infiltrated by inflammatory cells. Paradoxically, insulitis and diabetes are not well correlated, as observed in the NOD/WEHI strain of NOD mice, which shows insulitis without diabetes. These observations are consistent with the idea that insulitis may be necessary but not sufficient to induce diabetes (Charlton et al. 1989). Furthermore, TNFa induces the synthesis of amylin in β cells, without affecting insulin expression in the MIN6 cell line and islets from mice (Cai et al. 2011).

Interleukin-6 (IL-6) is a cytokine with pleiotropic biological effects that is secreted by adipocytes and the stromal fraction of adipose tissue. IL-6 expression has been correlated with insulitis and β-cell destruction in NOD mice. However, this is not the case for human islets. In fact, it has been observed in other models that IL-6 may protect β-cells from other inflammatory insults; at least, it is clear that it is not cytotoxic without other cytokines. Interestingly, several studies indicate that IL-6 inhibits insulin secretion in rodent β cells; in contrast, human islets are not

affected by this cytokine (Kristiansen and Mandrup-Poulsen 2005). We have also observed that IL-6 plasma levels are increased in Wistar rats with MS, developed by sucrose ingestion in drinking water (unpublished observation).

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin (NT) family of factors, which play important roles in the development of both the central and peripheral nervous systems. BDNF is produced by adipocytes and may be altered in MS and diabetes mellitus (Chaldakov 2011; Sornelli et al. 2009). Evidence in humans indicates that BDNF could play an important role in the body weight control and energy homeostasis (Rosas-Vargas et al. 2011). It has been suggested that BDNF plasma levels are decreased in obesity, showing an inverse correlation with body mass index. However, in T2DM, BDNF levels decrease, independently of obesity. Plasma BDNF is also inversely associated with fasting plasma glucose, but not with insulin, suggesting a contribution to glucose metabolism (Krabbe et al. 2007).

Direct effects of BDNF on β cells have not been extensively explored. It was described that chronic BDNF administration in db/db mice prevents exhaustion of β cells by maintaining the cellular organization in the islets and restoring the levels of insulin-secreting granules (Yamanaka et al. 2006).

Hyperinsulinemia and Insulin Resistance

Insulin resistance is a major sign in metabolic syndrome and an important warning for T2DM development, due to dysregulation of glucose and lipid metabolism (Saltiel and Kahn 2001). Mechanisms involved in the insulin-signaling elements that could be affected in insulin resistance include the phosphorylation–dephosphorylation processes, interaction with insulin-signaling negative regulator proteins, interaction with non-insulin-signaling proteins, interaction with lipid metabolites or oxidative stress mediators, proteolytic processes, or susceptibility to transcriptional modulation by other signaling pathways, among others. Moreover, several elements of the insulin-signaling network may play potential crosstalk roles with other signaling pathways.

Interaction of proteins from the insulin pathway with other signaling molecules such as SOCS is suggested as an important mechanism of insulin resistance. TNF-α, IL6, and even the insulin cascade enhance the transcription of SOCS genes. These proteins are suggested to play different roles in insulin resistance, including interaction with IR and competition with IRS proteins, inhibition of Janus kinase involved in insulin signaling, and targeting IRSs to proteasomal degradation (Howard and Flier 2006; Mlinar et al. 2007).

Evidence has showed that ROS, such as hydrogen peroxide, are involved in the normal regulation of cellular processes. However, chronic or increased production of ROS or a reduced capacity for their elimination could lead to

impairment of intracellular signaling and result either in inflammation or in insulin resistance. As mentioned before, IKKβ and JNK proteins have been proposed as oxidative stress-sensitive kinases and may play a role in the attenuation of insulin signaling. The resulting effect of activating oxidative stress-sensitive kinases is the serine/threonine phosphorylation of proteins such as IR and IRSs (Evans et al. 2005).

During MS, the β cells are continuously stimulated. In this condition, they secrete high amounts of insulin and eventually become exhausted and incapable to secrete sufficient amounts of hormone to maintain glucose homeostasis, leading to T2DM development. (see also chapter "► The β-Cell in Human Type 2 Diabetes").

The MS has been extensively studied in different animal models. We recently reported that adult male *Wistar* rats, treated for 2 or 6 months with 20 % sucrose in drinking water, develop MS, characterized by obesity, hypertriglyceridemia, hyperinsulinemia, hypertension, and insulin resistance.

After 2 months of treatment, the short-term/early effects of MS on β cells include an increase in the total amount and expression level of the GLUT2 protein (Larqué et al. 2011) and a discrete increment of calcium currents (unpublished observations). We consider that these cellular changes play an important role in hyperinsulinemia.

We also analyzed the long-term effects of MS on β-cell electrical activity after a 6-month treatment (Fig. 3). Electrophysiological studies of K_{ATP} single-channel activity demonstrated that the channel conductance was not modified. However, in isolated patches of membrane, the ATP sensitivity of K_{ATP} channels in MS rats increased with respect to control rats. The change in the Kd for ATP indicates that the channel closes at lower intracellular ATP concentrations in the MS β cells (Velasco et al. 2012).

Furthermore, we found three subpopulations of β cells, according to their calcium currents (Fig. 3). One half of the cells had low current density (we named them MS1), 35 % of the cells showed large currents (MS2), and 15 % of them showed no IBa^{2+} at all. We observed that during the metabolic syndrome, MS2 cells showed an increase (92 %) in the maximum peak of barium current density, which could explain hyperinsulinemia. However, MS1 cells begin to get exhausted, by showing less current and probably less insulin secretion (Velasco et al. 2012).

β-Cell Exhaustion and T2DM

Cytokines in obesity affect islet health and potentially contribute to islet inflammation in T2DM (Fig. 4). β cells show a low antioxidant defense and are more vulnerable to oxidative stress caused by high glucose and NEFAs levels (see also chapter "► Role of NADPH Oxidase in β Cell Dysfunction"). This can cause ER

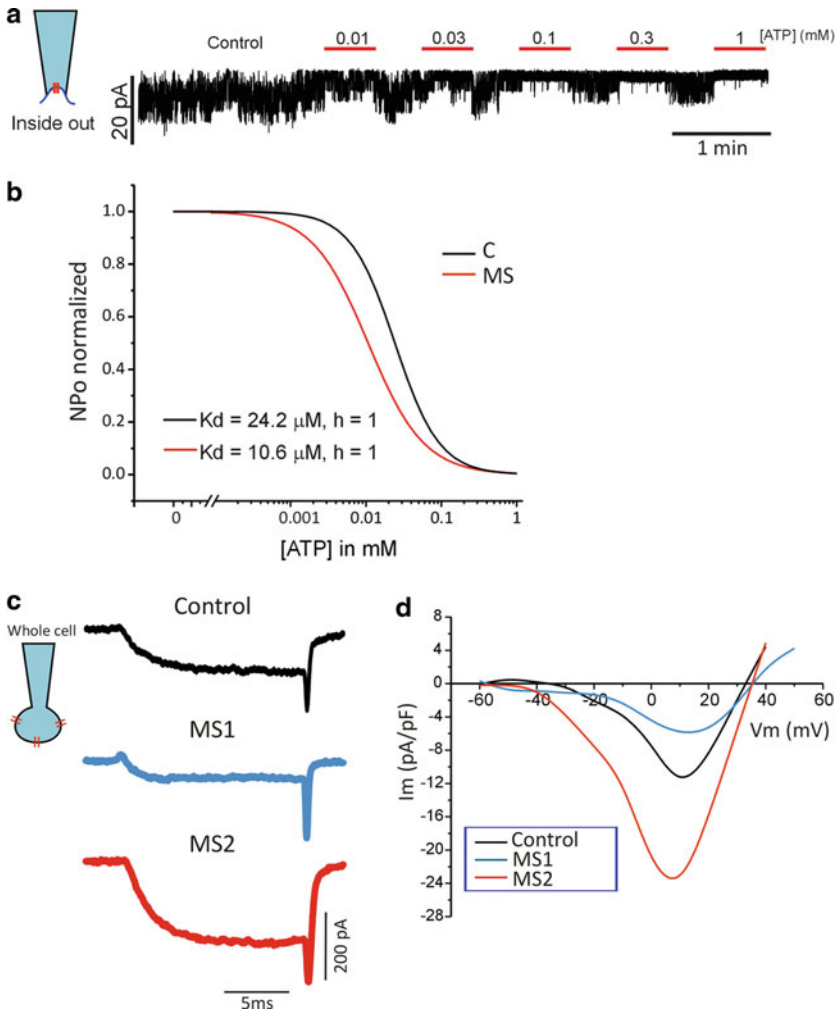

Fig. 3 Electrophysiological changes in β cells during metabolic syndrome. (**a**) Time course of K_{ATP} channels activity in metabolic syndrome registered with inside-out configuration in a cell membrane patch. K_{ATP} channel blockade in a concentration-dependent fashion is observed. (**b**) ATP concentration-response curve. (**c**) Barium currents observed at a +10 mV potential in normal β-cells, and in β-cells during metabolic syndrome. Two β-cell populations, named MS1 and MS2 were classified according to their current amplitude compared to controls. (**d**) Current to voltage relationship obtained from cells in Section C

stress and amplify cell dysfunction (Imai et al. 2013). A decrease in β-cell mass by 60–75 % is observed in T2DM (see also chapters "▶ Mechanisms of Pancreatic β-Cell Apoptosis in Diabetes" and "▶ The β-Cell in Human Type 2 Diabetes"). However, it is difficult to calculate this parameter in vivo. Also, the β-cell mass function of the surviving β cells is also important for the development of T2DM.

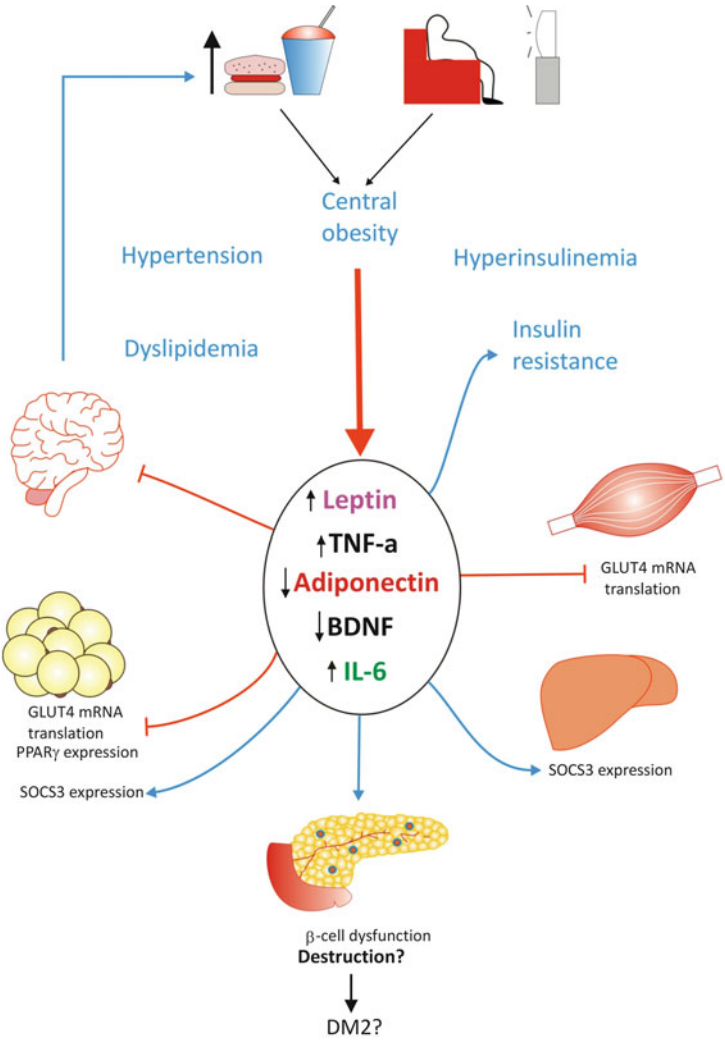

Fig. 4 Cytokines contribution to insulin resistance and β-cell dysfunction in the metabolic syndrome

β-cell exhaustion is not unlikely in a long-term metabolic disease considering the increased levels of ROS production (see also chapter "▶ Role of Mitochondriain β-cell Function and Dysfunction"), circulating pro-inflammatory cytokines, lipids, and hyperglycemic hyperinsulinemia. The oxidative stress is an inherent part of insulin secretion, signaling, and regulation, but β-cell dysfunction could arise in a glycolipotoxic context, along with a dysregulated ROS production as occurs in T2DM (Jezek et al. 2012; Somesh et al. 2013). Indeed, β cells face a challenging

environment in MS, which is plenty of stimulating signals that keep their metabolism activated and could cause a Ca^{2+} overload (see also chapter "► Calcium Signaling in the Islets").

Both the hyperexcitability and the exacerbation of several signaling pathways simultaneously could cause β-cell failure, because of desensitizing mechanisms and/or proapoptotic processes. Hypoglycemic agents, such as glibenclamide, which faces insulin resistance by increasing insulin secretion, eventually fail to control glucose levels in diabetic patients (Matthews et al. 1998). In vitro, they also impair β-cell performance and survival (Sawada et al. 2008) indicating that a chronic exposure to these agents could lead to β-cell failure or even death. Moreover, several of the signs that MS shares with diabetes could converge into toxic pathways and synergize their deleterious effects, as has been suggested for TRPA1 activation (Diaz-Garcia 2013).

Some new factors have recently been described with therapeutic potential in β-cell decrement in prediabetic and diabetic states. Betatrophin is a hormone secreted by the mouse liver that selectively promotes β-cell mass expansion, which could improve glucose tolerance (Yi et al. 2013). However, more basic research in β cells is needed to fully understand metabolic syndrome and β-cells failure but also how to prevent it.

Conclusions

- Pancreatic β-cell physiology is medullar for glucose homeostasis. β cells are sensitive to metabolic changes in the system; they are also especially vulnerable to ROS damage, and their replacement is low.
- Insulin signaling is important for the metabolism of carbohydrates, lipids, and proteins. When insulin resistance is developed, the nutrient homeostasis is affected.
- MS affects β-cell function even at early stages, and when inflammation and NEFAs levels rise, they promote, among other factors, an increase in ROS. One of the signs of MS is hyperinsulinemia, which is maintained until, depending on individual genetic traits, β cells are no longer capable of secreting enough insulin to maintain glucose levels in normal values and T2DM develops.
- The complete scenario of the individual presentation of MS is not well understood, and the time needed for β-cell exhaustion is not known, under this condition. Moreover, many of the effects of the different cytokines and adipokines on β cells are incompletely understood. Clearly more research is needed to have the whole picture of β-cell response to MS.
- Finally, the time and conditions needed to revert the pathophysiological changes of MS at different levels, including those in β cells, are not known. However, the main challenge for humans in this moment is to change their lifestyles, by having a moderate and healthy food consumption and by increasing their exercise habits. It will be difficult, but it is worth trying.

Cross-References

- (Dys)Regulation of Insulin Secretion by Macronutrients
- Electrophysiology of Islet Cells
- High-Fat Programming of β-Cell Dysfunction
- β-Cell function in obese-hyperglycaemic mice [*ob*/*ob* mice]
- The β-Cell in Human Type 2 Diabetes

References

Abedini A, Schmidt AM (2013) Mechanisms of islet amyloidosis toxicity in type 2 diabetes. FEBS Lett 587(8):1119–1127

Ahren B (2009) Islet G protein-coupled receptors as potential targets for treatment of type 2 diabetes. Nat Rev Drug Discov 8(5):369–385

Ahren B (2012) Islet nerves in focus – defining their neurobiological and clinical role. Diabetologia 55(12):3152–3154

Ahren B, Lundquist I (1981) Effects of selective and non-selective β-adrenergic agents on insulin secretion in vivo. Eur J Pharmacol 71(1):93–104

Alberti KG, Eckel RH et al (2009) Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 120(16):1640–1645

Aloe L (2011) Rita Levi-Montalcini and the discovery of NGF, the first nerve cell growth factor. Arch Ital Biol 149(2):175–181

Asante-Appiah E, Kennedy BP (2003) Protein tyrosine phosphatases: the quest for negative regulators of insulin action. Am J Physiol Endocrinol Metab 284(4):E663–E670

Bagger JI, Knop FK et al (2011) Glucagon antagonism as a potential therapeutic target in type 2 diabetes. Diabetes Obes Metab 13(11):965–971

Bays H, Mandarino L et al (2004) Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. J Clin Endocrinol Metab 89(2):463–478

Braun M, Ramracheya R et al (2010) Gamma-aminobutyric acid (GABA) is an autocrine excitatory transmitter in human pancreatic β-cells. Diabetes 59(7):1694–1701

Braun M, Wendt A et al (2004) Regulated exocytosis of GABA-containing synaptic-like microvesicles in pancreatic β-cells. J Gen Physiol 123(3):191–204

Byrne CD (2012) Dorothy Hodgkin Lecture 2012: non-alcoholic fatty liver disease, insulin resistance and ectopic fat: a new problem in diabetes management. Diabet Med 29(9):1098–1107

Cabrera O, Berman DM et al (2006) The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proc Natl Acad Sci USA 103(7):2334–2339

Cabrera-Vasquez S, Navarro-Tableros V et al (2009) Remodelling sympathetic innervation in rat pancreatic islets ontogeny. BMC Dev Biol 9:34

Cai K, Qi D et al (2011) TNF-α acutely upregulates amylin expression in murine pancreatic β cells. Diabetologia 54(3):617–626

Chakraborty S, Mukherjee B et al (2013) Pinpointing proline substitution to be responsible for the loss of amyloidogenesis in IAPP. Chem Biol Drug Des 82(4):446–452

Chaldakov G (2011) The metabotrophic NGF and BDNF: an emerging concept. Arch Ital Biol 149(2):257–263

Chaldakov GN, Fiore M et al (2010) Neuroadipology: a novel component of neuroendocrinology. Cell Biol Int 34(10):1051–1053
Chaldakov GN, Tonchev AB et al (2009) NGF and BDNF: from nerves to adipose tissue, from neurokines to metabokines. Riv Psichiatr 44(2):79–87
Charlton B, Bacelj A et al (1989) Cyclophosphamide-induced diabetes in NOD/WEHI mice. Evidence for suppression in spontaneous autoimmune diabetes mellitus. Diabetes 38(4):441–447
Cheng Z, Tseng Y et al (2010) Insulin signaling meets mitochondria in metabolism. Trends Endocrinol Metab 21(10):589–598
Covey SD, Wideman RD et al (2006) The pancreatic β cell is a key site for mediating the effects of leptin on glucose homeostasis. Cell Metab 4(4):291–302
DeFronzo RA, Okerson T et al (2008) Effects of exenatide versus sitagliptin on postprandial glucose, insulin and glucagon secretion, gastric emptying, and caloric intake: a randomized, cross-over study. Curr Med Res Opin 24(10):2943–2952
Denroche HC, Huynh FK et al (2012) The role of leptin in glucose homeostasis. Journal of Diabetes Investigation 3(2):115–129
Diaz-Garcia CM (2013) The TRPA1 channel and oral hypoglycemic agents: Is there complicity in β-cell exhaustion? Channels (Austin) 7(6):420–422
Dong H, Kumar M et al (2006) Gamma-aminobutyric acid up- and downregulates insulin secretion from β cells in concert with changes in glucose concentration. Diabetologia 49(4):697–705
Drews G, Krippeit-Drews P et al (2010) Electrophysiology of islet cells. Adv Exp Med Biol 654:115–163
Evans JL, Maddux BA et al (2005) The molecular basis for oxidative stress-induced insulin resistance. Antioxid Redox Signal 7(7–8):1040–1052
Expert Panel on Detection, E., and Treatment of High Blood Cholesterol in Adults (2002) Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. Circulation 106(25):3143–3421
Fadini GP, de Kreutzenberg SV et al (2011) The metabolic syndrome influences the response to incretin-based therapies. Acta Diabetol 48(3):219–225
Faradji V, Sotelo J (1990) Low serum levels of nerve growth factor in diabetic neuropathy. Acta Neurol Scand 81(5):402–406
Gautam D, Han SJ et al (2006) A critical role for β cell M3 muscarinic acetylcholine receptors in regulating insulin release and blood glucose homeostasis in vivo. Cell Metab 3(6):449–461
Gebre-Medhin S, Olofsson C et al (2000) Islet amyloid polypeptide in the islets of Langerhans: friend or foe? Diabetologia 43(6):687–695
Gezginci-Oktayoglu S, Bolkent S (2009) Exendin-4 exerts its effects through the NGF/p75NTR system in diabetic mouse pancreas. Biochem Cell Biol 87(4):641–651
Gezginci-Oktayoglu S, Karatug A et al (2012) The relation among NGF, EGF and insulin is important for triggering pancreatic β cell apoptosis. Diabetes Metab Res Rev 28(8):654–662
Graciano MF, Valle MM et al (2011) Regulation of insulin secretion and reactive oxygen species production by free fatty acids in pancreatic islets. Islets 3(5):213–223
Gromada J, Franklin I et al (2007) α-cells of the endocrine pancreas: 35 years of research but the enigma remains. Endocr Rev 28(1):84–116
Harwood HJ Jr (2012) The adipocyte as an endocrine organ in the regulation of metabolic homeostasis. Neuropharmacology 63(1):57–75
Henquin JC (2000) Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 49(11):1751–1760
Henquin JC (2011) The dual control of insulin secretion by glucose involves triggering and amplifying pathways in β-cells. Diabetes Res Clin Pract 93(Suppl 1):S27–S31
Henquin JC, Meissner HP (1984) Significance of ionic fluxes and changes in membrane potential for stimulus-secretion coupling in pancreatic β-cells. Experientia 40(10):1043–1052

Hiriart M, Aguilar-Bryan L (2008) Channel regulation of glucose sensing in the pancreatic β-cell. Am J Physiol Endocrinol Metab 295(6):E1298–E1306
Hiriart M, Vidaltamayo R et al (2001) Nerve and fibroblast growth factors as modulators of pancreatic β cell plasticity and insulin secretion. Isr Med Assoc J 3(2):114–116
Hotamisligil GS (2006) Inflammation and metabolic disorders. Nature 444(7121):860–807
Hotamisligil GS, Arner P et al (1995) Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. J Clin Invest 95(5):2409–2415
Howard JK, Flier JS (2006) Attenuation of leptin and insulin signaling by SOCS proteins. Trends Endocrinol Metab 17(9):365–371
Hristova M, Aloe L (2006) Metabolic syndrome – neurotrophic hypothesis. Med Hypotheses 66(3):545–549
Hunt KJ, Resendez RG et al (2004) National Cholesterol Education Program versus World Health Organization metabolic syndrome in relation to all-cause and cardiovascular mortality in the San Antonio Heart Study. Circulation 110(10):1251–1257
Huypens PR, Huang M et al (2012) Overcoming the spatial barriers of the stimulus secretion cascade in pancreatic β-cells. Islets 4(1):1–9
Imai Y, Dobrian AD et al (2013) Islet inflammation: a unifying target for diabetes treatment? Trends Endocrinol Metab 24(7):351–360
Jensen MV, Joseph JW et al (2008) Metabolic cycling in control of glucose-stimulated insulin secretion. Am J Physiol Endocrinol Metab 295(6):E1287–E1297
Jewell JL, Oh E et al (2010) Exocytosis mechanisms underlying insulin release and glucose uptake: conserved roles for Munc18c and syntaxin 4. Am J Physiol Regul Integr Comp Physiol 298(3):R517–R531
Jezek P, Dlaskova A et al (2012) Redox homeostasis in pancreatic β cells. Oxid Med Cell Longev 2012:932838
Kim SJ, Nian C et al (2012) GIP-overexpressing mice demonstrate reduced diet-induced obesity and steatosis, and improved glucose homeostasis. PLoS One 7(7):e40156
Koh DS, Cho JH et al (2012) Paracrine interactions within islets of Langerhans. J Mol Neurosci 48(2):429–440
Krabbe KS, Nielsen AR et al (2007) Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. Diabetologia 50(2):431–438
Kristiansen OP, Mandrup-Poulsen T (2005) Interleukin-6 and diabetes: the good, the bad, or the indifferent? Diabetes 54(Suppl 2):S114–S124
Larqué C, Velasco M et al (2011) Early endocrine and molecular changes in metabolic syndrome models. IUBMB Life 63(10):831–839
Larrieta ME, Vital P et al (2006) Nerve growth factor increases in pancreatic β cells after streptozotocin-induced damage in rats. Exp Biol Med (Maywood) 231(4):396–402
Leavens KF, Birnbaum MJ (2011) Insulin signaling to hepatic lipid metabolism in health and disease. Crit Rev Biochem Mol Biol 46(3):200–215
Lee YH, Magkos F et al (2011) Effects of leptin and adiponectin on pancreatic β-cell function. Metabolism 60(12):1664–1672
Leibiger IB, Leibiger B et al (2008) Insulin signaling in the pancreatic β-cell. Annu Rev Nutr 28:233–251
Li C, Matschinsky FM et al (2012) Amino acid-stimulated insulin secretion: the role of the glutamine-glutamate-α-ketoglutarate axis. Monogenic hyperinsulinemic hypoglycemia disorders. S. C. A. and D. L. D. D., Philadelphia, p 21
Lorenzo A, Razzaboni B et al (1994) Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. Nature 368(6473):756–760
MacDonald PE, Wheeler MB (2003) Voltage-dependent K^+ channels in pancreatic β cells: role, regulation and potential as therapeutic targets. Diabetologia 46(8):1046–1062
Maffei M, Halaas J et al (1995) Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1(11):1155–1161

Matthews DR, Cull CA et al (1998) UKPDS 26: sulphonylurea failure in non-insulin-dependent diabetic patients over six years. UK Prospective Diabetes Study (UKPDS) Group. Diabet Med 15(4):297–303

Mlinar B, Marc J et al (2007) Molecular mechanisms of insulin resistance and associated diseases. Clin Chim Acta 375(1–2):20–35

Navarro-Tableros V, Fiordelisio T et al (2007) Nerve growth factor promotes development of glucose-induced insulin secretion in rat neonate pancreatic β cells by modulating calcium channels. Channels (Austin) 1(6):408–416

Newsholme P, Bender K et al (2007) Amino acid metabolism, insulin secretion and diabetes. Biochem Soc Trans 35(Pt 5):1180–1186

Nishizawa M, Nakabayashi H et al (2013) Intraportal GLP-1 stimulates insulin secretion predominantly through the hepatoportal-pancreatic vagal reflex pathways. Am J Physiol Endocrinol Metab 305(3):E376–E387

Nolan CJ, Leahy JL et al (2006) β cell compensation for insulin resistance in Zucker fatty rats: increased lipolysis and fatty acid signalling. Diabetologia 49(9):2120–2130

Nolan CJ, Prentki M (2008) The islet β-cell: fuel responsive and vulnerable. Trends Endocrinol Metab 19(8):285–291

Nyman LR, Wells KS et al (2008) Real-time, multidimensional in vivo imaging used to investigate blood flow in mouse pancreatic islets. J Clin Invest 118(11):3790–3797

Ohara-Imaizumi M, Fujiwara T et al (2007) Imaging analysis reveals mechanistic differences between first- and second-phase insulin exocytosis. J Cell Biol 177(4):695–705

Osundiji MA, Evans ML (2013) Brain control of insulin and glucagon secretion. Endocrinol Metab Clin North Am 42(1):1–14

Phillips LK, Prins JB (2011) Update on incretin hormones. Ann N Y Acad Sci 1243:E55–E74

Pillay K, Govender P (2013) Amylin uncovered: a review on the polypeptide responsible for type II diabetes. Biomed Res Int 2013:826706

Polak M, Scharfmann R et al (1993) Nerve growth factor induces neuron-like differentiation of an insulin-secreting pancreatic β cell line. Proc Natl Acad Sci U S A 90(12):5781–5785

Prentki M, Matschinsky FM (1987) Ca^{2+}, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. Physiol Rev 67(4):1185–248

Prentki M, Matschinsky FM et al (2013) Metabolic signaling in fuel-induced insulin secretion. Cell Metab 18(2):162–85

Rask-Madsen C, Kahn CR (2012) Tissue-specific insulin signaling, metabolic syndrome, and cardiovascular disease. Arterioscler Thromb Vasc Biol 32(9):2052–2059

Reetz A, Solimena M et al (1991) GABA and pancreatic β-cells: colocalization of glutamic acid decarboxylase (GAD) and GABA with synaptic-like microvesicles suggests their role in GABA storage and secretion. EMBO J 10(5):1275–1284

Rhodes CJ, White MF et al (2013) Direct autocrine action of insulin on β-cells: does it make physiological sense? Diabetes 62(7):2157–2163

Rodriguez-Diaz R, Caicedo A (2013) Novel approaches to studying the role of innervation in the biology of pancreatic islets. Endocrinol Metab Clin North Am 42(1):39–56

Rodriguez-Diaz R, Dando R et al (2011) α cells secrete acetylcholine as a non-neuronal paracrine signal priming β cell function in humans. Nat Med 17(7):888–8892

Romanatto T, Roman EA et al (2009) Deletion of tumor necrosis factor-α receptor 1 (TNFR1) protects against diet-induced obesity by means of increased thermogenesis. J Biol Chem 284(52):36213–36222

Rorsman P, Renstrom E (2003) Insulin granule dynamics in pancreatic β cells. Diabetologia 46(8):1029–1045

Rosas-Vargas H, Martinez-Ezquerro JD et al (2011) Brain-derived neurotrophic factor, food intake regulation, and obesity. Arch Med Res 42(6):482–494

Rosenbaum T, Castanares DT et al (2002) Nerve growth factor increases L-type calcium current in pancreatic β cells in culture. J Membr Biol 186(3):177–184

Rosenbaum T, Sanchez-Soto MC et al (2001) Nerve growth factor increases insulin secretion and barium current in pancreatic β-cells. Diabetes 50(8):1755–1762

Rosenbaum T, Vidaltamayo R et al (1998) Pancreatic β cells synthesize and secrete nerve growth factor. Proc Natl Acad Sci U S A 95(13):7784–7788

Rosengren AH, Jokubka R et al (2010) Overexpression of α2A-adrenergic receptors contributes to type 2 diabetes. Science 327(5962):217–220

Ruiz de Azua I, Gautam D et al (2012) Critical metabolic roles of β-cell M3 muscarinic acetylcholine receptors. Life Sci 91(21–22):986–991

Ruohonen ST, Ruohonen S et al (2012) Involvement of α2-adrenoceptor subtypes A and C in glucose homeostasis and adrenaline-induced hyperglycaemia. Neuroendocrinology 96(1):51–59

Saltiel AR, Kahn CR (2001) Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414(6865):799–806

Sawada F, Inoguchi T et al (2008) Differential effect of sulfonylureas on production of reactive oxygen species and apoptosis in cultured pancreatic β-cell line, MIN6. Metabolism 57(8):1038–1045

Smismans A, Schuit F et al (1997) Nutrient regulation of gamma-aminobutyric acid release from islet β cells. Diabetologia 40(12):1411–1415

Somesh BP, Verma MK et al (2013) Chronic glucolipotoxic conditions in pancreatic islets impair insulin secretion due to dysregulated calcium dynamics, glucose responsiveness and mitochondrial activity. BMC Cell Biol 14:31

Sornelli F, Fiore M et al (2009) Adipose tissue-derived nerve growth factor and brain-derived neurotrophic factor: results from experimental stress and diabetes. Gen Physiol Biophys 28 Spec No:179–183

Stein DT, Stevenson BE et al (1997) The insulinotropic potency of fatty acids is influenced profoundly by their chain length and degree of saturation. J Clin Invest 100(2):398–403

Straub SG, Shanmugam G et al (2004) Stimulation of insulin release by glucose is associated with an increase in the number of docked granules in the β-cells of rat pancreatic islets. Diabetes 53 (12):3179–3183

Straub SG, Sharp GW (2012) Evolving insights regarding mechanisms for the inhibition of insulin release by norepinephrine and heterotrimeric G proteins. Am J Physiol Cell Physiol 302(12): C1687–C1698

Sumara G, Formentini I et al (2009) Regulation of PKD by the MAPK p38delta in insulin secretion and glucose homeostasis. Cell 136(2):235–248

Taborsky GJ Jr, Ahren B et al (2002) Autonomic mechanism and defects in the glucagon response to insulin-induced hypoglycaemia. Diabetes Nutr Metab 15(5):318–322, discussion 322–323

Taniguchi CM, Emanuelli B et al (2006) Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol 7(2):85–96

Togashi K, Hara Y et al (2006) TRPM2 activation by cyclic ADP-ribose at body temperature is involved in insulin secretion. EMBO J 25(9):1804–1815

Tomas E, Wood JA et al (2011) Glucagon-like peptide-1(9–36)amide metabolite inhibits weight gain and attenuates diabetes and hepatic steatosis in diet-induced obese mice. Diabetes Obes Metab 13(1):26–33

Tuduri E, Bruin JE et al (2013) Impaired Ca^{2+} Signaling in β-Cells Lacking Leptin Receptors by Cre-loxP Recombination. PLoS One 8(8):e71075

Ullrich S, Wollheim CB (1985) Expression of both α 1- and α 2-adrenoceptors in an insulin-secreting cell line. Parallel studies of cytosolic free Ca^{2+} and insulin release. Mol Pharmacol 28(2):100–106

Velasco M, Larque C et al (2012) Metabolic syndrome induces changes in K_{ATP}-channels and calcium currents in pancreatic β-cells. Islets 4(4):302–311

Vidaltamayo R, Sanchez-Soto MC et al (2002) Nerve growth factor increases sodium channel expression in pancreatic β cells: implications for insulin secretion. FASEB J 16(8):891–892
Vollenweider P (2003) Insulin resistant states and insulin signaling. Clin Chem Lab Med 41(9):1107–1119
Wang Q, Jin T (2009) The role of insulin signaling in the development of β-cell dysfunction and diabetes. Islets 1(2):95–101
Westermark P, Andersson A et al (2011) Islet amyloid polypeptide, islet amyloid, and diabetes mellitus. Physiol Rev 91(3):795–826
White MF (2003) Insulin signaling in health and disease. Science 302(5651):1710–1711
Xu XJ, Gauthier MS et al (2012) Insulin sensitive and resistant obesity in humans: AMPK activity, oxidative stress, and depot-specific changes in gene expression in adipose tissue. J Lipid Res 53(4):792–801
Yamanaka M, Itakura Y et al (2006) Protective effect of brain-derived neurotrophic factor on pancreatic islets in obese diabetic mice. Metabolism 55(10):1286–1292
Yi P, Park JS et al (2013) Betatrophin: a hormone that controls pancreatic β cell proliferation. Cell 153(4):747–758
Youos JG (2011) The role of α-, δ- and F cells in insulin secretion and action. Diabetes Res Clin Pract 93(Suppl 1):S25–S26
Zhang T, Li C (2013) Mechanisms of amino acid-stimulated insulin secretion in congenital hyperinsulinism. Acta Biochim Biophys Sin (Shanghai) 45(1):36–43

Apoptosis in Pancreatic β-Cells in Type 1 and Type 2 Diabetes

31

Tatsuo Tomita

Contents

Abstract

Apoptosis plays an important role in the pathophysiology of both type 1 and type 2 diabetes. In type 1 diabetes, β-cell death by apoptosis following autoimmune insulitis causes an absolute insulin deficiency triggered by an extrinsic receptor-mediated pathway, which activates a cascade of caspase family reaction. The etiology of type 2 diabetes is multifactorial, including obesity-associated insulin resistance, defective insulin secretion, and loss of β-cell mass through β-cell apoptosis. β-cell apoptosis is mediated through a milliard of caspase family cascade machinery in both type 1 and type 2 diabetes. The glucose-induced insulin secretion is the principle pathophysiology of diabetes and insufficient insulin secretion results in chronic hyperglycemia and diabetes. Recently, hyperglycemia-induced β-cell apoptosis has been extensively studied with regard to the balance of pro-apoptotic genes (Bad, Bid, and Bik) and the anti-apoptotic Bcl family toward apoptosis in in vitro isolated islets. Apoptosis can only occur when the concentration of pro-apoptotic Bcl-2 exceeds that of anti-apoptotic proteins at the mitochondrial membrane of the intrinsic pathway.

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_45,

The bulk of recent research on hyperglycemia-induced apoptosis on β-cells unveiled complex details of glucose toxicity on β-cells at a molecular level coupled with cell membrane potential by the K^+ and Ca^{2+} channels opening and closing. Further, animal models using knockout mice will shed light on our basic understanding of the pathophysiology of diabetes as a glucose metabolic disease complex, and on the balance of the anti-apoptotic Bcl family and pro-apoptotic genes. The cumulative knowledge will provide a better understanding of the metabolic control of glucose metabolism at a molecular level and will lead to eventual prevention and therapeutic application for type 1 and type 2 diabetes.

Keywords

Amyloid • Anti-apoptotic genes and proteins • Apoptosis • β-cells • Bcl family • Ca^{++} channel • Caspase • Glucokinase • Glucose-induced insulin secretion • Glucose toxicity • Immunocytochemistry • Insulin • Islet amyloid polypeptide • TUNEL assay • Type 1 and type 2 diabetes mellitus

Introduction

There is increasing evidence to support the notion that both type 1 and type 2 diabetes (DM) is modulated through β-cell apoptosis. Apoptosis is a complex biological phenomenon characterized by cell shrinkage, chromatic condensation, internucleosomal DNA fragmentation, and disassembly into membrane-encircled vesicles (apoptotic bodies) (Kerr et al. 1972). This programmed cell death is implicated in the remodeling of the normal endocrine pancreas after birth, and plays an important role in the development of final β-cell mass (Finegood et al. 1995).

The role of apoptosis in the physiology of normal pancreatic development has been demonstrated in neonatal pancreas, which has a threefold higher frequency of apoptotic cells than adult animals (Scaglia et al. 1997). Pancreatic β-cell apoptosis is also a pathological feature that is common to both type 1 and type 2 diabetes mellitus (DM). In type 1 DM (T1DM), β-cells are selectively destroyed by insulitis of T-lymphocytic infiltration in islets, and autoimmune destruction follows after the appearance of islet autoantibody, and results in insulin deficiency (Urusova et al. 2004). In type 2 DM (T2DM), insulin resistance with obesity leads to a glucose toxicity effect, which contributes to β-cell death by apoptosis (Mandrup-Poulsen 2001).

Defects in apoptotic regulatory machinery are implicated in a variety of pathological statuses: excess apoptosis is the underlying cause for β-cell loss for both T1DM and T2DM and inadequate apoptosis may contribute to the oncogenesis of pancreatic endocrine tumors (Lee and Pervaiz 2007). Of the two apoptosis pathways, the extrinsic (receptor-mediated) and the intrinsic (mitochondria-driven) pathways, it is the extrinsic pathway that is activated upon ligation of the cells'

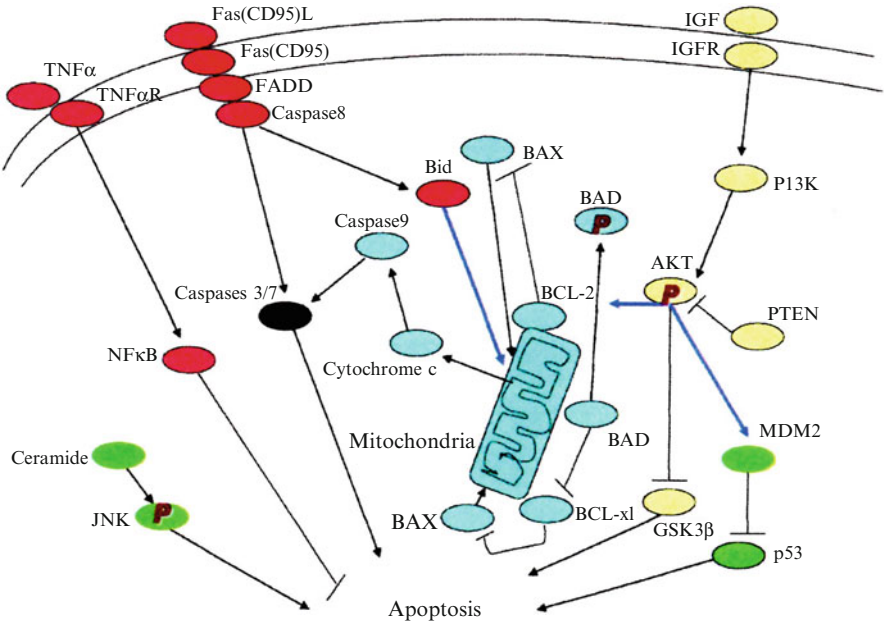

Fig. 1 There are the extrinsic (receptor-mediated, *red*) and intrinsic (mitochondria-driven, *blue*) apoptosis pathways as opposed to the survival proteins such as the P13/Akt signaling circuitry (*yellow*) (From Lee and Pervaiz (2007))

surface death receptor(s), which in turn activate(s) a downstream effector mechanism orchestrated by the caspase family of cysteine proteases (Fig. 1; Finegood et al. 1995; Lee and Pervaiz 2007; Green 2005; Emamaullee and Shapiro 2006). The prototype example of death signaling via the extrinsic pathway is the Fas death receptor, which instigates assembly of the death-inducing signaling complex (DISC), a multi-protein complex comprising the cytoplasmic aspects of the Fas receptor, the adaptor protein FADD (Fas-associated death domain containing protein), and procaspase-8 (Fig. 1; Finegood et al. 1995; Lee and Pervaiz 2007; Green 2005; Emamaullee and Shapiro 2006).

Caspase-3 is a converging point of the apoptotic pathway (Fig. 2; Emamaullee and Shapiro 2006) and its peptide inhibitors have been shown to prevent islet apoptosis and improve islet graft function (Nakano et al. 2004; Brandhorst et al. 2006; Cheng et al. 2008). Caspases are cysteine-containing aspartic acid-specific proteases that exist as zymogens in the soluble cytoplasm, endoplasmic reticulum, mitochondrial intermembrane space, and nuclear matrix (Nicholson and Thornberry 1997). Apoptosis induced by ligation of cell surface receptors like Fas (CD 95) or TNF receptors, "death receptors," represents a pathway controlled by caspases (Chandra et al. 2001). Ligand binding of the receptor causes assembly of a series of proteins of the death-inducing signaling complex, which then activates an apical caspase, procaspase-8 (Peter and Krammer 1998). The resulting events

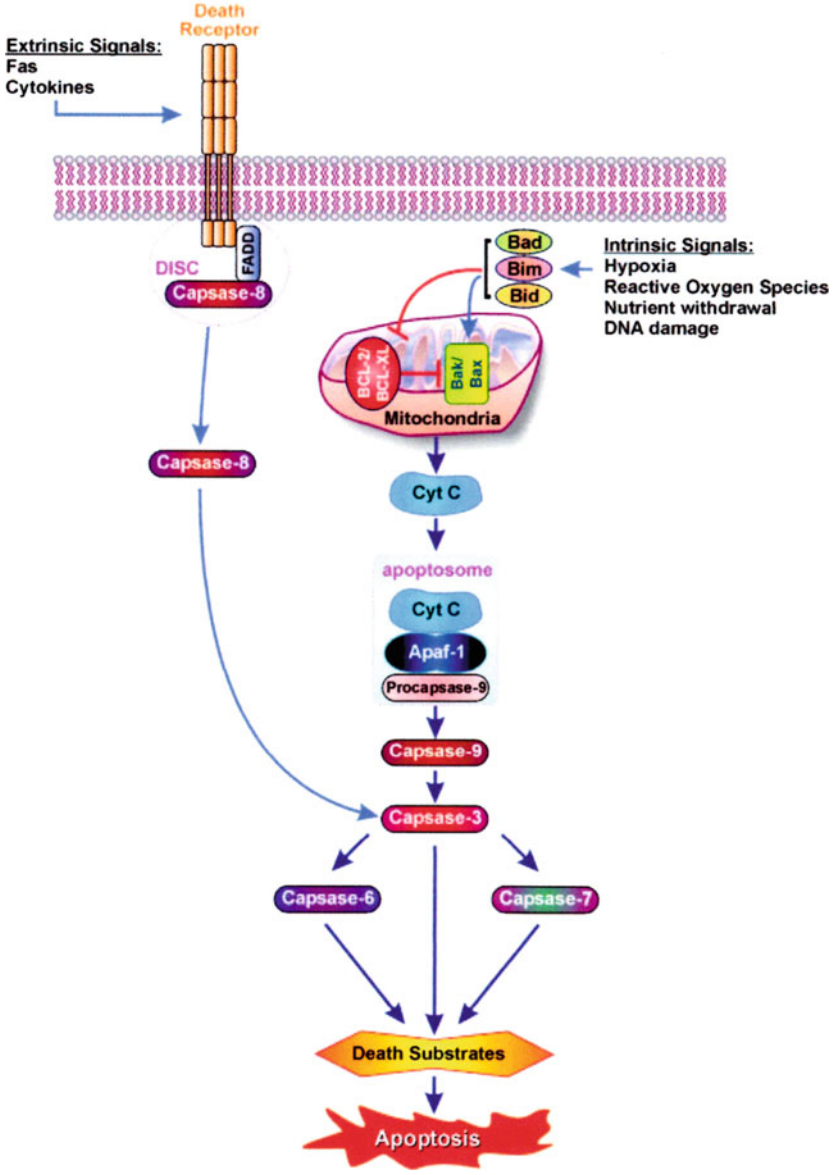

Fig. 2 Extrinsic and intrinsic pathways lead to apoptosis via cytochrome c and "apoptosome". Extrinsic pathway: Fas-Fas L binding leads to the death-inducing signaling complexes (DISC) where the DISC-caspase-8 complex is activated, leading to caspase-3 activation. Intrinsic pathway: pro-apoptotic proteins (Bad, Bid, Bik, Bim) become activated and translocate to the mitochondria, where they bind or inactivate Bcl proteins or form pores in the mitochondrial membrane, which facilitates the release of cytochrome c into the cytosol. Once cytochrome c accumulates in the cytosol, it forms a complex with apocaspase-9 and Apaf-1 to make the "apoptosome", which in turn activates caspase-3. From Emamaulee and Shapiro (2006)

proceed in cascades that caspase-8 induces activation of caspase-3 (Chandra et al. 2001). One of these proteins is a caspase-dependent endonuclease (CAD), which is freed from its inhibitor (ICAD) by caspase-3 and subsequently cuts DNA into oligonucleosomal (180-bp) fragments (Peter and Krammer 1998; Sakahira et al. 1998).

Apoptosis manifests in two major execution programs, downstream of the death signal, the caspase pathway (Gross et al. 1999), and upstream of irreversible cellular damage reside the Bcl family members, which are proteins with both pro-apoptotic and anti-apoptotic properties, playing a pivotal role in the life and death of cells (Figs. 1 and 2) (Green 2005; Emamaullee and Shapiro 2006; Federici et al. 2001). Anti-apoptotic members of the Bcl family, including Bcl-2 and Bcl-xL, blunt intrinsic death signaling by blocking the recruitment of pro-apoptotic members to the mitochondria (Green 2005).

The cumulative data support the notion that high glucose might modulate the balance of the pro-apoptotic caspase family and anti-apoptotic Bcl proteins toward apoptosis, thus leading to β-cell death (Emamaullee and Shapiro 2006; Federici et al. 2001).

Apoptosis in Pancreatic β-Cells by Immunological and Immunocytochemical Study

Apoptosis is a cause of absolute β-cell deficiency in T1DM and relative β-cell deficiency in T2DM. In T1DM, a rapid β-cell loss occurs by T-cell mediated autoimmunity through the interaction of helper (CD 4^+) and cytotoxic ($CD8^+$) cells under the influence of MHC loci such as HLA DR-3 and HLA DR-4, and non-MHC determinants (Lee and Pervaiz 2007). "Insulitis" is surrounded by peri-islet inflammation associated with pro-inflammatory cytokines (Il-β, TNF-σ, interferon-ϒ) release by monocytes, Fas-ligand (CD 178) and autoreactive T-cells, leading to destruction of the β-cells and the onset of hyperglycemia (Hui et al. 2004). β-cells from newly diagnosed T1DM patients have increased cell surface expression of Fas (CD 95), which is then delivered via the Fas L(CD178) expressed on the infiltrating T lymphocytes (Stassi et al. 1997). Expression of dominant-negative Fas or neutralizing antibody to Fas significantly blocks apoptosis, manifests adequate β-cell function, blocks the adoptive transfer of diabetes by primed T-cells, and impedes the course of T1DM development (Willcox et al. 2008). With immunocytochemical staining of 29 cases of pancreata from the subjects with T1DM who died within 18 months of diagnosis, Willcox et al. (2008) showed the following findings: CD 8^+ cytotoxic T cells were the most abundant population in insulitis; CD 68^+ macrophages were also present during insulitis, whereas CD 4^+ cells were present in the islet infiltrates, but were less numerous than CD 8^+ cells or CD 68^+ cells. Both CD 8^+ and CD 68^+ cells may contribute to β-cell death during early insulitis, whereas CD 20^+ cells increased in number during late insulitis, since CD 20^+ cells were recruited late during insulitis (Allison et al. 2005). It is presumed that β-cell damage and destruction are mediated by both CD 8^+ and CD 4^+ (Eisenbarth and Kotzin 2003). Natural killer cells

(NK cells) do not appear to be required for β-cell death (Willcox et al. 2008). Dysregulation of apoptosis is a central defect in diverse murine autoimmune diseases, including the NOD mouse model for human T1DM (Willcox et al. 2008). Mutations in Fas (CD 95) or Fas L (CD 178) have been identified to render lymphoid cells resistant to apoptosis (Hayashi and Faustman 2003). The MHC region of the genome contains immune response genes, which are important for T-cell education and for antigen presentation by both MHC class I and II molecules. Studies of both humans and rodents have suggested that the centrally located MHC class II genes confer the greatest statistical risk for autoimmune diseases. Cellular abnormalities in the expression of maturation markers or in antigen presentation have been detected in NOD mice and human T1DM (Hayashi and Faustman 2003; Rabinovitch and Skyler 1998). The defects include reduced expression of the maturation antigen CD 45 and reduced the abundance of conformational correct complexes of MHC class I molecules and self-peptides on the cell surface (Faustman et al. 1989). Human autoimmune diseases are associated with impairment of antigen processing controlled by the MHC. Cytosolic extracts of lymphocytes from humans with T1DM exhibit altered patterns of the cleavage of test substrates by the proteasome (Hayashi and Faustman 2003). These results are followed by the generation of peptides, which are poorly suited for assembly with MHC class I molecules (Faustman et al. 1991). Clinical studies have shown that the antigen presentation defect correlates with disease expression in identical twins with T1DM (Faustman et al. 1991; Jansen et al. 1995). The certain MHC class II haplotypes, HLA-DR3 or -DR4, or both, are positively associated with 95 % of T1DM cases compared with 20 % of the general population, whereas HLA-DR2 is negatively associated with T1DM (Cucca et al. 2001).

T2DM is characterized by insulin resistance, defective insulin secretion, loss of β-cell mass with increased β-cell apoptosis and islet amyloid deposits (Haataja et al. 2008). In T2DM, insulin resistance with obesity precedes insulin deficiency and plays a considerable role (Reaven 1988), followed by a failure of β-cell insulin production against the progressive insensitivity to insulin. β-cell mass fluctuates according to the body's need for insulin:

1. β-cell mass can increase during insulin resistance
2. Progressive β-cell loss is present in T2DM
3. β-cell deficiency correlates with glucose intolerance
4. β-cell death may directly lead to insulin deficiency when loss of 60 % or more is accompanied by the presence of insulin resistance with obesity (Butler et al. 2003a)

B-cell mass is regulated by a balance of β-cell replication and apoptosis, and islet hyperplasia and new islet formation from exocrine pancreatic ducts (Bonner-Weir 2000a; Leonardi et al. 2003; Marchetti et al. 2004). Elevated caspase-3 and -8 are activated in β-cells from T2DM subjects, which can be inhibited by the anti-diabetic agent metform (Marchetti et al. 2004). Hyperglycemia-induced β-cell apoptosis has been implicated and has been studied

mainly inT2DM (Butler et al. 2003a). Butler et al. extensively studied 124 cases of pancreata from autopsy, including 91 in obese patients (BMI > 27 kg/m^2 : 41 cases – T2DM, 15 cases– impaired fasting glucose, and 35 cases – non-DM cases) and 33 lean patients (BMI <25 kg/m^2 : 16 cases – type 2 DM, 17 cases – non-diabetic cases). The authors measured relative β-cell mass volume using Image-Pro Plus software (Media Cybermetric, Silber Springs, MD, USA), the frequency of β-cell apoptosis by TUNEL (terminal deoxynucleotydyl transferase-mediated dUTP nick-end labeling), and the replication index using Ki-67 immunocytochemical staining (Butler et al. 2003a). With the use of TUNEL staining, only discernible cells with TUNEL-positive nuclei were included as positive cells (Butler et al. 2003a). Obese patients with impaired fasting glucose and T2DM subjects showed 40 % and 63 % less β-cell volume compared with non-diabetic obese and lean controls respectively (Butler et al. 2003a). The frequency of β-cell replication was very low at 0.04–0.06 % of β-cell mass, but the frequency of β-cell apoptosis by TUNEL was increased tenfold in lean patients with DM (0.47 % of the β-cell area) and threefold in obese patients with DM (0.31 % of the β-cell area) compared with the respective non-diabetic control subjects (Butler et al. 2003a). It appears that β-cell replication by Ki-67 is underestimated and that β-cell apoptosis by TUNEL is overestimated, since the replication and apoptosis rates should be about the same in order to maintain the β-cell mass in a delicate balance. The authors conclude in T2DM that β-cell mass is decreased and that the mechanism underlying the β-cell loss is increased β-cell apoptosis (Butler et al. 2003a).

Another Immunocytochemical marker for apoptosis is cleaved caspase-3. Each caspase family protease becomes active when the precursor is cleaved into a large subunit with a molecular mass of ~20 kDa and a small subunit with a molecular mass of ~10 kDa, which then forms a tetramer consisting of two large and two small units (Hirata et al. 1998; Tewari et al. 1995). One of these cleaved caspases is present on activated caspase-3, a ubiquitously distributed caspase that is a main effector caspase of the apoptotic cascade within cells (Marchetti et al. 2004; Hirata et al. 1998). The commercially available polyclonal anti-cleaved caspase-3 detects endogenous levels of the large (17/19 kDa) cleaved caspase-3 resulting from cleavage adjacent to Asp 175 and does not recognize the full length or other cleaved caspases (Cleaved caspase-3 (Asp 175) antibody 2006; Gown and Willingham 2002). Recently, involvement of caspase-3 in both T1DM and T2DM was implicated: in T1DM, Fas(CD 95)-Fas L (CD 178) may be critical for β-cell destruction, as apoptosis in the β-cell clone expressing the human Fas β-cell line is mediated by elevated caspase-3-like activity in tissue culture (Martin and Green 1995) and the frequency of β-cell apoptosis in T2DM pancreatic tissues from autopsy was increased using TUNEL, as described before (Butler et al. 2003a). Our group studied 16 cases of T2DM pancreata compared with 10 control pancreata using rabbit antihuman cleaved caspase-3 (Cell Signaling Technology, Beverly, MA, USA) for immunocytochemicalstaining: the control islets revealed that 4.7 % of total islet cells were cleaved caspase-3-positive islet cells, with large and small

islets positive at 4.1 % and 7.0 % respectively, whereas type 2 DM islets showed a higher level of positive cells at 8.7 % of total islet cells, with large and small islets positive at 7.7 % and 12 % respectively, about twice the control values (Table 1; Tomita 2010a). A double immunostaining for insulin and cleaved caspase-3 supports the notion that β-cell nuclei in the degranulated cytoplasm were positive for cleaved caspase-3 (Tomita 2010a). Cleaved caspase-3-positive islet cells were more frequent in the islets containing fewer amyloid deposits than in the islet cells containing more amyloid deposits; the latter corresponded to the end-stage T2DM islets, which have completed the apoptosis process (Tomita 2010a). Thus, the higher number of cleaved caspase-3 positive islets from T2DM subjects may implicate an accelerated apoptotic cascade, accompanied by increasing amyloid deposits, before proceeding to ultimate β-cell death by overwhelming interstitial amyloid deposits (Tomita 2010a).

We performed cleaved caspase-3 immunocytochemical staining in 8 cases of T1DM pancreata compared with 8 controls. T1DM islets showed higher amounts of cleaved caspase-3-positive cells at 16 % of the total islet cells, with large and small islets positive at 14 %and 17 % respectively, at 3.3, 3.6 and 2.4 times that of the corresponding control values (Table 2; Tomita 2010b). The T1DM islets were a mixture of major small-sized islets consisting of β-cell-poor and σ-cell-rich islets with more caspase-3-positive cells and occasional large islets, consisting of non-β-cells and σ-, δ-, and PP-rich islets with moderately increased caspase-3-positive cells (Fig. 3a, b; Table 2; Tomita 2010b). These increased caspase-3 positive islet cells in T1DM pancreas may correspond to a more accelerated apoptosis cascade than in T2DM islets before entirely exhausting the β-cell mass by apoptosis (Tomita 2010b).

In adult islets, β-cells have an estimated life-span of about 60 days (Bonner-Weir 2000b). Under normal conditions, 0.5 % of control adult β-cells were reportedly to undergo apoptosis (Rhodes 2005). By cleaved caspase-3 immunostaining, about 5 % of β-cells are positive for this apoptosis marker (Martin and Green 1995). Thus, there is a wide range of 0.5–5 % of apoptotic islet cells in the control islets according to TUNEL and cleaved caspase immunocytochemical staining (Butler et al. 2003a; Tomita 2010a; b).

Each of the two immunocytochemical staining methods for apoptosis has advantages and disadvantages. The TUNEL assay is very sensitive and widely used, but it is prone to some pitfalls. The TUNEL technique can label non-apoptotic nuclei showing signs of active gene transcription (Tomita 2010a; Bonner-Weir 2000b; Rhodes 2005; Barett et al. 2001). Tumor necrosis (Sava et al. 2001) and autolysis generate a significantly higher number of DNA ends, which can be positively labeled under certain conditions (Duan et al. 2003). The technical problem of TUNEL is mostly related to DNA strand breaks associated with excessive levels of protease digestion, fixation, and processing procedures (Duan et al. 2003). Therefore, techniques that detect DNA fragmentation are not specific to apoptosis and frequently generate erroneously higher results (Duan et al. 2003; Butler et al. 2003b). Duan et al. carefully studied apoptosis in histological sections of prostatic cancer cell line PC-3 using both TUNEL and cleaved caspase-3

Table 1 Cleaved capase-3 immunostaining in type 2 diabetic islets (From Tomita (Tomita 2010a), with permission)

		Large islets			Small islets			Total islets		
Diabetic subjects, case no.	Age/sex, history[a]	Positive cells	Islet cells	Positive[b] %	Positive cells	Islet cells	Positive[b] %	Positive cells	Islet cells	Positive[b] %
1	49/M_1	3.5	61.4	5.7 (18)	3.1	26.0	11.9 (12)	3.3	87.3	7.1 (30)
2	54/M_2	3.1	65.7	4.8 (14)	2.7	23.4	11.0 (16)	2.9	43.2	6.7 (30)
3	57/M_2	13.8	53.9	25.6 (17)	7.3	24.4	29.9 (13)	11.0	41.1	26.8 (30)
4	62/M_3	5.9	69.0	8.5 (15)	3.1	25.3	12.4 (15)	4.5	47.2	9.5 (30)
5	62/F_3	4.8	89.2	5.4 (18)	2.4	26.3	9.2 (12)	4.0	41.1	9.5 (30)
6	62/F_2	3.5	45.2	5.1 (20)	2.0	24.0	8.3 (10)	3.0	53.2	5.1 (30)
7	62/F_2	3.9	62.9	6.2 (17)	2.4	21.5	11.2 (13)	3.2	45.4	7.0 (30)
8	63/M_3	2.4	57.6	4.2 (16)	2.1	26.3	7.8 (14)	2.3	43.0	5.3 (30)
9	63/F_2	3.4	58.9	5.8 (21)	2.2	26.6	8.4 (9)	3.1	S9.2	6.2 (30)
10	64/M_2	5.9	56.7	10.4 (12)	2.4	21.4	11.1 (18)	3.8	35.8	10.7 (30)
II	66/M_3	2.9	73.1	4.0 (20)	2.3	23.S	10.1 (10)	2.7	36.3	4.8 (30)
12	71/F_4	3.4	71.4	4.8 (18)	2.8	22.7	12.5 (12)	3.2	51.9	6.2 (30)
13	72/F_3	3.6	72.4	5.5 (17)	2.3	21.2	10.6 (13)	3.0	47.1	6.4 (30)
14	75/F_4	6.1	59.0	10.3 (16)	2.9	22.2	12.9 (14)	4.6	41.8	10.9 (30)
15	76/F_4	5.6	56.1	10.0 (18)	2.9	19.1	15.1 (12)	4.0	40.5	10.9 (30)
16	77/1M_3	4.4	68.9	6.3 (19)	2.1	23.8	8.8 (11)	3.5	52.4	6.7 (30)
	Mean	4.8	63.8	7.7[c]	2.8	23.6	12.0[c]	3.9	49.8	8.7 (30)[c]
	SE	0.67	2.55	1.31	0.31	0.55	1.29	0.50	3.97	1.31 (30)
Controls ($n = 10$)										
	Mean	2.9	71.1	4.1[c]	1.7	24.4	7.0[c]	2.6	55.7	4.7 (30)[c]
	SE	0.23	4.30	0.28	0.10	1.10	0.36	0.18	2.78	0.33 (30)

[a]History of diabetes: 1, <5 years; 2, 5–10 years; 3, 11–15 years; 4, > 15 years
[b]Numbers in parentheses are the numbers of islets examined
[c]*p* values calculated with the paired type 2 diabetic and corresponding control values: $p < 0.001$
F female, *M* male, *SE* standard error

Table 2 Cleaved caspase-3 immunostaining in type 1 diabetic islets (From Tomita (Tomita 2010b), with permission)

		Large islets			Small islets			Total islets		
Diabetic subjects		Positive cells	Islet cells	Positive %	Positive cells	Islet cells	Positive %	Positive cells	Islet cells	Positive %
Case	Age/sex									
1	18/M	7.3	37.3	19.9 (12)	5.4	22.7	23.7 (18)	6.1	28.6	21.3 (30)
2	35/F	9.2	62.1	14.8 (18)	3.3	38.3	8.7 (12)	6.8	48.6	14.0 (30)
3	43/F	2.8	44.9	6.3 (13)	2.3	14.1	16.3 (17)	2.5	30.3	8.4 (30)
4	50/F	21.8	73.3	29.7 (20)	6.4	27.2	23.5 (10)	16.7	57.9	28.8 (30)
5	61/F	7.7	43.1	18.0 (15)	5.4	27.2	19.9 (15)	6.6	35.1	18.7 (30)
6	75/F	3.6	50.6	7.1 (9)	5.4	37.4	14.5 (21)	S.S	41.4	13.2 (30)
7	75/F	5.1	SI.6	9.8 (18)	3.7	28.1	13.1 (12)	4.5	42.2	10.7 (30)
8	75/F	4.4	44.3	9.9 (15)	3.5	29.4	11.9 (15)	3.9	39.4	9.9 (30)
	Mean	7.7[a]	50.9	14.4[a]	4.5[a]	26.8	16.5[a]	6.6[a]	40.4[a]	15.7 (30)[a]
	SE	2.1	3.75	2.80	0.50	2.60	1.94	1.50	3.40	2.45 (30)
Controls ($n = 8$)										
	Mean	2.9[a]	72.4	4.0[a]	1.7[a]	25.0	6.8[a]	2.7[a]	58.5[a]	4.7 (30)[a]
	SE	0.29	5.32	0.39	0.13	1.20	0.43	0.19	2.91	0.40 (30)

Numbers in parenthesis are the numbers of islets examined

[a] p values calculated with the corresponding control values are: $p < 0.001$

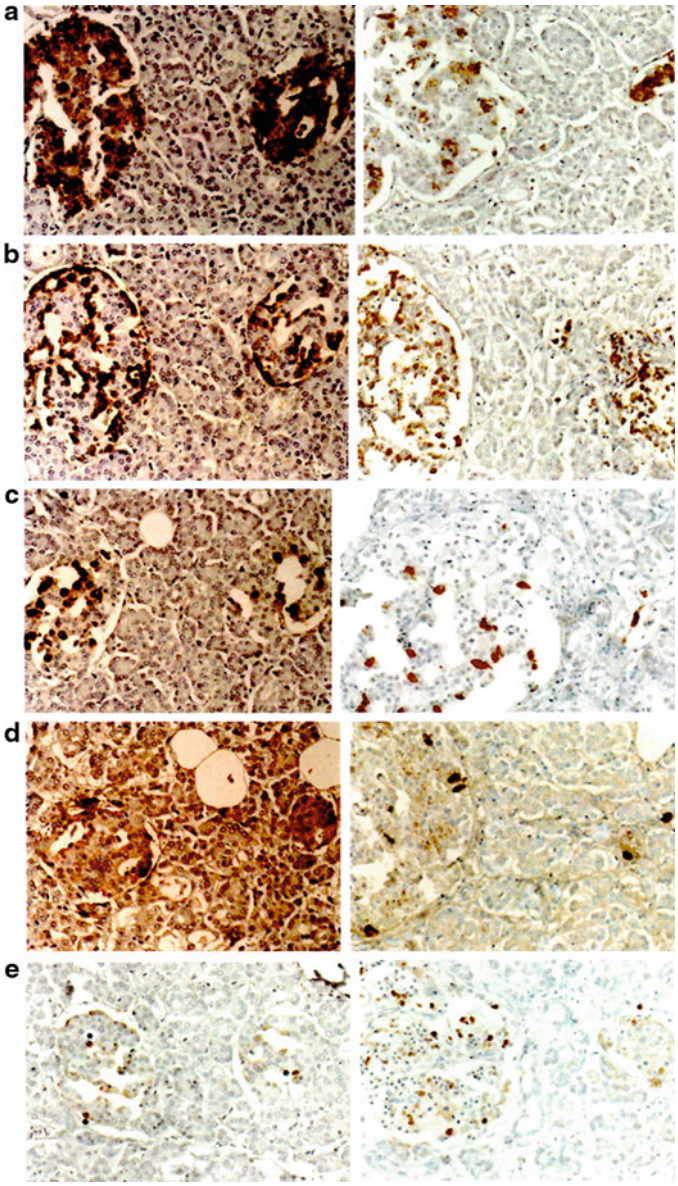

Fig. 3 Normal islets (*left column*), and β-cell-less and σ-cell-rich type 1 diabetic islets (*right column*) by cleaved caspase-3 immunostaining. (*Left column*) Normal islets consist of major β-cells (80 %), next major σ-cells (10 %) and minor δ-cells (<7 %), and PP cells (<5 %). There is about 5 % cleaved caspase-3-positive nuclear staining for normal islet cell nuclei. (*Right column*) β-cell-poor (3–20 %) and σ-cell-rich (80 %) type 1 diabetic islets are the major islet cells with relatively increased δ-cells (>7 %) and PP cells (>5 %). About 17 % of islet cell nuclei are positive for cleaved caspase-3. (**a**) Insulin, (**b**) glucagon, (**c**) SRIF, (**d**) pancreatic polypeptide, (**e**) cleaved caspase −3 immunostained (From Tomita (2010b), with permission)

immunostaining (Duan et al. 2003). TUNEL staining depends on the optimal concentration of terminal deoxynucleotydyl transferase (TdT), with which less diluted (1:7) solution positively stained the majority (>90 %) of the transplanted cancer cells compared with an optimally diluted solution (1:16) stained adequate numbers (< 2 %) of TUNEL-positive cancer cells (Duan et al. 2003). By comparing TUNEL and cleaved caspase-3 immunostaining, the authors concluded that cleaved caspase-3 immunostaining was an easy, sensitive, and reliable method for detecting and quantifying apoptosis and that a good correlation of apoptotic indices existed between caspase-3 immunostaining and the TUNEL assay (Duan et al. 2003). We agree with Duan et al. that cleaved caspase-3 immunostaining is an easier and more reliable immunostaining method than TUNEL, although the former is not as commonly used as the latter. Cleaved caspase-3 immunostaining also has its own pitfalls, as does immunocytochemical staining in general: good fixation with optimal tissue preservation and proper tissue processing are mandatory using an adequate concentration of antibody for optimal cleaved caspase-3 immunostaining, and the stained sections have to be critically evaluated for discernible nuclear positive staining by excluding false-positive staining in tissue debris and autolytic tissues (Sava et al. 2001; Duan et al. 2003; Butler et al. 2003b; Tomita 2009).

Our group studied cleaved caspase-3 immunostaining in 37 cases of pancreatic endocrine tumors (PETs) (Tomita 2009): among 15 cases of insulinomas, 5 cases were positive and 10 cases were negative for cleaved caspase-3 (67 %). Among non-β cell PETs, 2 out of 2 glucagonomas (100 %), 6 out of 9 pancreatic polypeptidomas (67 %), 10 out of 12 gastrinomas (83 %), and 3 out of 3 non-functioning PETs (100 %) were negative for cleaved caspase-3, with a total of 21 out of 24 non-β cell tumors (88 %) being negative. This results supports the notion that 88 % of non-β cell PETs are potentially malignant according to the absence of cleaved caspase-3 immunostaining (Tomita 2009) and that negative cleaved caspa-3 immunostaining may be a candidate for being a malignant marker for PETs (Tomita 2009). So far, positive activated caspase-3 immunocytochemical staining appears to be a good marker for β-cell apoptosis.

Amyloid Theory on β-Cell Apoptosis in Type 2 Diabetes

The amyloid deposit in pancreatic islets is a characteristic finding for T2DM (Ehrlich and Ratner 1961; Hoppener et al. 2000; Hull et al. 2004). The chief constituent of amyloid deposits is islet amyloid polypeptide (IAPP) (Butler et al. 2003b; Ehrlich and Ratner 1961; Hoppener et al. 2000; Hull et al. 2004; Westermark et al. 1986; Cooper et al. 1987). IAPP is concomitantly co-secreted with insulin from β-cells into the blood in response to glucose-induced insulin secretion (Cooper et al. 1987; Kahn et al. 1999). IAPP hyposecretion is well established in T1DM and insulin-requiring T2DM (Cooper et al. 1987; Kahn et al. 1999):a synthetic IAPP, pramlintide (Cucca et al. 2001; Haataja et al. 2008;

Reaven 1988) (pro-hIAPP) has been used for treating both T1DM and insulin-requiring T2DM, together with insulin injection, for better glycemic control (Kruger et al. 1999; Weyer et al. 2001; Buse et al. 2002; Fineman et al. 2002). We studied the transformation of soluble IAPP in β-cell granules to insoluble amyloid deposits in islet stroma by using immunocytochemical staining for IAPP (Tomita 2012): a ratio of IAPP-positive cells to insulin-positive cells was 43 % in control islets compared with 25 % inT2DM islets, in support of the decreased IAPP serum levels in T2DM, since the source of serum IAPP is the β-cell granule (Tomita 2012). Pancreatic extracts from normal humans contain less IAPP, at 10 % that of insulin, and the fasting serum IAPP level in non-obese controls is 2.0 μmol/l, 5 % that of the insulin level of 48 μmol/l (Clark and Nilsson 2004). As IAPP-positive β-cell cytoplasm decreased, stromal islet amyloid deposits increased in T2DM islets (Tomita 2012). In advanced stages of islets with amyloid deposits, weakening IAPP-immunostaining in the residual β-cells co-existed with the adjacent fine amyloid fibrils, which were positively immunostained for IAPP (Fig. 4f; Tomita 2012). This finding suggests that disappearing soluble IAPP from β-granules are transformed to amyloid fibrils in the adjacent islet stroma. Freshly prepared IAPP oligomers can form non-selective iron permeable membrane pores, leading to increased Ca^{2+}concentration, endoplasmic reticulum stress, and apoptosis (Haataja et al. 2008). In early stages of islet amyloidosis, round to sickle-shaped β-cell cytoplasm without a nucleus was strongly immunopositive for IAPP (Fig. 4f; Tomita 2012). This cytoplasm probably represents an early fibrillar form of amyloidogenic β-cell cytoplasmic proteins consisting of smaller IAPP polymers, which may subsequently form extracellular amyloid β-sheets. IAPP has the propensity to form membrane-permeable toxic oligomers, but it remains unclear why amyloidogenic proteins form oligomers in vivo, what their exact structure is, and to what extent these oligomers play a role in the cytotoxicity that is now often called unfolded protein disease (Haataja et al. 2008; Ehrlich and Ratner 1961; Clark and Nilsson 2004; Clark et al. 1988; Mirzabekov et al. 1996; O'Brien et al. 2010; Jurgens et al. 2011).

According to the toxic oligomer theory, β-cells in T2DM are killed through IAPP-induced damage of the β-cell membrane (Clark et al. 1988; Mirzabekov et al. 1996; O'Brien et al. 2010; Jurgens et al. 2011; Anguiano et al. 2002; Jansen et al. 1999; Engel et al. 2008). These toxic oligomers (not monomers or mature amyloid fibrils) eventually form the end product of β-sheets containing IAPP, Aβ, synuclein, transthyretin, and prion protein, and share a common epitope (Kayed et al. 2003; Tomita 2005). Amyloid p is a universal immunological and immunocytochemical marker for all forms of amyloidosis (Kayed et al. 2003; Tomita 2005). Freshly prepared intermediate IAPP polymers (25–6,000 IAPP molecules) have a toxic effect on β-cells, but do not exhibit a toxic effect on σ, δ, and PP islet cells (Haataja et al. 2008; Ehrlich and Ratner 1961; Tomita 2012; Clark and Nilsson 2004). Water-soluble IAPP with low molecular weight in β-cell granules is readily and densely immunostained for IAPP, whereas water-insoluble amyloid fibrils containing IAPP polymers are only weakly immunostained (Ehrlich and Ratner 1961; Kahn et al. 1999). The cause of this lack of strong IAPP immunostaining for

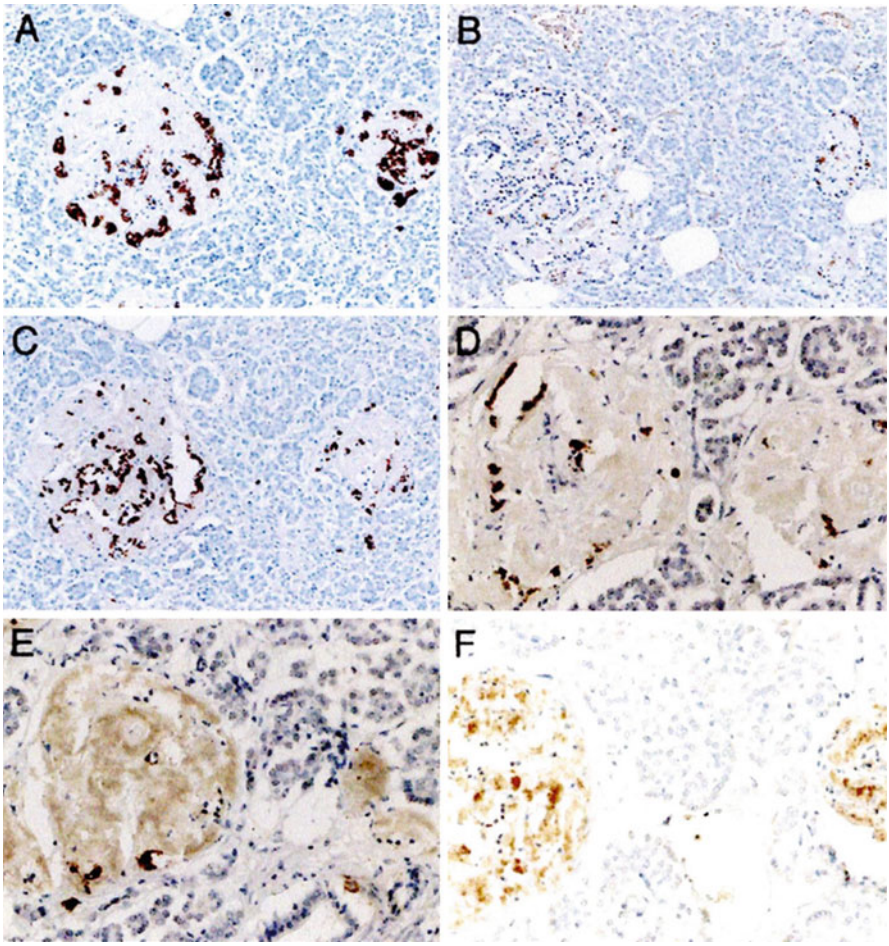

Fig. 4 Amyloid deposits in type 2 diabetic islets. Diabetic islets are occupied by amyloid deposits in 95 % (**a**–**c**) and are also occupied by > 99 % (**d**–**f**). The residual β-cells with plump cytoplasm (**a**) are minor cells and σ-cells with small and compact cytoplasm (**c**) are major islet cells. IAPP immunostaining with 1:400 diluted IAPP antibody reveals weak staining in the islets occupied by amyloid at 95 % (**b**) whereas islets occupied by amyloid > 99 % reveal stronger IAPP immunostaining with round to sickle-shaped positive immunostaining in the β-cell cytoplasm (**f**). Thus, IAPP immunostaining revealed various immunostaining for IAPP-depleted islets (**b**) to a mixture of dense IAPP-positive cytoplasm and weaker interstitial fibers to darker interstitial fibers (**e**) in the same patient. (**a**) and (**d**) Insulin, (**c**) glucagon, (**b**), (**e**), and (**f**) IAPP antibody at 1:400 immunostained for IAPP (From Tomita (2012a), with permission)

amyloid fibrils is not clear, but one likely reason is that the unexposed epitope of IAPP within the water-insoluble amyloid fibrils forms β-sheet conformation, into which IAPP antibody cannot penetrate to bind (Anguiano et al. 2002). Antibodies generated to this epitope using toxic $A\beta_{1-40}$ bind toxic oligomers generated from

the other amyloidogenic proteins in cell culture, and block the cytotoxic effects of each of these diverse oligomers (Kayed et al. 2003; Ritzel and Butler 2003).

Butler and his associates tested the hypothesis that β-cells are preferably vulnerable to hIAPP-induced apoptosis with isolated human islets in tissue culture: apoptotic cells by TUNEL were increased fivefold after incubation with 40 μmol/l hIAPP compared with control islets (Ritzel and Butler 2003). Further, in T2DM islets, the apoptotic cells in islets were adjacent to each other and contained two separate nuclei, suggesting that cells might undergo apoptosis shortly after mitosis (Ritzel and Butler 2003). More studies are needed to answer the question whether amyloid deposits are the cause or the result of T2DM or amyloid deposits are both the cause and the results of T2DM.

Hyperglycemia-Induced Apoptosis in β-Cells

Glucose is the main fuel that stimulates insulin secretion and mechanisms of glucose-induced insulin secretion is the fundamental principle for the pathophysiology of insulin secretion (Tomita et al. 1974; Tomita and Scarpelli 1977), and chronic hyperglycemia causes β-cell glucose toxicity and eventually leads to β-cell apoptosis (Federici et al. 2001). Glucose-induced insulin secretion with isolated perifused islets typically presents both an initial insulin secretion within several minutes and a second larger sustained secretion in about 20 min after exposure to a high glucose medium, in which the initial small secretion occurs before glucose is metabolized (Tomita et al. 1974; Tomita and Scarpelli 1977). This early peak of insulin secretion has brought about the glucose sensor theory by Matschinsky et al., who proposed glucokinase (GK, hexokinase IV) as a glucose sensor (Begoya et al. 1986; Shimizu et al. 1988; Matschinsky 1990, 1995; Matschinsky et al. 1993). GK constitutes a key component of mammalian glucose-sensing machinery (Matschinsky 1990, 1995; Matschinsky et al. 1993). In the liver, GK controls glycogen synthesis and glucose output, whereas in pancreatic islets it regulates insulin secretion (McDonald et al. 2005). Subsequent studies showed that glucose-sensing mechanisms in the β-cells are divided into the two components: proximal events of glucose entry and metabolism, and the distal mechanisms of insulin secretion, spanning from mitochondrial signal generation and initiation of electrical activity to the ultimate effectors of β-granule exocytosis (McDonald et al. 2005). The proximal sensing and metabolic signal generation includes the following:

1. Glucose equilibrates rapidly across the β-cell membrane owing to expression of the high-capacity, low-affinity glucose-transporter-2.
2. After glucose has entered the β-cells, it is phosphorylated to glucose-6-phosphate by the high K_MGK, which constitutes the flux-determining step of glycolysis and is considered a glucose sensor.
3. Once the glucose is phosphorylated, it is metabolized by glycolysis to pyruvate, NADH, and ATP (Innedian 1993; De Vos et al. 1995).

Pyruvate is the main end product of glycolysis in β-cells and is essential for mitochondrial ATP synthesis, and is suggested to be an important modulator of insulin secretion (McDonald et al. 2005). In the mitochondrial matrix, pyruvate is oxidized by pyruvate dehydrogenase to form acetyl-CoA. Acetyl-CoA enters the TCA cycle to undergo additional oxidation steps, generating CO_2 and reducing equivalents, $FADH_2$ and NADH (Innedian 1993; De Vos et al. 1995; Newgard and McGarry 1995). Oxidation of reducing equivalents by the respiratory chain is coupled with the extrusion of protons from the matrix to the outside of the mitochondria, thereby establishing the electrochemical gradient across the inner mitochondrial membrane (Fridlyand and Philson 2010). The final electron acceptor of these reactions is molecular oxygen (Fridlyand and Philson 2010). The distal sensing of metabolic signals includes the following:

1. In the absence of stimulatory glucose (<5 mmol/l) rodent β-cells are electrically silent, with a resting membrane potential of ~70 mV.
2. Reduction of the resting K^+ conductance by stimulated glucose leads to membrane depolarization and initiation of electrical activity characterized by slow wave depolarization.
3. ATP-sensitive K^+ channels set the β-cell membrane potential and closure of these leads to depolarization.
4. Membrane depolarization triggers action potential firing and opening voltage-dependent Ca^{2+} channels, leading to Ca^{2+} influx, which triggers β-granule exocytosis.
5. Action potential is terminated by the opening of voltage-dependent K^+ channels, which limit Ca^{2+} entry and thus insulin release (McDonald et al. 2005; Innedian 1993; De Vos et al. 1995; Newgard and McGarry 1995; Fridlyand and Philson 2010).

A comprehensive mathematical model of β-cell sensitivity to glucose predicts the special role of the mitochondrial control mechanism in insulin secretion and reactive oxygen species (ROS) generation in the β-cells (McDonald et al. 2005; Newgard and McGarry 1995; Fridlyand and Philson 2010). A failure of the insulin secretory machine results in insulin deficiency and subsequent hyperglycemia. Mutations in the GK gene lead to impaired insulin secretion in maturity onset diabetes of the young T2DM (MODY2), an autosomal-dominant DM, and this fact further supports the role of GK as a glucose sensor in diabetes (Porter and Barette 2005; Rossetti et al. 1990; DeFrenzo 1999). The individuals with mutations within the GCK gene on chromosome 7p (GCK-MODY2) have an abnormal glucose sensing with stable mild hyperglycemia and do not predispose to complications of diabetes (Froguel et al. 1992). The bulk of detailed information on hyperglycemia-induced apoptosis has been derived from controlled cultured human and rodent islets in vitro (Federici et al. 2001; Leahy et al. 1992). In order to study glucose toxicity as a deleterious effect of chronic hyperglycemia on β-cells in vitro, Federici et al. cultured 400 isolated human islets per batch for 5 days in a low-glucose (5.5 mmol/L) and a high-glucose medium (16.6 mmol/L) for studying any possible high glucose effects on Bcl-2 (β-cell lymphoma 2) family gene expression by RT-PCR (Federici et al. 2001).

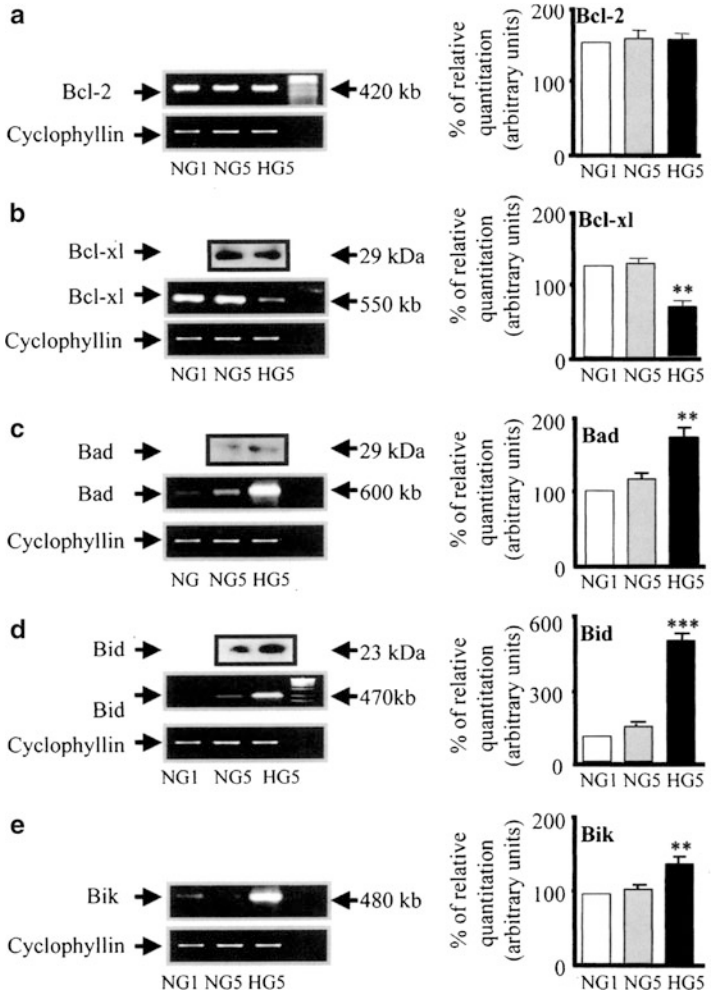

Fig. 5 Bcl family gene regulation in human islets cultured in high (HG, 16.7 mM) versus normal glucose (NG, 5.5 mM). (**a**) Bcl-2, (**b**) Bcl-xL, (**c**) Bad, (**d**) Bid, and (**e**) Bik RNA. *NG1* incubated in NG for 1 day, *NG5* incubated in NG for 5 days, *HG5* incubated in HG for 5 days (From Federici et al.(2001))

1. Bcl-2 was highly expressed in both low- and high-glucose media and expression did not change between a high- and a low-glucose condition (Fig. 5a) (Federici et al. 2001).
2. However, Bcl-xL was reduced by 45 % in high-glucose cultured islets compared with low-glucose cultured islets (Fig. 5b; Federici et al. 2001), supporting the notion that a reduction in Bcl-xL protein expression was due to high glucose exposure (Fig. 5b; Federici et al. 2001).
3. Bad, Bid, and Bik were expressed in low-glucose medium at low levels. Bad gene expression was greatly increased with a high-glucose medium and the

Bad protein level increased 80 % as well compared with low-level glucose (Fig. 5c–e; Federici et al. 2001).

Bid gene expression was markedly increased with high-glucose medium and so was Bik protein (Fig. 5d–e; Federici et al. 2001). Thus,anti-apoptotic Bcl-2 was unaffected by high glucose, but the pro-apoptotic genes, Bad, Bid, and Bik markedly increased in high-glucose cultured islets (Fig. 5c–e; Federici et al. 2001). These data support the notion that chronic high-glucose incubation in vitro modulates the balance of pro-apoptotic and anti-apoptotic Bcl proteins toward apoptosis, thus leading to eventual β-cell death (Federici et al. 2001).

Recent Studies on β-Cell Apoptosis

The main studies on apoptotic Bcl proteins were performed in artificially forced overexpression experiments in vitro (Zhou et al. 2000; Ou et al. 2005; Saldeen 2000; Chan and Yu 2004; Hengartner 2000). Caspases are activated in a hierarchy order, in which initiator caspases (caspase-8 and -10) function to cleave effector caspases (caspase-3 and -7), the latter in turn degrade a number of intercellular protein substrates and lead to the classical morphological changes of apoptosis (Figs. 1 and 2). Extracellular events present during the inflammatory response through the release of cytokines, including INF-σ, Il-1β, and interferon-ϒ by infiltrating leukocytes or direct cytotoxic T-cell engagement, can initiate apoptosis (Emamaullee and Shapiro 2006). These intrinsic cues function via surface molecules in the death receptor pathway, where specific ligand-receptor binding such as TNF–TNF receptor binding, Fas (CD 95)-Fas L (CD 178) binding lead to receptor clustering, adaptor molecule recruitment, and formation of DISC) (Fig. 2; Emamaullee and Shapiro 2006). Caspase-8 associates with DISC complex, where it is activated, released, and leads to effector activation for caspase-3 (Boatright and Salvesen 2003). Intracellular cues such as DNA damage, hypoxia, nutrient deprivation or reactive oxygen species (ROS) function via the mitochondrial pathway, which is tightly modulated by the Bcl-2 proteins (Fig. 2; Emamaullee and Shapiro 2006). In healthy cells, pro-apoptotic Bcl-2 proteins (Bim, Bid, Bad, Bax, and Bak) are present in the mature form, while anti-apoptotic Bcl-2 proteins (Bcl-2 and Bcl-xL) are constitutively active and reside in the outer membrane of mitochondria (Fig. 2; Emamaullee and Shapiro 2006; Hui et al. 2004; Boatright and Salvesen 2003). Following an intrinsic cue, pro-apoptotic Bcl proteins become activated and translocate to the mitochondria, where they result in inactivation of anti-apoptotic Bcl-2 proteins or form pores in the mitochondrial membrane, which facilitates the release of cytochrome c into the cytosol (Fig. 2; Emamaullee and Shapiro 2006). When cytochrome c accumulates in the cytosol, it forms a complex with procaspase-9 and Apaf-1 to form the "apoptosome," which in turn activates caspase-3 (Fig. 2; Emamaullee and Shapiro 2006). Both intrinsic and extrinsic signaling cascades converge at the point of caspase-3 activation, which is often considered the "point of no return" in apoptosis (Emamaullee and Shapiro 2006).

Apoptosis can only occur when the concentration of pro-apoptotic Bcl proteins exceeds that of anti-apoptotic proteins at the mitochondrial membrane of the intrinsic pathway (Emamaullee and Shapiro 2006).

Recent studies unveiled new roles for certain Bcl-2 family members in other physiological pathways, including glucose metabolism (Hengartner 2000), Ca^{2+} homeostasis (Daniel et al. 2003, 2008; Karbowski et al. 2006), and mitochondrial morphology (Daniel et al. 2008; Karbowski et al. 2006). BAD nucleates a core complex at the mitochondria-containing GK, the product of the gene associated with MODY2 (Shimizu et al. 1988). BAD resides in a GK-containing complex that regulates glucose-driven mitochondrial respiration (Daniel et al. 2008). Daniel et al. studied new insights into the role of BAD in glucose-stimulated insulin secretion by β-cells from $Bad^{-/-}$ compared with $Bad^{+/+}$ islets (Fig. 6) (Daniel et al. 2008): perifused islets from $Bad^{-/-}$ mice secreted significantly less insulin in response to 25 mM of glucose compared with Bad $^{+/+}$ mouse islets, at about 40 % for the first-phase secretion (0–15 min) and 60 % for the second-phase secretion (15–40 min) (Fig. 6a, b; Daniel et al. 2008); however, the total insulin secretion by 30 mM KCl (Fig. 6a; Daniel et al. 2008), glucose-induced (25 mM) changes in the ATP/ADP ratio (Fig. 6c; Daniel et al. 2008) and insulin secretion in response to 10 mM KIC and 25 mM tolbutamide (Fig. 6d; Daniel et al. 2008) were compatible in both $Bad^{+/+}$ and $Bad^{-/-}$ islets. GK activity in homogenates of $Bad^{-/-}$ islets was about 25 % that of $Bad^{+/+}$ islets (Fig. 6e; Daniel et al. 2008). Insulin secretion by $Bad^{-/-}$ islets was considerably lower than that of $Bad^{+/+}$ islets in response to glucose concentration of 15–25 mM (Fig. 6f; Daniel et al. 2008). A signature of β-cell dysfunction associated with impaired GK activity is a loss on glucose sensing (Fig. 6e, f; Daniel et al. 2008) and $Bad^{-/-}$ islets require more glucose to secrete insulin than wild-type islets (Fig. 6d, f; Daniel et al. 2008). Glucose-induced changes in mitochondrial membrane potential are significantly reduced in $Bad^{-/-}$ β-cells (Daniel et al. 2008). The reduction does not cause a global impairment of mitochondrial respiratory chains, as both genotypes (Bad $^{+/+}$ and Bad $^{-/-}$) show comparable changes in membrane potential to KIC (Daniel et al. 2008). Basal $[Ca^{2+}]_i$ at 3 mM glucose is compatible for both genotypes, indicating that basic control mechanisms for Ca^{2+} handling are presented in $Bad^{-/-}$ cells (Daniel et al. 2008). $Bad^{-/-}$ islets do not present a stepwise increase in insulin secretion when exposed to incrementally increased glucose concentration (Fig. 6f; Daniel et al. 2008). The efficiency of glucose and other fuel secretagogues to stimulate insulin secretion correlates with their capacity to hyperpolarize the mitochondrial potential (Karbowski et al. 2006; Antinozzi et al. 2002). In β-cells, the characteristic features of glucose-driven mitochondrial respiration correspond to those of glucose phosphorylation by GK (Liang et al. 1996; Berggren and Larson 1994). Glucose-induced changes in the mitochondrial membrane potential were significantly reduced in $Bad^{-/-}$ β-cells and the average $[Ca^{2+}]_i$ response to 11 mM glucose was significantly lower in $Bad^{-/-}$ β-cells (Daniel et al. 2008). The BH3 domain of BAD is an amphipathic σ-helix, which binds to Bcl-2 and Bcl-xL and neutralizes the apoptotic activity of BAD (Daniel et al. 2008). An intact BH3 domain is required for glucose-stimulated insulin secretion by its binding to Bcl-2 and Bcl-xL. Treatment with BAD $SAHB_A$

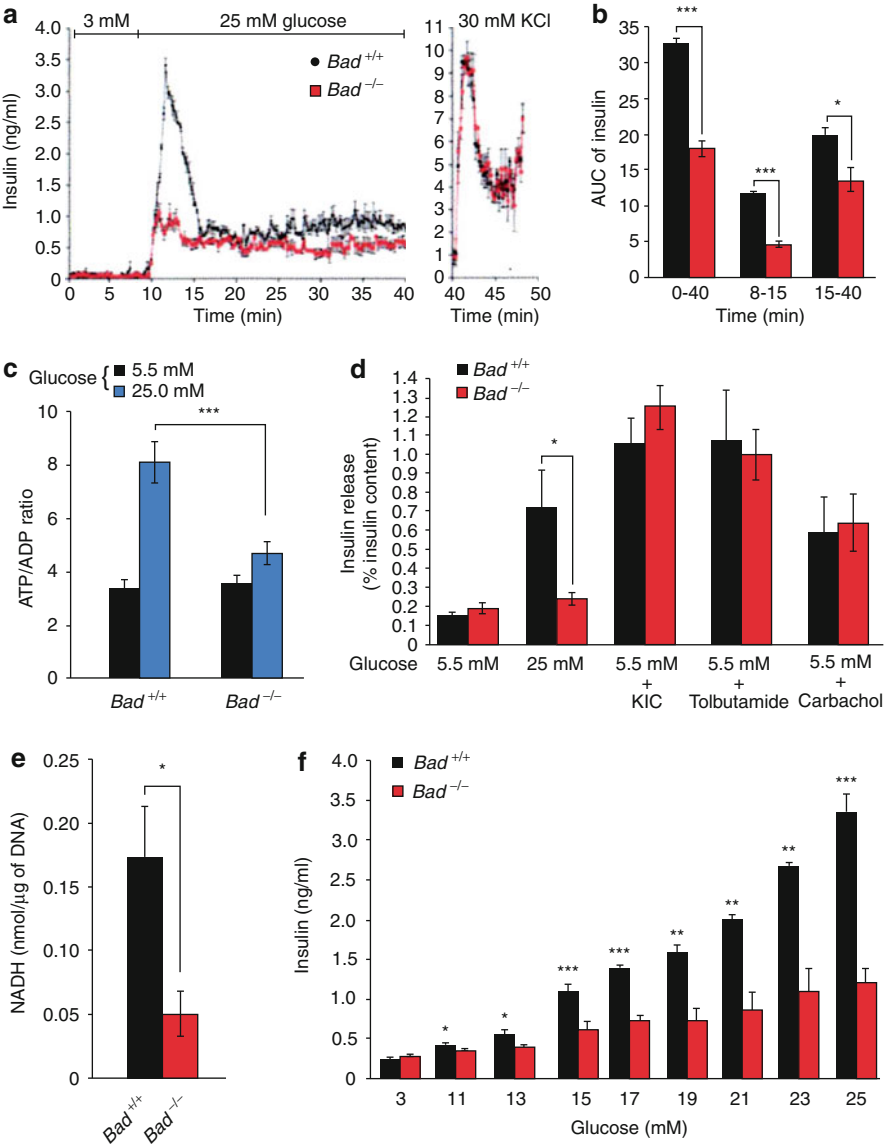

Fig. 6 Characterization of the insulin secretion defect in Bad$^{-/-}$ islets. (**a**) Perifused islets from Bad$^{-/-}$ mice (*red*) with 25 mM glucose secreted significantly less insulin compared with Bad$^{+/+}$ islets (*black*). (**b**) Insulin secretion throughout the perifusion (0–40 min), first phase (8–15 min) and second phase (15–40 min). (**c**) Glucose-induced changes in the ATP/ADP ratio in Bad$^{+/+}$ and Bad$^{-/-}$ islets – 5.5 mM (*black*), 25 mM (*blue*). (**d**) Insulin secretion in response to glucose 5.5 mM and 25 mM, 10 mM σ-ketoisocaproate (KIC), 0.25 mM tolbutamide and carbachol. (**e**) GK activity in homogenates of primary islets isolated from Bad$^{+/+}$ (*black*) and Bad$^{-/-}$ mice (*red*). (**f**) Insulin secretion by Bad$^{+/+}$ (*black*) and Bad$^{-/-}$ (*red*) islets perifused with incrementally increasing concentrations of glucose (From Daniel et al. (2003))

(stabilized σ-helix of Bcl-2 domain) restored the secretion defect in $Bad^{-/-}$ islets (Daniel et al. 2008), underscoring the sequence specificity of the BAD SAHB effect. Mutating the conserved leucine and aspartic acid residues of the BAD BH3 sequence (BAD $SAHB_{A(L,D\rightarrow A)}$) abrogated its effect on insulin secretion (Daniel et al. 2008). In their extensive elegant study, Daniel et al. identified GK as a novel and direct physiological target of the BAD BH3 domain in β-cells and that phosphorylation within the BH3 domain drives the metabolic functionality of BAD and serves as a physiological switch of its apoptotic and metabolic effects (Daniel et al. 2008). They demonstrated that Bad plays a physiological role in β-cells, aside from its role in β-cell apoptosis, and specifically that Bad phosphorylated at serine 155 promotes glucose-stimulated insulin secretion via interactions with GK. The therapeutic application of BAD $SAHB_s$ and other BAD BH3 may be applied in restoring insulin secretion. A phosphorylated BAD SAHB that activates glucose-stimulated insulin secretion, but does not affect the survival function of Bcl-xL, may serve as a prototype therapeutic in diabetes and islet transplantation (Daniel et al. 2003, 2008; Karbowski et al. 2006; Antinozzi et al. 2002; Liang et al. 1996; Berggren and Larson 1994).

Recently, Luciani et al. have demonstrated that chemical and genetic loss-of-function of anti-apoptotic Bcl-2 and Bcl-xL significantly augments glucose-dependent metabolic and Ca^{2+}signals by extending the role of Bcl-2 and Bcl-xL through suppressing glucose signaling in pancreatic β-cells (Luciani et al. 2013). Prolonged Bcl antagonism induced dose- and time-dependent cell death in human and mouse islet cells (Fig. 7; Luciani et al. 2013). The enhancement of β-cell glucose response by Bcl-2 was studied using genetically ablated mice as a genetic loss-of-function approach. Real-time PCR confirmed the loss of $Bcl\text{-}2^{-/-}$ β-cells and $Bcl\text{-}2^{+/-}$ islets compared with $Bcl^{+/+}$ control islets with no compensatory increase in Bcl-xL (Fig. 7a; Luciani et al. 2013). $Bcl\text{-}2^{-/-}$ and $Bcl^{+/-}$ β-cells showed enhanced sensibility to glucose (Fig. 7b, c; Luciani et al. 2013). Intact islets from $Bcl\text{-}2^{-/-}$ mice also showed increased Ca^{2+} and metabolic NAD(P)H response to glucose (Fig. 7e, f; Luciani et al. 2013). Perifused $Bcl\text{-}2^{-/-}$ islets to glucose revealed significantly increased insulin secretion compared with $Bcl\text{-}2^{+/+}$ islets (Fig. 7g; Luciani et al. 2013). Loss of Bcl-2 had no effect on responses to depolarization with KCl (Fig. 7d, h; Luciani et al. 2013). The immediate augmentation of glucose-induced Ca^{2+} responses in Bcl-2 heterozygous β-cells indicates that effects were dependent on gene dosage (Fig. 7b, c; Luciani et al. 2013). Inducible deletion of Bcl-xL mouse β-cells also increased glucose-stimulated NAD(H)P and Ca^{2+} responses and resulted in an improved in vivo glucose tolerance in the Bcl-xL knockout mice (Luciani et al. 2013). These results suggest that prosurvival Bcl proteins normally dampen the β-cell response to glucose and physiology of β-cells (Luciani et al. 2013). Bcl proteins directly affect mitochondrial proteins in the β-cells. The study of anti-apoptotic activates of Bcl-2 and Bcl-xL has shown that they interact with mitochondria via their BH4 domain (Luciani et al. 2013; Real et al. 2004). A cell-permeable Bcl-xL BH4 domain peptide triggers cytosolic and mitochondrial Ca^{2+} fluctuations in β-cells, which may result from direct mitochondrial actions of the BH4 domain and endoplasmic reticulum Ca^{2+} release

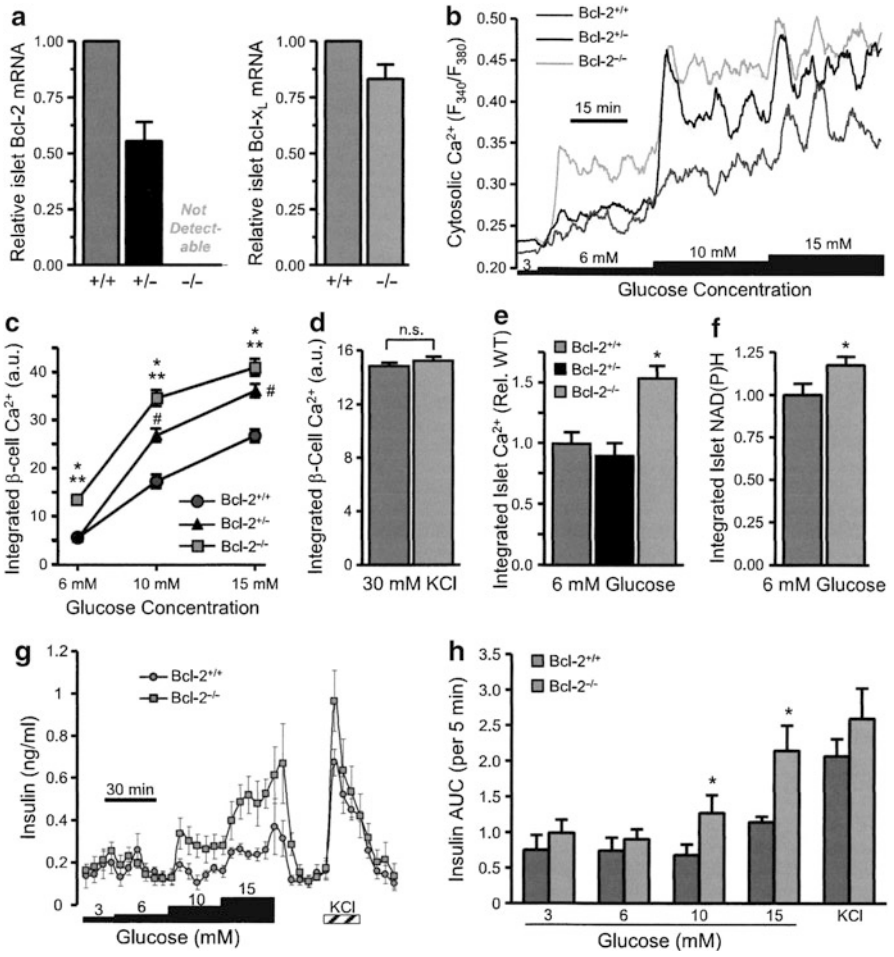

Fig. 7 Loss of Bcl-2 enhances β-cell glucose responses. (**a**) Quantitative PCR quantification of Bcl-2 and Bcl-xL mRNA levels in islets from Bcl-$2^{+/-}$ and Bcl-$2^{-/-}$ islets compared with Bcl-$2^{+/+}$ islets. (**b**) Average cytosolic Ca^{2+} levels of dispersed islet cells from Bcl-$2^{+/+}$, Bcl-$2^{+/-}$, and Bcl-$2^{-/-}$ islets. (**c**) Incremental area under the curve of Ca^{2+} responses by Bcl-$2^{+/+}$, Bcl-$2^{+/-}$, and Bcl-$2^{-/-}$ islets. (**d**) Integrated cytosolic Ca^{2+} responses of Bcl-$2^{-/-}$ and Bcl-$2^{+/+}$ β-cells depolarized with 30 mM KCl. (**e**) and (**f**) Integrated Ca^{2+} and NAD(P)H autofluorescence increases of intact islet cells, normalized Bcl-$2^{+/+}$ control islet cells. (**g**) Insulin secretion profiles of perifused islets from Bcl-$2^{+/+}$ and Bcl-$2^{-/-}$ islets. (**h**) Quantified area under the curve of insulin secretion profiles by Bcl-$2^{+/+}$ and Bcl-$2^{-/-}$ islets (From Luciani et al. (2013))

(Real et al. 2004). Bcl-xL can lower acetyl-CoA levels independently of Bax and Bak (Yi et al. 2011). Anti-apoptotic Bcl-2 family proteins can modulate β-cell function, and thus have implications for the pathophysiology of DM. The reduction in Bcl-2 and Bcl-xL under prediabetic conditions can affect β-cell function and insulin hypersecretion is an early marker of human DM (Tsujimoto and Shimizu 2007;

Rong et al. 2009; Simonson 1990). These results suggest that endogenous Bcl-2 and Bcl-xL suppress the β-cell response to glucose. The emerging evidence places Bcl family proteins at the intersection of β-cell function and survival. The involvement of apoptosis-regulating proteins provides fertile ground for future insights into the pathophysiology of diabetes and other diseases. GK is a novel and direct physiological target of the Bad BH3 domain in β-cells and genetic evidence combined with the pharmacological activity of novel stapled Bad BH3 peptides indicates that phosphorylation within the BH3 domain drives the metabolic function of Bad and serves as a physiological switch between its apoptotic and metabolic effects (Real et al. 2004). The molecular targeting of GK activation holds therapeutic promise and is leading to the development of several GK activator compounds; further, there is a therapeutic application of Bad, SAHBs, and other Bad BH3 mimics in restoring insulin secretion (Luciani et al. 2013).

The recent reports on β-cell apoptosis have come from well-funded laboratories with many researchers of different disciplines to cooperate in this competitive area of research. The current and ongoing research is focusing on insulin secretion coupled with glucose metabolism using isolated islets from genetically ablated mice. These studies explore the effects of Bcl-2 and Bcl-xL proteins versus anti-apoptotic proteins, Bim, Bid, Bad, and Bik as pro-apoptotic proteins (Daniel et al. 2008). Another genetic approach by McKenzie showed that in wild-type mice, islets were exposed to 33.3 mmol/L glucose or 50 mmol/L ribose in tissue culture for 5 days, which showed increased DNA fragmentation and cytochrome c release. The pan caspase-inhibitor qVD.oph significantly inhibited both ribose- and glucose-induced islet cell DNA fragmentation and cytochrome c release, supporting the notion that islet cell killing occurred through a caspase-dependent apoptotic process (McKenzie et al. 2010). The islets from RIP Bcl-2 transgenic mice did not reveal increased DNA fragmentation and cytochrome c release. These results implicate the Bcl-2 regulated apoptotic pathway in glucose-induced β-cell killing (McKenzie et al. 2010).

Glucose induces insulin secretion through its glucose sensor and metabolism, and insulin secretion in turn modulates glucose metabolism through its myriad of glycolytic reactions in mitochondria and K^+ and Ca^{2+} channel opening and closing at the β-cell membrane. Chronic hyperglycemia also induces a myriad of reactions causing glucose toxicity in β-cells. How to prevent and reverse β-cell apoptosis by modulating the balance of the Bcl family and apoptotic genes against apoptosis may lead to prevention and therapeutic application for both T1DM and T2DM (Luciani et al. 2013).

Cross-References

- ▶ Immunology of β-Cell Destruction
- ▶ Islet Structure and Function in the GK Rat
- ▶ Mechanisms of Pancreatic β-Cell Apoptosis in Diabetes and Its Therapies

► Microscopic Anatomy of the Human Islet of Langerhans
► The β-Cell in Human Type 2 Diabetes

References

Allison J, Thomas HE, Catterall T, Catterall T, Kay TW, Strasser A (2005) Transgenic expression of dominant-negative Fas-associated death domain protein in β cells protects against Fas ligand-induced apoptosis and reduces spontaneous diabetes in nonobese diabetic mice. J Immunol 175:293–301

Anguiano M, Nowak RJ, Lansbury PT Jr (2002) Protofibrillar islet amyloid polypeptide permeabilizes synthetic vesicles by a pore-like mechanisms that may be relevant to type II diabetes. Biochemistry 41:11338–11343

Antinozzi PA, Ishihara H, Newgard CB, Wollheim CB (2002) Mitochondrial metabolism sets the maximal limit of fuel-stimulated insulin secretion in a model pancreatic β cell: a survey of four fuel secretagogues. J Biol Chem 277:11746–11755

Barett KL, Willingham JM, Garvin AJ, Willingham MC (2001) Advances in cytochemical methods for detection of apoptosis. J Histochem Cytochem 49:821–832

Begoya FJ, Matschinsky F, Shimizu T, O'Neil JJ, Appel MC (1986) Differential regulation of glucokinase activity in pancreatic islets. J Biol Chem 261:10760–10764

Berggren PO, Larson D (1994) Ca^{2+} and pancreatic β-cell function. Biochem Soc Trans 22:12–18

Boatright KM, Salvesen GS (2003) Mechanisms of caspase activation. Curr Opin Cell Biol 15:725–731

Bonner-Weir S (2000a) Islet growth and development in the adult. J Mol Endocrinol 24:297–302

Bonner-Weir S (2000b) Life and death of the pancreatic β cells. Trends Endocrinol Metab 11:375–378

Bonner-Weir S, O'Brien TD (2008) Islets in type 2 diabetes in honor of Dr Robert C Turner. Diabetes 57:2899–2904

Brandhorst D, Kumarasamy V, Maatoui A, Aht A, Bretzel RG, Brandhorst H et al (2006) Porcine islet graft function is affected by pretreatment with a caspase-3 inhibitor. Cell Transplant 15:311–317

Buse JB, Weyer C, Maggs DC (2002) Amylin replacement with Pramlintide in type 1 and type 2 diabetes: a physiological approach to overcome a barrier with insulin therapy. Clin Diabetes 20:137–144

Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC (2003a) β cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 52:102–110

Butler AE, Janson J, Soeller WC, Butler PC (2003b) Increased β-cell apoptosis prevents adoptive increase in β-cell mass in mouse model of type 2 diabetes: evidence for role of islet amyloid formation rather than direct action of amyloid. Diabetes 52:2304–2314

Chan SI, Yu VC (2004) Proteins of the Bcl-2 family in apoptosis signaling: from mechanic insights to therapeutic opportunities. Clin Exp Pharmacol Physiol 31:119–128

Chandra J, Zhivotovsky B, Zaitsev S, Juntti-Berggren H, Berggren PO, Orreni S (2001) Role of apoptosis in pancreatic β-cell death in diabetes. Diabetes 50(Suppl 1):S44–S47

Cheng G, Ahu L, Mahato RI (2008) Caspase gene silencing for inhibiting apoptosis in insulin cells and human islets. Mol Pharmacol 5:1093–1102

Clark A, Nilsson MR (2004) Islet amyloid: a complication of islet dysfunction or an aetiological factor in type 2 diabetes? Diabetologia 47:157–169

Clark A, Wells CA, Buley ID, Cruickshank JK, Vanhegan RI et al (1988) Islet amyloid, increased α-cells, reduced β-cells and exocrine fibrosis quantitative changes in the pancreas in type 2 diabetes. Diabetes Res 9:151–159

Cleaved caspase-3 (Asp 175) antibody (2006). vol 9661. Cell Signaling Technology, Beverly, MA, Cat. # 9661 pp 1–7

Cooper GJ, Willis AC, Clark A, Tumer RC, Sim RB, Reid KB (1987) Purification and characterization of a peptide from amyloid-rich pancreas of type 2 diabetic pancreas. Proc Natl Acad Sci U S A 84:8628–8632

Cucca F, Lampis R, Congia M, Angius E, Nutland S et al (2001) A correlation between the relative predisposition of MHC class II alleles to type 1 diabetes and their structure of their proteins. Hum Mol Gen 10:2025–2037

Daniel NN, Gramm CF, Scorrano L, Zhang CY, Kraus S et al (2003) BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis. Nature 424:952–956

Daniel NN, Walensky LD, Zhang CY, Choi CS, Fisher JK et al (2008) Dual role of proapoptotic BAD in insulin secretion and β cell survival. Nat Med 14:144–153

De Vos A, Heimberg H, Quatier E, Huypens P, Bouwens L et al (1995) Human and rat β cells differ in glucose transporter but not in glucokinase gene expression. J Clin Invest 96:2489–2495

DeFrenzo RA (1999) Pharmacologic therapy for type 2 diabetes mellitus. Ann Int Med 131:281–303

Duan WR, Garmer DS, Williams SD, Funkes-Shippy C, Spath I, Blomme EAG (2003) Comparison of immunohistochemistry of activated caspase-3 and cleaved cytokeratin 18 with the TUNEL method for quantification of apoptosis in histological section of PC-3 subcutaneous xenografts. J Pathol 199:221–228

Ehrlich JC, Ratner IM (1961) Amyloidosis of the islets of Langerhans. A restudy of islet hyaline in diabetic and non-diabetic individuals. Am J Pathol 38:49–59

Eisenbarth GS, Kotzin BL (2003) Enumerating autoreactive T cells in peripheral blood: a big step in diabetes prediction. J Clin Invest 111:179–181

Emamaullee JA, Shapiro AM (2006) Interventional strategies to prevent β-cell apoptosis in islet transplantation. Diabetes 55:1907–1914

Engel MFM, Khemtemourian I, Kleijer CC, Meeklik HJ, Jacobs J et al (2008) Membrane damage by human islet amyloid polypeptide through fibril growth at the membrane. Proc Natl Acad Sci U S A 105:6033–6038

Faustman D, Eisenbarth G, Daley J, Beitmyer J (1989) Abnormal T lymphocytes subsets intype1 diabetes mellitus. Analysis with anti-2H4 andanti-4B4 antibodies. Diabetes 38:1462–1468

Faustman DL, Li X, Lim HY, Eisenbarth G, Avruch J, Guo J (1991) Linkage of family major histocompatibility complex class I to autoimmune diseases. Science 254:1756–1761

Federici M, Hrivbal M, Perego L, Ranalli M, Caradonna Z et al (2001) High glucose causes apoptosis in cultured human pancreatic islets of Langerhans. A potential role for regulation of specific Bcl family genes toward an apoptotic cell death program. Diabetes 50:1290–1301

Finegood D, Scaglia L, Bonner-Weir S (1995) Dynamics of β-cell mass in the growing rat pancreas: estimation with a simple mathematical model. Diabetes 44:249–256

Fineman M, Weyer C, Maggs DG, Strobel S, Koltermano G (2002) The human amylin analog, pramlintide, reduces postprandial hyperglucagonemia in patients with type 2 diabetes mellitus. Horm Metab Res 34:504–508

Fridlyand L, Philson LH (2010) Glucose sensing in the pancreatic β cells: a computational systems analysis. Theor Biol Med Model 7:1–44

Froguel P, Vaxillaire M, Sun F, Valho G, Zouali H et al (1992) Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin -dependent diabetes mellitus. Nature 356:162–164

Gown AM, Willingham MC (2002) Improved detection of apoptotic cells in archival paraffin sections: immunohistochemistry using antibodies to cleaved caspase3. J Histochem Cytochem 50:449–454

Green D (2005) Apoptotic pathways: ten minutes to death. Cell 121:671–674

Gross A, McDonnell JM, Korsmeyer SJ (1999) Bcl-2 family members and the mitochondria in apoptosis. Genes Dev 13:1899–1911

Haataja L, Gurlo T, Huang CJ, Butler PC (2008) Islet amyloid in type 2 diabetes and the toxic oligomer hypothesis. Endocr Rev 29:303–316

Hayashi T, Faustman DL (2003) Role of defective apoptosis in type 1 diabetes and other autoimmune diseases. Rec Prog Horm Res 58:131–153
Hengartner MO (2000) The biochemistry of apoptosis. Nature 407:770–776
Hirata H, Takahashi A, Kobayashi S, Yonehara S, Sawai H et al (1998) Caspases are activated in a branched protease cascade and control distinct down-stream processes in Fas-induced apoptosis. J Exp Med 187:587–600
Hoppener JW, Ahren B, Lips CJ (2000) Islet amyloid and type 2 diabetes mellitus. N Eng J Med 343:411–419
Hui H, Dotta F, De Maria U, Perfetti R (2004) Role of caspases in the regulation of apoptotic pancreatic islet β-cell death. J Cell Physiol 200:177–200
Hull RI, Westermark GT, Westermark P, Kahn SE (2004) Islet amyloid: a critical entity in the pathogenesis of type 2diabetes. J Clin Endocrinol Metab 89:3629–3643
Innedian BB (1993) Mammalian glucokinase and its gene. Biochem J 293:1–13
Jansen A, Van Hagen M, Drexhage HA (1995) Defective mutation and function of antigen-presenting cells in type1 diabetes. Lancet 345:491–492
Jansen J, Ashley RH, Harrison D, McIntyre S, Butler PC (1999) The mechanisms of islet amyloid polypeptide toxicity is membrane disruption by intermediate-sized toxic amyloid particle. Diabetes 48:491–498
Jurgens CA, Toukatly MN, Flinger CL, Udayasankar J (2011) Subramanian, SL et al: β-cell loss and β-cell apoptosis inhuman type2 diabetes are related to islet amyloid deposition. Am J Pathol 178:2632–2640
Kahn SE, Andrikopoulos S, Verchere CBN (1999) Islet amyloid: a long-recognized but underappreciated pathological feature of type 2 diabetes. Diabetes 48:241–253
Karbowski M, Norris KL, Cleland NM, Jeong SY, Youle RJ (2006) Role of Bax and Bak in mitochondrial morphogenesis. Nature 443:658–662
Kayed R, Head E, Thompson JI, McIntyre TM, Milton SC et al (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300:486–489
Kerr JP, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-range implications in tissue kinetics. Br J Cancer 26:239–257
Kruger DF, Gatcomb PM, Owen SK (1999) Clinical implication of amylin and amylin deficiency. Diabetes Educ 25:389–397
Leahy JL, Bonner-Weir S, Weir CC (1992) β-cell dysfunction induced by chronic hyperglycemia: current ideas on mechanisms of impaired glucose-induced insulin secretion. Diabetes Care 15:442–455
Lee SC, Pervaiz S (2007) Apoptosis in pathophysiology of diabetes mellitus. Int J Biochem 39:497–504
Leonardi O, Mints G, Hussain MA (2003) β-cell apoptosis in the pathogenesis of human type 2 diabetes mellitus. Eur J Endocrinol 149:99–102
Liang Y, Bai N, Doliba C, Wang L, Barner DK, Matschinsky FM (1996) Glucose metabolism and insulin release in mouse HC9 cells, as model of wild-type pancreatic β cells. Am J Physiol 270: E846–E857
Luciani DS, White SA, Widenmaier SB, Saran VV, Taghizadeh F et al (2013) Bcl-2 and Bcl-xL suppress glucose signaling in pancreatic β-cells. Diabetes 62:170–182
Mandrup-Poulsen T (2001) β-cell apoptosis: stimuli and signaling. Diabetes 50(Suppl 1):S58–S63
Marchetti P, Guerra S, Marselli L, Marselli L, Lupi R, Masini M et al (2004) Pancreatic islets from type 2 diabetic patients have functional defects and increased apoptosis that are ameliorated by metformin. J Clin Endocrinol Metab 89:5535–5541
Martin SJ, Green DR (1995) Protease activation during apoptosis: death by a thousand cuts? Cell 82:349–452
Matschinsky FM (1990) Glucokinase as glucose sensor and metabolic signal generator in pancreatic β-cell and hepatocytes. Diabetes 39:647–652
Matschinsky FM (1995) Banting lecture. A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. Diabetes 1995(45):223–241

Matschinsky F, Liang Y, Kesavan P (1993) Glucokinase as pancreatic β cell sensor and diabetes. J Clin Invest 92:2092–2098
McDonald PE, Joseph JW, Rorsman P (2005) Glucose-sensing mechanisms in pancreatic β-cells. Phil Trans R Soc 360:2211–2225
McKenzie MD, Jamieson E, Jansen ES, Scott CL, Huang DCS et al (2010) Glucose induces pancreatic islet cell apoptosis that requires the BH3-only proteins Bim and Puma and multi-BH domain protein Bax. Diabetes 59:644–652
Mirzabekov TA, Lin MC, Kagan BL (1996) Pore formation by the cytotoxic islet amyloid peptide amylin. J Biol Chem 27:1988–1992
Nakano M, Matsumoto I, Sawada T, Ansite J, Oberbroeckling J et al (2004) Caspase-3 inhibitor prevents apoptosis of human islets immediately after isolation and improves islet graft function. Pancreas 29:104–109
Newgard CB, McGarry JD (1995) Metabolic coupling factors in pancreatic β-cell signal transduction. Ann Rev Biochem 64:689–719
Nicholson DW, Thornberry NA (1997) Caspases: killer proteases. Trends Biochem Sci 22:299–306
O'Brien TD, Glabe CG, Butler PC (2010) Evidence for proteotoxicity in β cells in type 2 diabetes: toxic islet amyloid oligomers from intracellularly in the secretory pathway. Am J Pathol 176:861–869
Ou D, Wang X, Metzger DL, James RFL, Pozzilli P et al (2005) Synergetic inhibition of tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis inhuman pancreatic β cells by Bcl-2 and X-linked inhibitor of apoptosis. Hum Immunol 66:274–284
Peter ME, Krammer PH (1998) Mechanisms of CD95 (APO-1/Fas)-mediated apoptosis. Curr Opin Immunol 10:545–551
Porter JR, Barette TG (2005) Monogenic syndromes of abnormal glucose homeostasis: clinical review and relevance to the understanding of the pathology of insulin resistance and β-cell failure. J Med Genet 42:893–902
Rabinovitch A, Skyler JS (1998) Prevention of type 1 diabetes. Med Clin North Am 82:739–755
Real PJ, Cao Y, Wang R, Nikolovska-Coleska Z, Sanz-Ortiz J et al (2004) Breast cancer cells can evade apoptosis-mediated selective killing by a novel small molecule inhibitor of Bcl-2. Cancer Res 64:7947–7953
Reaven G (1988) Role of insulin resistance in human disease. Diabetes 37:1595–1607
Rhodes CJ (2005) Type 2 diabetes – a matter of β-cell life and death? Science 307:380–384
Ritzel RA, Butler PC (2003) Replication increases β-cell vulnerability to human islet amyloid polypeptide-induced apoptosis. Diabetes 52:1701–1708
Rong YP, Bultynck G, Aromolaran AS, Zhong F, Parys JB et al (2009) The BH4 domain of Bcl-2 inhibits ER calcium release and apoptosis by binding the regulatory and coupling domain of the IP3 receptor. Proc Natl Acad Sci U S A 106:14397–14402
Rossetti I, Giaccari A, DeFrenzo RA (1990) Glucose toxicity. Diabetes Care 13:610–630
Sakahira H, Enari M, Nagata S (1998) Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature 391:96–99
Saldeen J (2000) Cytokines induce both necrosis and apoptosis via a common Bcl-2-inhibitable pathway in rat insulin-producing cells. Endocrinology 141:2003–2010
Sava V, Caates JP, Hall PA (2001) Analysis of apoptosis in tissue sections. Endocr Mol Biol 174:347–359
Scaglia L, Cahill CJ, Finegood DT (1997) Apoptosis participates in the remodeling of endocrine pancreas in the neonatal rat. Endocrinology 138:1736–1741
Shimizu T, Knowles BB, Matschinsky FM (1988) Control of glucose phosphorylation and glucose usage in clonal insulinoma cells. Diabetes 37:563–568
Simonson DC (1990) Hyperinsulinemia and its sequence. Horm Metab Res 22(Suppl):17–25
Stassi GD, De Maria R, Trucco G, Rudert W, Testi R et al (1997) Nitric oxide primes pancreatic β cells for Fas (CD95)-mediated destruction in insulin-dependent diabetes mellitus. J Exp Med 186:1193–1200

Tewari M, Quan LT, O'Rourke K, Dosmoyers K, Zeng Z, Beidler DR et al (1995) Yama/cp32β, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. Cell 81:801–809

Tomita T (2005) Amyloidosis of pancreatic islets in primary amyloidosis (AL type). Pathol Int 55:223–227

Tomita T (2009) Cleaved caspase-3 immunocytochemical staining for pancreatic islets and pancreatic endocrine tumors. A potential marker for biological malignancy. Islets 2:82–88

Tomita T (2010a) Immunocytochemical localisation of cleaved caspase-3 in pancreatic islets from type 2 diabetic subjects. Pathology 42:432–437

Tomita T (2010b) Immunocytochemical localization of cleaved casspase-3 in pancreatic islets from type 1 diabetic subjects. Islets 2:24–29

Tomita T (2012) Islet amyloid polypeptide in pancreatic islets from type 2 diabetic subjects. Islets 4:223–232

Tomita T, Scarpelli DG (1977) Interaction of cyclic AMP and alloxan on insulin secretion in isolated rat islets perifused in vitro. Endocrinology 100:1327–1333

Tomita T, Lacy PE, Matschinsky FM, McDaniel M (1974) Effect of alloxan on insulin secretion in isolated rat islets perifused in vitro. Diabetes 23:517–524

Tsujimoto Y, Shimizu S (2007) VDAC regulation by the Bcl-2 family of proteins. Cell Death Differ 7:1174–1181

Urusova IA, Farila L, Hui H, D'Amico E, Perfetti R (2004) GLP-inhibition of pancreatic islet cell apoptosis. Trends Endocrinol Metab 15:27–33

Westermark P, Westermark C, Wilander E, Sletten K (1986) A novel peptide in the calcitonin gene related peptide family as an amyloid fibril protein in the endocrine pancreas. Biochem Biophys Res Commun 140:827–831

Weyer C, Maggs DG, Young AA, Kohlman OG (2001) Amylin replacement with pramlintide as an adjunct to insulin therapy in type 1 and type 2 diabetes mellitus: a physiological approach toward improved metabolic control. Curr Pharm Des 7:1353–1373

Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG (2008) Analysis of islet inflammation in human type 1 diabetes. Clin Exp Immunol 155:173–181

Yi CH, Pan H, Seebacher J, Jang F, Hyberts SG, Hoffron GJ et al (2011) Metabolic regulation of protein N-α acetylation by Bcl-xL promotes cell survival. Cell 146:607–620

Zhou YP, Pena JC, Roe MW, Mittal A, Levisetti M et al (2000) Overexpression of Bcl-xL in β cells prevents cell death but impairs mitochondrial signal for insulin secretion. Am J Physiol Endocrinol Metab 278:E340–E351

Mechanisms of Pancreatic β-Cell Apoptosis in Diabetes and Its Therapies

32

James D. Johnson, Yu H.C. Yang, and Dan S. Luciani

Contents

Abstract

Diabetes occurs when β-cells no longer function properly or have been mostly destroyed. Pancreatic β-cell loss by apoptosis and other modes of death contributes to both autoimmune type 1 diabetes and type 2 diabetes. Programmed pancreatic β-cell death can be induced by multiple stresses in both major types of diabetes. There are also several rare forms of diabetes, including Wolcott-Rallison

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_14,

syndrome, Wolfram syndrome, as well as some forms of maturity onset diabetes of the young that are caused by mutations in genes that may play important roles in β-cell survival. The use of islet transplantation as a treatment for diabetes is also limited by excessive β-cell death. Mechanistic insights into the control of multiple modes of β-cell death are therefore important for the prevention and treatment of diabetes. Indeed, a substantial quantity of research has been dedicated to this area over the past decade. In this chapter, we will review the factors that influence the propensity of β-cells to die and the mechanisms of programmed cell death involved in the initiation and progression of diabetes.

Keywords

Clinical islet transplantation • Autoimmune diabetes • Glucotoxicity and lipotoxicity • Endoplasmic reticulum stress • Gene-environment interactions • Mitochondrial death pathway

List of Abbreviations

GLP-1	Glucagon-like peptide 1
MODY	Maturity onset diabetes of the young
NOD	Nonobese diabetic
UPR	Unfolded protein response
VNTR	Variable number of tandem repeats

Introduction to β-Cell Death

A person's functional β-cell mass determines, to a large extent, their glucose homeostasis and susceptibility to diabetes. Functional β-cell mass is the product of β-cell number, β-cell size, and the ability of individual β-cells to secrete mature insulin in a correct manner (Fig. 1)(Szabat et al. 2012).

It has become increasingly evident that β-cell apoptosis contributes to the development of both type 1 diabetes (autoimmune diabetes) and type 2 diabetes (adult-onset diabetes), as well as to more rare forms of the disease such as the various types of maturity onset diabetes of the young (MODY) (Oyadomari et al. 2002; Mathis et al. 2001; Leonardi et al. 2003; Johnson 2007; Donath and Halban 2004). Basal β-cell apoptosis also plays a role in the remodeling and development of the normal endocrine pancreas. For example, β-cells undergo a wave of apoptosis around the time of birth (Mathis et al. 2001), which is followed by a proliferation-driven postnatal expansion of β-cell mass (Meier et al. 2008). At all stages of life, β-cell replication and death are tightly controlled by intrinsic and extrinsic factors that control how β-cell mass adjusts to meet metabolic demand (Bell and Polonsky 2001). Only when a combination of genetic and environmental influences causes this balance to fail does diabetes develop. Despite major advances in recent years, the nature of the gene-environment interactions that promote β-cell apoptosis in diabetes remains unclear, as do many aspects of the apoptotic pathways

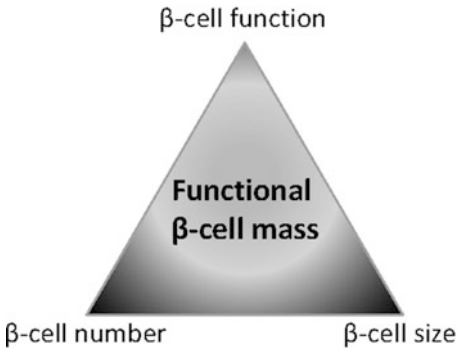

Fig. 1 Factors that dictate the functional β-cell mass

involved. In the following article, we will review some of the central mechanisms that have been implicated in the control β-cell apoptosis to date, as well as current therapeutic efforts that target these pathways.

Increased β-Cell Death as a Trigger and Mediator of Type 1 Diabetes

Type 1 diabetes is an autoimmune disease in which the pancreatic β-cells are gradually destroyed, but the initial trigger for this destruction and the exact mechanisms of β-cell death remain enigmatic. Like necrosis, excessive apoptosis is capable of initiating an immune response in susceptible individuals. It has been suggested that a perinatal wave of β-cell apoptosis may promote the presentation of β-cell autoantigens and thus provoke an autoimmune response against β-cells (Mathis et al. 2001; Liadis et al. 2005; Trudeau et al. 2000).

Clues to the cause and pathobiology of type 1 diabetes also come from the analysis of its genetics. In most cases, genes linked to type 1 diabetes are known to play specific roles in the immune system. IDDM1 is the human leukocyte antigen system superlocus containing the major histocompatibility complex genes. This region of the human and nonobese diabetic (NOD) mouse genome confers the majority of the risk for type 1 diabetes. Interestingly, the insulin gene itself (IDDM2) is the second most significant type 1 diabetes gene in humans. The genetic alterations are not in the coding sequence of insulin, but in an upstream regulatory region called the "variable number of tandem repeats" or VNTR (Pugliese et al. 1997; Bennett and Todd 1996; Barratt et al. 2004). At-risk alleles appear to reduce the expression of the insulin gene in the thymus where it is thought to play a role in tolerance (Pugliese et al. 1997). At the same time, VNTR sequences that confer diabetes risk increase insulin mRNA in the islets. High doses of insulin can have deleterious effects on the survival of β-cells under some culture conditions (Johnson et al. 2006a; Guillen et al. 2008). If this was also the case in vivo, one might expect that the VNTR could increase type 1 diabetes risk via direct effects on β-cell apoptosis. Recent in vivo evidence has demonstrated that insulin gene dose can

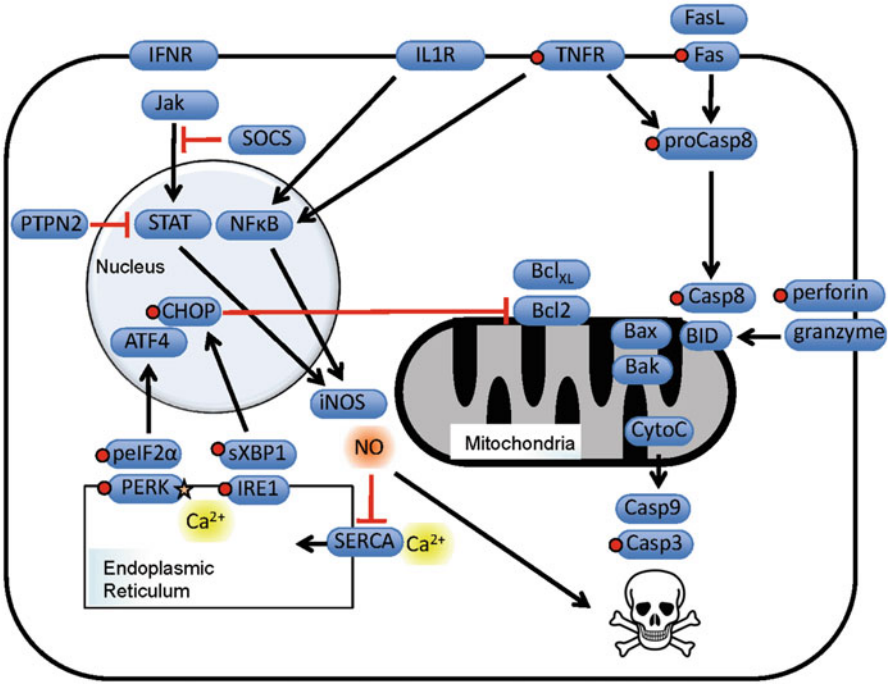

Fig. 2 Molecular mechanisms controlling β-cell apoptosis in type 1 diabetes. Shown is a partial description of signaling cascades that modulate β-cell survival in type 1 diabetes. Protein products of genes that are linked to human diabetes are denoted with a *star*. Genes that have been implicated in β-cell apoptosis or β-cell mass using in vivo or molecular loss-of-function experiments (i.e., knockout mice) are denoted with a *dot*

modulate β-cell mass (Mehran et al. 2012) and mathematical modeling has supported the feasibility of anti-apoptotic autocrine/paracrine insulin action (Wang et al. 2013).

Genome-wide association studies have identified several single nucleotide polymorphisms that contribute to the risk of type 1 diabetes. While it had long been assumed that most of these genes are involved exclusively in immune cells, it is now clear that virtually all of the currently known type 1 diabetes susceptibility genes are expressed in pancreatic β-cells as well (Eizirik et al. 2012), where they could theoretically modulate cell survival. For example, PTPN2 modulates β-cell apoptosis via effects on BIM and the transcription factor STAT1 (Moore et al. 2009; Santin et al. 2011; Colli et al. 2010). GLIS3 is unique in that it may modulate β-cell fate in the context of both type 1 diabetes and type 2 diabetes (Nogueira et al. 2013). Thus, genes that confer risk to type 1 diabetes may also affect β-cell death directly.

The mechanisms by which β-cells are selectively killed by the immune system have been studied extensively and appear to involve multiple pathways (Fig. 2).

One mechanism is the activation of "death receptors", Fas and tumor necrosis factor receptor, by their respective ligands. Interestingly, Fas expression is negligible in normal β-cells and it may be upregulated by cytokines such as IL-1

(Thomas et al. 2009). Activation of Fas by FasL converts pro-caspase-8 to active caspase-8 (Mathis et al. 2001). Caspase-8 then acts via the proapoptotic BH3-only Bcl family member Bid to promote mitochondrial outer membrane permeabilization and cytochrome c release (McKenzie et al. 2008). Bid may do so by interacting directly with the proapoptotic effector Bcl protein Bax and activate its channel forming functions in the outer mitochondrial membrane (Lovell et al. 2008).

Another pathway of β-cell apoptosis in type 1 diabetes involves perforin and granzyme B, cytotoxic components released by $CD8^+$ T cells. Mouse models suggest $CD8^+$ T cells to be major effectors of immune mediated β-cell death and perforin knockout mice on a NOD background have reduced diabetes incidence compared with NOD controls (Thomas et al. 2009). Granzyme B cleaves multiple substrates in the target cell, including Bid and studies with islets from Bid knockout mice demonstrate that Bid is also key in this β-cell death cascade (Estella et al. 2006).

The involvement of other Bcl family members in type 1 diabetes and its animal models is less clear. Pancreatic islets isolated from Bax knockout mice are partially protected from death receptor-triggered β-cell apoptosis, in agreement with Bax being the downstream effector of mitochondrial outer membrane permeabilization following Bid activation (McKenzie et al. 2008). Efforts to block diabetes using transgenic mice overexpressing Bcl-2 under the control of the rat insulin promoter provided mixed results (Thomas et al. 2009). To date, no in vivo loss-of-function experiments have demonstrated an essential role for anti-apoptotic Bcl-2 or Bcl-x_L in basal β-cell survival (Luciani et al. 2013). Interestingly, Bcl family proteins also play key roles in β-cell metabolic function (Luciani et al. 2013; Danial et al. 2008), making studies into the joint role of these proteins especially important.

While the Bcl proteins collectively control mitochondrial outer membrane permeabilization and cytochrome c release, the majority of the β-cell "execution" steps are triggered by the activation of effector caspases, such as caspase-3. These proteases also coordinate the semi-ordered disassembly of β-cells with members of the calpain family of calcium-activated proteases. Pancreatic β-cell apoptosis is promoted by caspase-3 and caspase-9, essential mediators in the intrinsic pathway of apoptosis. In cell culture models, β-cell death can be abrogated with inhibitors of caspase-3 activity (Yamada et al. 1999). In vivo, mice lacking caspase-3 in their β-cells are protected from type 1 diabetes (Liadis et al. 2005). Interestingly, isolated islets from β-cell-specific caspase-8 knockout mice are protected from Fas-induced apoptosis, but have increased "basal" apoptosis and glucose intolerance in the absence of frank diabetes (Liadis et al. 2007). These results suggest that the action of caspases can be context-dependent in the β-cell.

Pancreatic β-Cell Death as a Complication of Diabetes: Glucose Toxicity

Pancreatic β-cells are exquisitely sensitive to metabolic stress, since they must transduce changes in blood glucose levels into insulin release via glycolytic and mitochondrial ATP production (Bell and Polonsky 2001). Since hyperglycemia and

hyperlipidemia both are hallmarks of the diabetic state, β-cell apoptosis is also likely to be an important complication of diabetes. This downward spiral likely plays a significant role in the rapid reduction in functional β-cell mass that precipitates the onset of both type 1 and type 2 diabetes. Chronically elevated glucose induces β-cell apoptosis via multiple mechanisms, including modulating the gene expression of multiple Bcl family members (Federici et al. 2001). Toxic high levels of reactive oxygen species are produced by hyperactive mitochondria and β-cells contain relatively low levels of some key antioxidant proteins (Federici et al. 2001; Kaneto et al. 2005; Robertson et al. 2004). Moreover, elevated Ca^{2+} levels associated with overworked β-cells are toxic to the cells (Maedler et al. 2004; Efanova et al. 1998). This excitotoxicity may be the cause of the eventual clinical failure of long-term sulfonylurea treatment, which depolarizes β-cells by directly closing K_{ATP} channels (Maedler et al. 2005). Prolonged hyperglycemia may also activate Fas-mediated β-cell apoptosis (Maedler and Donath 2004) and pathways controlled by the proapoptotic protein TXNIP (Chen et al. 2008). Moreover, chronic hyperglycemia increases secretory demand, which has been speculated to cause ER stress due to the increased requirement for protein synthesis and processing (see below).

Programmed β-Cell Death as a Contributing Factor in Type 2 Diabetes

It is established that pancreatic β-cell death is a key event in type 1 diabetes, but evidence has only recently emerged supporting an important role for β-cell apoptosis in the pathobiology of type 2 diabetes (Leonardi et al. 2003; Donath and Halban 2004; Rhodes 2005; Shu et al. 2008; Jeffrey et al. 2008; Cnop et al. 2005; Johnson et al. 2004; Butler et al. 2003a, b) (Fig. 3).

Type 2 diabetes is a disease of gene-environment interactions, with obesity and hyperlipidemia being the main manifestations of the "environment." Obesity is associated with inflammation and insulin resistance in a multitude of key metabolic tissues, including liver, fat, and muscle (Ozcan et al. 2004, 2006). In the majority of obese people, an expansion of β-cell mass and workload can effectively compensate for the increased insulin secretory demand (Rhodes 2005; Butler et al. 2003a, b). However, if this compensatory increase in β-cell mass and function fails, the obese individual will progress to frank type 2 diabetes. Compared to weight-matched controls, patients with type 2 diabetes have been reported to exhibit a 60 % reduction in β-cell mass associated with significantly increased β-cell apoptosis and ER stress (Butler et al. 2003a, b). A disruption of islet architecture and an accumulation of amyloid deposits are also associated with type 2 diabetes (Haataja et al. 2008). It is clear that much work remains to be done to distinguish cause and effect relationships between these pathologies. Moreover, the concepts of β-cell health and β-cell dedifferentiation, intermediates between dysfunction and death, are gaining increased attention.

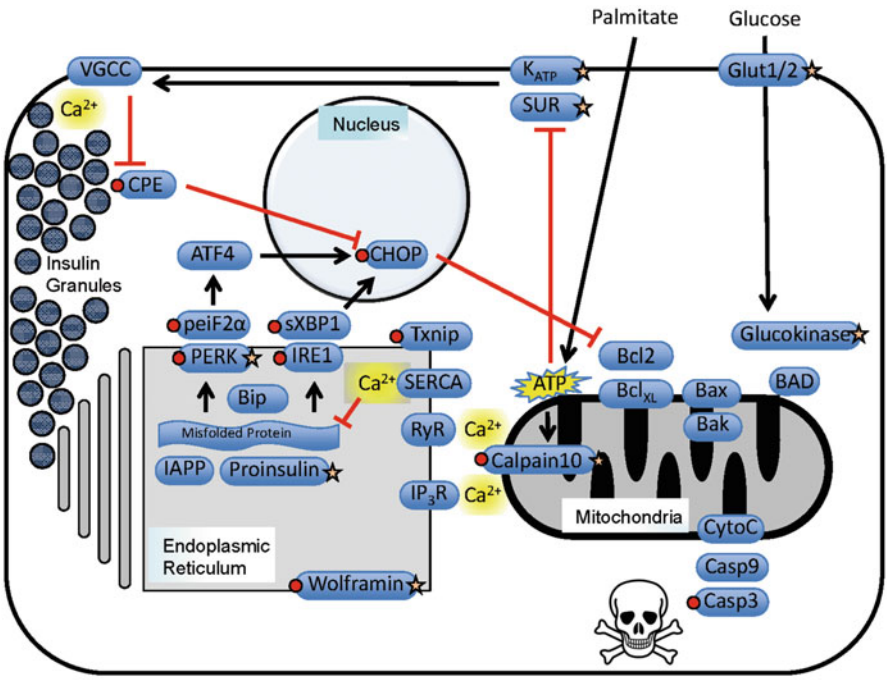

Fig. 3 Molecular mechanisms controlling lipid- and glucose-induced β-cell apoptosis in type 2 diabetes. Shown is a partial description of signaling cascades that modulate β-cell survival. Protein products of genes that are linked to human diabetes are denoted with a *star*. Genes that have been implicated in β-cell apoptosis or β-cell mass using in vivo loss-of-function experiments (i.e., knockout mice) are denoted with a *dot*

Mechanisms of β-Cell Apoptosis in Type 2 Diabetes: ER Stress

Pancreatic β-cells are the body's only source of blood-borne insulin and therefore must produce and secrete large amounts of this hormone as well as other hormones such as amylin. This high secretory demand makes them susceptible to secretory pathway stress, especially if demand is increased by insulin resistance. Elevated protein flux through the ER and Golgi can result in misfolded proteins and activation of the unfolded protein response (UPR) (Oyadomari et al. 2002; Eizirik et al. 2008; Harding and Ron 2002). Three main ER-resident signaling molecules, PERK, ATF6, and IRE1, act as sensors to trigger cellular adaptation responses or ultimately β-cell apoptosis if the stress is not alleviated. Important components of the initial "rescue response" are the PERK-triggered and eIF2α-mediated regulation of protein translation as well as an increased ER-associated degradation of misfolded proteins. When these rescue efforts fail, apoptosis is triggered. The relative sensitivity of β-cells to ER stress-induced cell death is illustrated by

humans and mice with mutations in PERK, since other cells in the body can be largely unaffected (Harding and Ron 2002). The transcription factor CHOP is a major mediator of ER stress-induced apoptosis downstream of PERK and ATF6. Mice lacking the CHOP gene are resistant to β-cell apoptosis following ER stress and are protected from developing diabetes under these conditions (Eizirik et al. 2008; Song et al. 2008). Importantly, there is now increasing evidence of ER stress in islets of human type 2 diabetes patients (Eizirik et al. 2008; Laybutt et al. 2007), suggesting that ER stress does in fact contribute to β-cell apoptosis during the progression of type 2 diabetes. It is less clear whether β-cell ER stress can initiate β-cell death prior to the onset of type 2 diabetes.

Mechanisms of β-Cell Apoptosis in Type 2 Diabetes: Lipotoxicity

Obesity is thought to trigger type 2 diabetes by causing hyperlipidemia and insulin resistance. These events impose increased secretory demand on individual β-cells, which can activate the UPR, as outlined above. Moreover, elevated fatty acids, such as palmitate, have direct toxic effects on the β-cell via activation of a number of relatively separate apoptosis-inducing events, including the generation of ceramide and excessive reactive oxygen species. Palmitate activates the caspase-3-dependent mitochondrial apoptosis pathway (Jeffrey et al. 2008). Some investigators have shown that the activation of caspase-3 by palmitate is synergistic with the detrimental effects of high glucose (El-Assaad et al. 2003; Prentki and Nolan 2006), but it also triggers β-cell apoptosis in the absence of elevated glucose levels (Jeffrey et al. 2008). Palmitate also decreases the expression of the anti-apoptotic Bcl-2 protein (Lupi et al. 2002). The type 2 diabetes susceptibility gene, calpain-10, is also implicated in palmitate-induced β-cell death, since islets lacking calpain-10 have ~30% less apoptosis and mice with transgenic overexpression of calpain-10 are more susceptible to palmitate toxicity (Johnson et al. 2004). Moreover, palmitate has been demonstrated to directly act on the distal components of the insulin processing machinery of the β-cell. Specifically, palmitate induces a rapid, Ca^{2+}-dependent degradation of carboxypeptidase E, the final enzyme required for the conversion of proinsulin into mature insulin (Jeffrey et al. 2008). Carboxypeptidase E is also reduced in high fat-fed mice and the transgenic MKR mouse model of insulin resistance (Lu et al. 2008). A decrease in carboxypeptidase E is sufficient to induce CHOP-dependent ER stress and β-cell apoptosis in vivo and in vitro. It is unclear how reduced carboxypeptidase E modulates β-cell apoptosis, but two possibilities can be considered. In one scenario, a backlog of unprocessed insulin induces the UPR from inside the cell. It is also possible that a reduction in local release of mature insulin could impair β-cell survival. Substantial evidence suggests local insulin levels at the right concentration may help protect β-cells against ER stress and apoptosis (Johnson et al. 2006a, b; Mehran et al. 2012; Johnson and Alejandro 2008; Martinez et al. 2008), and islets from patients with type 2 diabetes exhibit reductions in several critical insulin signaling components (Gunton et al. 2005). To add to the complexity, insulin may modulate the levels of CPE

(Chu et al. 2011). Fatty acids, including palmitate, also modulate secretory pathway stress by partially depleting ER Ca^{2+} stores (Gwiazda et al. 2009). Although an incomplete ER Ca^{2+} reduction alone is not sufficient to induce ER stress, this event activates PERK and it is likely that this could potentiate ER stress induced by other factors (Gwiazda et al. 2009). Other systems involved in β-cell lipotoxicity include de novo lipogenesis, the ubiquitin proteasome system, lipophagy, and subcellular lipid distribution/flux (Chu et al. 2010, 2012; Pearson et al. 2014; Boslem et al. 2011, 2012, 2013; Preston et al. 2009). This is an intense area of investigation with new breakthroughs on the horizon.

Mechanisms of β-Cell Apoptosis in Type 2 Diabetes: Proinflammatory Cytokines

There is emerging evidence that proinflammatory cytokines and immune cell infiltration of the islet are common factors in type 1 diabetes and type 2 diabetes. The type 2 diabetic milieu of increased hyperglycemia and hyperlipidemia appears to stimulate the production of IL-1β from islets themselves. This has been suggested to have local inflammatory effects and advance subsequent islet infiltration by macrophages to promote apoptosis in type 2 diabetes (Ehses et al. 2007). There is evidence that proapoptotic cytokines (IL-1β, TNFα, IFNγ) can act through nitric oxide to decrease the expression of the SERCA pumps that load Ca^{2+} into the ER, which in turn impairs Ca^{2+}-dependent protein processing and promotes ER stress-induced β-cell apoptosis (Eizirik et al. 2008; Cardozo et al. 2005). This is in addition to changes in ER Ca^{2+}-release channels seen in the diabetic state (Lee et al. 1999). Cytokines might thus promote similar types of β-cell apoptosis in type 1 and type 2 diabetes, but the extent to which overlapping pathways are involved has been questioned (Cnop et al. 2005). Interested readers are referred to some excellent reviews on this topic (Eizirik et al. 2013; Eizirik and Grieco 2012).

Genetic Factors Affecting β-Cell Apoptosis in Type 2 Diabetes

Type 2 diabetes is a polygenic disease, with dozens of genes being implicated via both candidate studies and unbiased genome-wide approaches. Some of the first gene candidates studied for their role in type 2 diabetes were ones that play important roles in β-cell function. These included the components of the ATP-sensitive potassium channels (KCNJ11, ABCC8). PPARγ was also linked to type 2 diabetes risk, and recent experiments point to a role for PPARγ in β-cell apoptosis (Lin et al. 2005). The first type 2 diabetes susceptibility gene discovered by unbiased linkage mapping was calpain-10 (Horikawa et al. 2000), although this association is not seen in all populations. In the β-cell, calpain-10 likely plays a proapoptotic role in addition to a role promoting insulin secretion (Marshall et al. 2005). Additional in vivo studies are required to determine the detailed roles of the calpain-10 gene, which encodes for 8 splice variants, in the maintenance of glucose homeostasis.

Newer genome-wide association studies have found about 20 single nucleotide polymorphisms that show significant and reproducible associations with type 2 diabetes (Lyssenko and Groop 2009). The susceptibilities conferred by these loci are greater than those of the candidate genes or calpain-10. Most of these genes are expressed in the endocrine pancreas, suggesting β-cells can be considered the main target of the genetic component in type 2 diabetes. In European populations the strongest association is associated with TCF7L2, a transcription factor involved in the development and survival of islet cells and enteroendocrine cells of the gut. In vitro studies implicate TCF7L2 in β-cell apoptosis associated with increased caspase-3 cleavage and decreased Akt activity (Shu et al. 2008). Pancreatic β-cell function is also reduced in patients with TCF7L2 polymorphisms (Lyssenko et al. 2008). It is important to realize that each of the top 20 diabetes-linked genes has minimal effects on their own and that their combined effects are not synergistic. Also, their net contribution cannot explain the apparent heredity of type 2 diabetes, suggesting either that heredity has been overestimated or that epigenetic factors are dominant in the development of type 2 diabetes. The epigenetics of β-cell death in type 2 diabetes will be an important area for investigation in the future, given the persistent effects of fetal and early nutrition on β-cell function and survival.

The Role of β-Cell Apoptosis in Rare Forms of Diabetes

Although the common forms of type 1 and type 2 diabetes are polygenic, several rare forms of diabetes are caused by mutations in single genes. In most cases, these genes are important for β-cell survival or function. Monogenic causes of diabetes include mutations in proinsulin that prevent its proper folding, cause ER stress and β-cell death, and result in early-onset diabetes (Stoy et al. 2007). Wolcott-Rallison syndrome is caused by mutations in the ER stress-sensing protein PERK (Eizirik et al. 2008). ER stress-induced β-cell apoptosis may also be the cause of diabetes in Wolfram syndrome (Riggs et al. 2005). Several of the six MODY genes may also influence β-cell survival. The prime example here appears to be Pdx-1 (MODY4). Mice lacking one allele of Pdx-1 have increased β-cell apoptosis, caspase-3 activation, a reduction in the Bcl-x_L to Bax ratio, and 50% decrease in β-cell mass evident at 1 year of age (Johnson et al. 2003). This increase in apoptosis might reflect the fact that full expression of Pdx-1 is required for the pro-survival effects of insulin and incretin hormones in the β-cell (Johnson et al. 2006a; Li et al. 2005). Other MODY genes have also been linked to β-cell apoptosis, including HNF1a (Johnson 2007; Wobser et al. 2002). Pancreatic β-cells expressing a dominant-negative HNF1a exhibit caspase-3- and Bcl-x_L-dependent apoptosis (Wobser et al. 2002b). Collectively, the genes implicated in monogenic diabetes illustrate the critical importance of β-cell function and survival in human glucose homeostasis.

Islet Engraftment and β-Cell Death in Islet Transplantation

Islet transplantation is severely limited by β-cell death at several stages of this clinical treatment. Since islets are isolated from cadaveric donors, a number of factors reduce the viability of islets even before they are isolated, including the age and health status of the donor as well as organ ischemia and the time from donor death to islet harvest. The process of islet isolation itself also causes significant β-cell death, by both necrosis and apoptosis. Islets are then cultured, typically at high density, and this is associated with a 2–20 % apoptosis rate, which is markedly higher than what is observed in vivo (Dror et al. 2007, 2008a). The implantation of islets into the liver is associated with rapid β-cell death, with only a fraction of islets engrafting with sufficient microvasculature. During and after the process of engraftment, β-cells also experience toxicity from the immunosuppressant drugs that are currently required to prevent allo- and auto-rejection of the transplant. A side-by-side comparison of three clinically significant immunosuppressant drugs revealed distinct differences in the mechanisms by which they impair β-cell function and survival (Johnson et al. 2009). Clinically relevant doses of rapamycin and mycophenolate mofetil increased caspase-3-dependent apoptosis and CHOP-dependent ER stress in human islets, but did not have direct effects on glucose-stimulated insulin secretion. On the other hand, FK506, which had direct deleterious effects on insulin secretion, but caused relatively modest induction of caspase-3 activation and ER stress, resulted in the worst graft function in vivo when transplanted into STZ-diabetic NOD/scid mice. Treating islet cultures with the glucagon-like peptide 1 (GLP-1) agonist exenatide ameliorated the effects of these drugs on human β-cell function and survival (Johnson et al. 2009).

Thus, islet transplantation is associated with a cluster of related stresses including hypoxia and nutrient deprivation. The specific mechanisms that mediate β-cell death from hypoxia remain to be fully elucidated, but likely involves hypoxia-inducible factors (HIF)(Miao et al. 2006). Interestingly, von Hippel-Lindau factor and HIF1b have also been implicated in β-cell function (Gunton et al. 2005; Zehetner et al. 2008). Pancreatic β-cells can undergo programmed cell death under hypoglycemic conditions, and this environment appears to regulate the expression of HIF1b (Dror et al. 2008b). Interestingly, the RyR2 Ca^{2+} channel and calpain-10 appear to be involved in β-cell death in hypoglycemia as well (Johnson et al. 2004). In adult islets, these genes form a network that also includes presenilin, notch, neurogenin-3, and Pdx-1. This gene network appears to influence the basal rate of apoptosis, specifically under low glucose conditions (Dror et al. 2007, 2008a). Whether hypoglycemia, nutrient deprivation, or hypoxia is involved in the progression of diabetes is not well understood. Such a scenario might occur under conditions where genetic or acquired defects in the extensive intra-islet vascular network restrict the delivery of oxygen and nutrients to the β-cells (Clee et al. 2006).

Survival Factors that Prevent β-Cell Apoptosis

A large number of endogenous and exogenous growth factors have been shown to promote β-cell survival, in vitro or in vivo. Some of the key factors will be discussed here (Fig. 4).

Examples of such anti-apoptotic signaling cascades are those activated by the gut hormones GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), which were first investigated for their positive effects on glucose-stimulated insulin secretion. The new diabetes drug Byetta acts by mimicking GLP-1 and has been shown to protect rodent β-cells from apoptosis when administered at high doses (Li et al. 2005). It is likely that other hormones that increase cAMP and activate RyR Ca^{2+} channels would also have anti-apoptotic effects on β-cells. It has also been suggested that inhibiting dipeptidyl peptidase-4, an enzyme that degrades GLP-1, might increase β-cell mass by preventing apoptosis

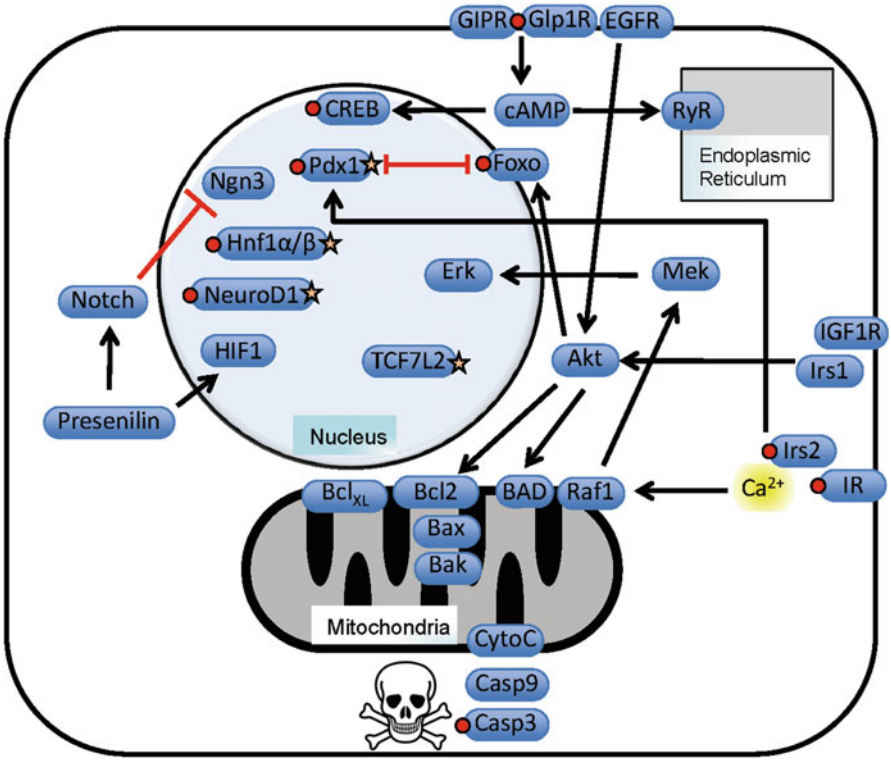

Fig. 4 Molecular mechanisms controlling basal β-cell apoptosis and survival factor signaling cascades. Shown is a partial description of signaling cascades that modulate β-cell survival. Protein products of genes that are linked to human diabetes are denoted with a *star*. Genes that have been implicated in β-cell apoptosis or β-cell mass using in vivo loss-of-function experiments (i.e., knockout mice) are denoted with a *dot*

and increasing proliferation. Nevertheless, caution is critical since this ubiquitous enzyme has many targets.

Many other β-cell growth factor systems, including hepatocyte growth factor, fibroblast growth factors, parathyroid hormone-related protein, gastrin, delta/notch, netrin, and Slit-Robo also promote β-cell survival (Dror et al. 2007; Garcia-Ocana et al. 2001; Yang et al. 2011, 2013). The Slit-Robo system is interesting as it is one of the only examples discovered to date of a local secreted factor that is required for β-cell survival (Yang et al. 2013). One of the most important local endogenous β-cell growth factors appears to be insulin itself (Johnson et al. 2006a, b; Johnson and Alejandro 2008; Alejandro and Johnson 2008; Beith et al. 2008; Ohsugi et al. 2005; Kulkarni et al. 1999a; Hennige et al. 2003; Otani et al. 2004; Ueki et al. 2006; Okada et al. 2007). Based on knockout mouse studies, the insulin receptor even appears more important than the IGF-1 receptor (Ueki et al. 2006). Insulin acts via a complex series of signaling events, including both the PI3-kinase/Akt pathway and the Raf-1/Erk pathway (Johnson et al. 2006a; Johnson and Alejandro 2008; Alejandro and Johnson 2008; Ueki et al. 2006). We have recently shown that 14-3-3 proteins play an important role in coordinating β-cell survival signaling (Lim et al. 2013). Akt acts on multiple downstream targets, including Bad. In addition to stimulating Erk, Raf-1 can also dephosphorylate and inactivate Bad at the mitochondria. Interestingly, signaling through IRS-2 rather than IRS-1 appears to play a role in β-cell survival (Kulkarni et al. 1999b). While constitutive insulin signaling seems to be essential for β-cell survival under stressful conditions, excessive concentrations of insulin may be deleterious (Johnson and Alejandro 2008). Further work is needed to understand the ideal way to harness this and other endogenous anti-apoptotic signaling pathways. Ongoing studies have employed high-throughput imaging methodologies to identify generation and stress-specific β-cell survival factors from large libraries of endogenous biologic factors.

The Role of Other β-Cell Death Modalities: Beyond β-Cell Apoptosis

Numerous intrinsic and extrinsic signals are required for the maintenance of functional β-cell mass by providing pro-survival and pro-death signals. Mechanistic studies on the initiation and progression of β-cell death can make significant contributions to the prevention and treatment of type 1 diabetes and type 2 diabetes, in addition to improving the success of islet transplantations. Programmed cell death via apoptosis has been well characterized as an important mechanism of β-cell death (Mathis et al. 2001; Cnop et al. 2005). However, there are other commonly characterized forms of cell death distinguished by morphological and biochemical features, including necrosis and autophagy (Fig. 5) (Kroemer et al. 2009; Galluzzi et al. 2009, 2012).

The redundancy of signaling molecules involved in the temporal cascade of events leading to β-cell apoptosis, autophagy, and necrosis has not been well

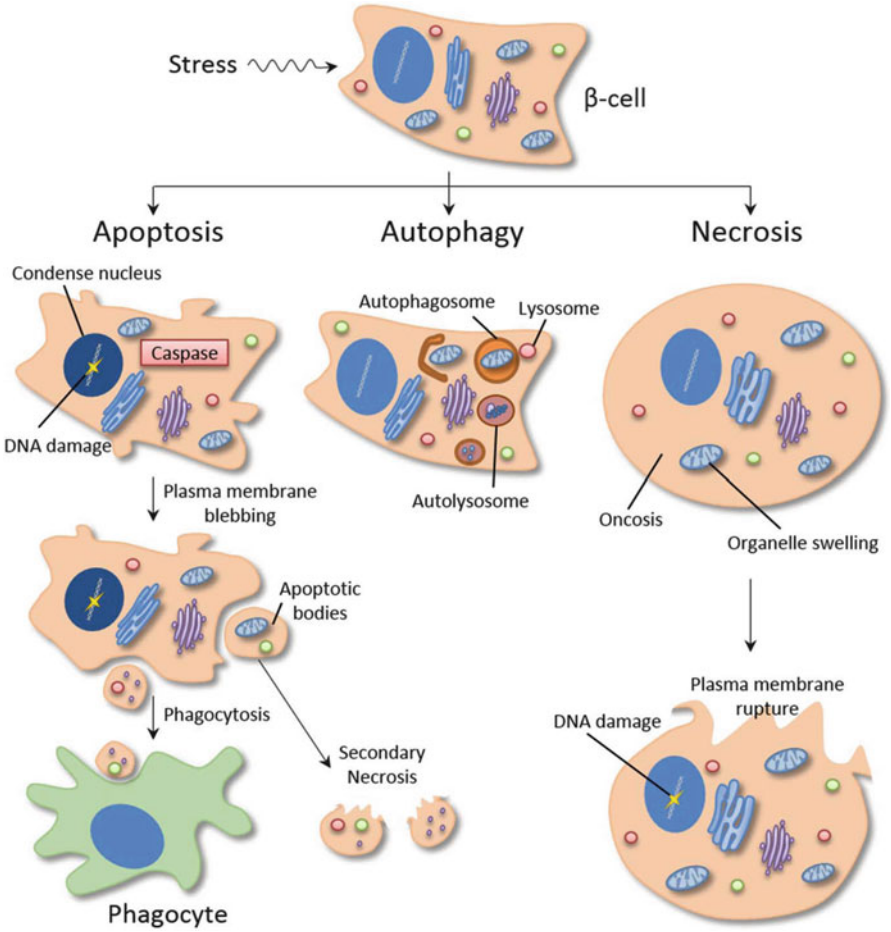

Fig. 5 Schematic of common β-cell death pathways. Prolonged exposure to stress conditions, including cytokine exposure hyperglycemia, hyperlipidemia, oxidative stress, and ER stress, can lead to β-cell death. Apoptosis, autophagy-mediated, and necrosis are the most well-characterized modes of cell death

characterized on a single cell level, resulting in the underappreciation of non-apoptotic forms of cell death (Yang et al. 2013b). Understanding the redundancy and exclusiveness of different mechanisms of cell death has important implications for the detection and therapeutic manipulation of cell death.

Autophagy is a catabolic process often favoring cell survival under conditions of nutrient deprivation, hypoxia, ER stress, pathogen infection, and DNA damage (Kroemer et al. 2009, 2010; Fleming et al. 2011; Levine and Yuan 2005). These conditions are relevant to the initiation and progression and diabetes. Also, in islet transplantation, islet cells are exposed to hypoxia and nutrient deprivation prior

to vascularization and engraftment. The formation of double membrane vacuoles that sequester damaged organelles and harmful cytoplasmic contents, termed autophagosomes, is a defining feature of autophagy, which concludes with the delivery of the contents to the lysosome for degradation and recycling (Kroemer et al. 2010; Levine and Yuan 2005). Autophagic cell death is simply characterized by the lack of chromatin condensation and accumulation of autophagosomes and does not necessarily implicate autophagy as the cause of cell death (Kroemer et al. 2009). Ablation of free fatty acid-induced autophagy leads to a lack of compensatory β-cell hyperplasia and impaired glucose tolerance (Ebato et al. 2008). Diminished maintenance of functional β-cell mass by autophagy may increase the susceptibility to β-cell death under basal and stressed conditions and consequently affect diabetes initiation and progression (Ebato et al. 2008; Jung et al. 2008; Levine and Kroemer 2008). The mutual inhibition between apoptosis and autophagy further supports the involvement of autophagy in maintaining β-cell health (Kroemer et al. 2010; Kang et al. 2011).

β-Cell death via necrosis has also been implicated in the pathogenesis of diabetes (Fujimoto et al. 2010; Steer et al. 2006). Necrosis is often defined as cell death lacking the characteristics of apoptosis or autophagy (Yang et al. 2013b). In addition, key morphological features of necrosis include plasma membrane rupture and swelling of cytoplasmic organelles (Kroemer et al. 2009; Golstein and Kroemer 2007). Although initially believed to be an uncontrolled form of cell death leading to the release of inflammatory cellular contents, there is accumulating evidence supporting the notion that necrotic cell death is regulated by a defined set of signaling events induced by oxidative stress, loss of Ca^{2+} homeostasis, or ischemia (Kroemer et al. 2009; Golstein and Kroemer 2007; Fink and Cookson 2005). In fact, apoptosis and necrosis may share common signaling pathways involving mitochondrial membrane permeabilization through activation of proapoptotic Bcl-2 family members (Golstein and Kroemer 2007; Kim et al. 2003). Receptor-interacting protein kinase 1 (RIP1)-dependent necrosis is a well-characterized case of regulated necrosis that can be activated upon binding of tumor necrosis factor α (TNFα) to TNF receptor 1 in the absence of caspase-8 activity (Galluzzi et al. 2012; Vandenabeele et al. 2010). Consequently, RIP1 is deubiquitinated and associates with RIP3 to activate necrotic cell death. Upon exposure to stress, inhibition of the apoptotic signaling cascade by direct inhibition of caspase activation or depletion of ATP (which is required for caspase activation) can favor necrotic cell death (Kim et al. 2003; Leist et al. 1997; Eguchi et al. 1997). This suggests that multiple modes of cell death can coexist within the same cell and they have the potential to substitute for each other. The complex interplay between different modes of cell death further complicates the development of therapeutics for preventing β-cell death.

Understanding the molecular processes behind cell death may reveal novel therapeutic targets. In addition to the complex interplay between apoptosis, necrosis, and autophagy, other pathways can also proceed. The diversity of the molecular pathways mediating cell death has led to the characterization of new modalities of

cell death that sometimes share similar features. Mitotic catastrophe is initiated by aberrant mitosis leading to cell death during mitosis or interphase via apoptosis or necrosis (Kroemer et al. 2009). Anoikis is an intrinsic apoptotic response of adherent cells to the detachment from extracellular matrix interactions (Galluzzi et al. 2012; Frisch and Francis 1994). Parthanatos is a caspase-independent cell death pathway involving DNA damage induced by over-activation of poly-ADP-ribose polymerases (PARPs), which can further result in ATP and NAD^+ depletion, PAR accumulation, loss of mitochondrial membrane potential, and subsequently AIF release (David et al. 2009; Luo and Kraus 2012). Pyroptosis is a caspase-1-mediated cell death pathway that exhibits morphological features of apoptosis and/or necrosis. The activation of caspase-1 leads to the mature processing of inflammatory cytokines interleukin-1β (IL-1β) and IL-18 (Fink and Cookson 2005). It remains controversial whether these new modalities constitute unique cell death subroutines or whether they represent specific cases of apoptosis and/or necrosis. Elucidating the context-dependent distribution of various mechanisms of cell death may determine the success of targeted therapeutic interventions to control β-cell death. It is conceivable that therapies for promoting β-cell survival may require inhibition of all forms of cell death through targeted inhibition of upstream events or combinatorial therapies.

β-Cell Apoptosis as a Therapeutic Target in Diabetes: Future Directions

The protection of existing β-cells and the regeneration of new ones is a major goal in diabetes research. Therapeutic strategies to protect β-cells could have an immediate impact on clinical islet transplantation, where close to half of the islets transplanted into the liver die before becoming engrafted. In future years we also expect drugs may be developed that improve endogenous β-cell survival in vivo. These treatments would theoretically slow the progression of, or perhaps reverse, type 1 diabetes or type 2 diabetes. Once the exact molecular defects are better known, specific components of the β-cell apoptosis system could be targeted more selectively. For diabetes caused by β-cell ER stress, so-called molecular chaperones might be useful to decrease unfolded proteins in the ER. In cases where diabetes is associated with apoptosis controlled by cellular metabolism, we expect that direct interventions at the level of β-cell mitochondria might be of benefit. Since islet amyloid formation can be found in type 2 diabetes and in transplantation, chemical inhibitors of this process might have therapeutic potential (Potter et al. 2009). A thorough understanding of survival signaling pathways induced by endogenous β-cell growth factors will hopefully provide new targets for intervention, based on the β-cell's own defenses. Moreover, unbiased and high-throughput methods promise to accelerate the pace at which we discover the mechanisms of β-cell apoptosis and treatments that target β-cell apoptosis in diabetes.

Cross-References

Interested readers are encouraged to view other articles on this platform. For example, there is an entire chapter devoted to the effects of glucose, insulin signaling, and immune cells on β-cell survival:

- ▶ IGF-1 and insulin-receptor signaling in insulin secreting cells: from function to survival
- ▶ Immunology of β-Cell Destruction
- ▶ Inflammatory Pathways Linked to β Cell Demise in Diabetes

There is also information on β-cell dysfunction in:

- ▶ (Dys)Regulation of Insulin Secretion by Macronutrients
- ▶ High Fat Programming of β-Cell Dysfunction
- ▶ Role of Mitochondria in β-Cell Function and Dysfunction

A discussion on possible approaches to detect and treat β-cell death can be found in:

- ▶ Clinical Approaches to Preserve β-Cell Function in Diabetes
- ▶ Current Approaches and Future Prospects for the Prevention of β-Cell Destruction in Autoimmune Diabetes
- ▶ In Vivo Biomarkers for Detection of β Cell Death

References

Alejandro EU, Johnson JD (2008) Inhibition of raf-1 alters multiple downstream pathways to induce pancreatic β-cell apoptosis. J Biol Chem 283(4):2407–2417

Barratt BJ et al (2004) Remapping the insulin gene/IDDM2 locus in type 1 diabetes. Diabetes 53 (7):1884–1889

Beith JL, Alejandro EU, Johnson JD (2008) Insulin stimulates primary β-cell proliferation via Raf-1 kinase. Endocrinology 149(5):2251–2260

Bell GI, Polonsky KS (2001) Diabetes mellitus and genetically programmed defects in β-cell function. Nature 414(6865):788–791

Bennett ST, Todd JA (1996) Human type 1 diabetes and the insulin gene: principles of mapping polygenes. Annu Rev Genet 30:343–370

Boslem E et al (2011) A lipidomic screen of palmitate-treated MIN6 β-cells links sphingolipid metabolites with endoplasmic reticulum (ER) stress and impaired protein trafficking. Biochem J 435(1):267–276

Boslem E, Meikle PJ, Biden TJ (2012) Roles of ceramide and sphingolipids in pancreatic β-cell function and dysfunction. Islets 4(3):177–187

Boslem E et al (2013) Alteration of endoplasmic reticulum lipid rafts contributes to lipotoxicity in pancreatic β-cells. J Biol Chem 288(37):26569–26582

Butler AE et al (2003a) Increased β-cell apoptosis prevents adaptive increase in β-cell mass in mouse model of type 2 diabetes: evidence for role of islet amyloid formation rather than direct action of amyloid. Diabetes 52(9):2304–2314

Butler AE et al (2003b) β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 52(1):102–110

Cardozo AK et al (2005) Cytokines downregulate the sarcoendoplasmic reticulum pump Ca^{2+} ATPase 2b and deplete endoplasmic reticulum Ca^{2+}, leading to induction of endoplasmic reticulum stress in pancreatic β-cells. Diabetes 54(2):452–461

Chen J et al (2008) Thioredoxin-interacting protein: a critical link between glucose toxicity and β-cell apoptosis. Diabetes 57(4):938–944
Chu KY et al (2010) ATP-citrate lyase reduction mediates palmitate-induced apoptosis in pancreatic β cells. J Biol Chem 285(42):32606–32615
Chu KY et al (2011) Differential regulation and localization of carboxypeptidase D and carboxypeptidase E in human and mouse β-cells. Islets 3(4):155–165
Chu KY et al (2012) Ubiquitin C-terminal hydrolase L1 is required for pancreatic β cell survival and function in lipotoxic conditions. Diabetologia 55(1):128–140
Clee SM et al (2006) Positional cloning of Sorcs1, a type 2 diabetes quantitative trait locus. Nat Genet 38(6):688–693
Cnop M et al (2005) Mechanisms of pancreatic β-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes 54(Suppl 2):S97–S107
Colli ML et al (2010) MDA5 and PTPN2, two candidate genes for type 1 diabetes, modify pancreatic β-cell responses to the viral by-product double-stranded RNA. Hum Mol Genet 19(1):135–146
Danial NN et al (2008) Dual role of proapoptotic BAD in insulin secretion and β cell survival. Nat Med 14(2):144–153
David KK et al (2009) Parthanatos, a messenger of death. Front Biosci 14:1116–1128
Donath MY, Halban PA (2004) Decreased β-cell mass in diabetes: significance, mechanisms and therapeutic implications. Diabetologia 47(3):581–589
Dror V et al (2007) Notch signalling suppresses apoptosis in adult human and mouse pancreatic islet cells. Diabetologia 50(12):2504–2515
Dror V et al (2008a) Glucose and Endoplasmic Reticulum Calcium Channels Regulate HIF-1β via Presenilin in Pancreatic β-Cells. J Biol Chem 283(15):9909–9916
Dror V et al (2008b) Glucose and endoplasmic reticulum calcium channels regulate HIF-1β via presenilin in pancreatic β-cells. J Biol Chem 283(15):9909–9916
Ebato C et al (2008) Autophagy is important in islet homeostasis and compensatory increase of β cell mass in response to high-fat diet. Cell Metab 8(4):325–332
Efanova IB et al (1998) Glucose and tolbutamide induce apoptosis in pancreatic β-cells. A process dependent on intracellular Ca^{2+} concentration. J Biol Chem 273(50):33501–33507
Eguchi Y, Shimizu S, Tsujimoto Y (1997) Intracellular ATP levels determine cell death fate by apoptosis or necrosis. Cancer Res 57(10):1835–1840
Ehses JA et al (2007) Increased number of islet-associated macrophages in type 2 diabetes. Diabetes 56(9):2356–2370
Eizirik DL, Grieco FA (2012) On the immense variety and complexity of circumstances conditioning pancreatic β-cell apoptosis in type 1 diabetes. Diabetes 61(7):1661–1663
Eizirik DL, Cardozo AK, Cnop M (2008) The role for endoplasmic reticulum stress in diabetes mellitus. Endocr Rev 29(1):42–61
Eizirik DL et al (2012) The human pancreatic islet transcriptome: expression of candidate genes for type 1 diabetes and the impact of pro-inflammatory cytokines. PLoS Genet 8(3): e1002552
Eizirik DL, Miani M, Cardozo AK (2013) Signalling danger: endoplasmic reticulum stress and the unfolded protein response in pancreatic islet inflammation. Diabetologia 56(2): 234–241
El-Assaad W et al (2003) Saturated fatty acids synergize with elevated glucose to cause pancreatic β-cell death. Endocrinology 144(9):4154–4163
Estella E et al (2006) Granzyme B-mediated death of pancreatic β-cells requires the proapoptotic BH3-only molecule bid. Diabetes 55(8):2212–2219
Federici M et al (2001) High glucose causes apoptosis in cultured human pancreatic Islets of Langerhans – a potential role for regulation of specific Bcl family genes toward an apoptotic cell death program. Diabetes 50(6):1290–1301
Fink SL, Cookson BT (2005) Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. Infect Immun 73(4):1907–1916

Fleming A et al (2011) Chemical modulators of autophagy as biological probes and potential therapeutics. Nat Chem Biol 7(1):9–17
Frisch SM, Francis H (1994) Disruption of epithelial cell-matrix interactions induces apoptosis. J Cell Biol 124(4):619–626
Fujimoto K et al (2010) Loss of Nix in Pdx1-deficient mice prevents apoptotic and necrotic β cell death and diabetes. J Clin Invest 120(11):4031–4039
Galluzzi L et al (2009) Guidelines for the use and interpretation of assays for monitoring cell death in higher eukaryotes. Cell Death Differ 16(8):1093–1107
Galluzzi L et al (2012) Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. Cell Death Differ 19(1):107–120
Garcia-Ocana A et al (2001) Using β-cell growth factors to enhance human pancreatic Islet transplantation. J Clin Endocrinol Metab 86(3):984–988
Golstein P, Kroemer G (2007) Cell death by necrosis: towards a molecular definition. Trends Biochem Sci 32(1):37–43
Guillen C et al (2008) Biphasic effect of insulin on β cell apoptosis depending on glucose deprivation. FEBS Lett 582(28):3855–3860
Gunton JE et al (2005) Loss of ARNT/HIF1β mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. Cell 122(3):337–349
Gwiazda KS et al (2009) Effects of palmitate on ER and cytosolic Ca^{2+} homeostasis in β-cells. Am J Physiol Endocrinol Metab 296(4):E690–E701
Haataja L et al (2008) Islet amyloid in type 2 diabetes, and the toxic oligomer hypothesis. Endocr Rev 29(3):303–316
Harding HP, Ron D (2002) Endoplasmic reticulum stress and the development of diabetes: a review. Diabetes 51(Suppl 3):S455–S461
Hennige AM et al (2003) Upregulation of insulin receptor substrate-2 in pancreatic β cells prevents diabetes. J Clin Invest 112(10):1521–1532
Horikawa Y et al (2000) Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nat Genet 26(2):163–175
Jeffrey KD et al (2008) Carboxypeptidase E mediates palmitate-induced β-cell ER stress and apoptosis. Proc Natl Acad Sci U S A 105(24):8452–8457
Johnson JD (2007) Pancreatic β-cell apoptosis in maturity onset diabetes of the young. Can J Diabetes 31(1):001–008
Johnson JD, Alejandro EU (2008) Control of pancreatic β-cell fate by insulin signaling: the sweet spot hypothesis. Cell Cycle 7(10):1343–1347
Johnson JD et al (2003) Increased islet apoptosis in $Pdx1^{+/-}$ mice. J Clin Invest 111(8):1147–1160
Johnson JD et al (2004) RyR2 and calpain-10 delineate a novel apoptosis pathway in pancreatic islets. J Biol Chem 279(23):24794–24802
Johnson JD et al (2006a) Insulin protects islets from apoptosis via Pdx1 and specific changes in the human islet proteome. Proc Natl Acad Sci U S A 103(51):19575–19580
Johnson JD et al (2006b) Suppressed insulin signaling and increased apoptosis in CD38-null islets. Diabetes 55(10):2737–2746
Johnson JD, et al. (2009) Different effects of FK506, rapamycin, and mycophenolate mofetil on glucose-stimulated insulin release and apoptosis in human islets. Cell Transpl 18 (8):833–845
Jung HS et al (2008) Loss of autophagy diminishes pancreatic β cell mass and function with resultant hyperglycemia. Cell Metab 8(4):318–324
Kaneto H et al (2005) Oxidative stress and pancreatic β-cell dysfunction. Am J Ther 12 (6):529–533
Kang R et al (2011) The Beclin 1 network regulates autophagy and apoptosis. Cell Death Differ 18 (4):571–580
Kim JS, He L, Lemasters JJ (2003) Mitochondrial permeability transition: a common pathway to necrosis and apoptosis. Biochem Biophys Res Commun 304(3):463–470

Kroemer G et al (2009) Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ 16(1):3–11
Kroemer G, Marino G, Levine B (2010) Autophagy and the integrated stress response. Mol Cell 40 (2):280–293
Kulkarni RN et al (1999a) Tissue-specific knockout of the insulin receptor in pancreatic β cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell 96(3): 329–339
Kulkarni RN et al (1999b) Altered function of insulin receptor substrate-1-deficient mouse islets and cultured β-cell lines. J Clin Invest 104(12):R69–R75
Laybutt DR et al (2007) Endoplasmic reticulum stress contributes to β cell apoptosis in type 2 diabetes. Diabetologia 50(4):752–763
Lee B et al (1999) Glucose regulates expression of inositol 1,4,5-trisphosphate receptor isoforms in isolated rat pancreatic islets. Endocrinology 140(5):2173–2182
Leist M et al (1997) Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. J Exp Med 185(8):1481–1486
Leonardi O, Mints G, Hussain MA (2003) β-cell apoptosis in the pathogenesis of human type 2 diabetes mellitus. Eur J Endocrinol 149(2):99–102
Levine B, Kroemer G (2008) Autophagy in the pathogenesis of disease. Cell 132(1):27–42
Levine B, Yuan J (2005) Autophagy in cell death: an innocent convict? J Clin Invest 115 (10):2679–2688
Li Y et al (2005) β-Cell Pdx1 expression is essential for the glucoregulatory, proliferative, and cytoprotective actions of glucagon-like peptide-1. Diabetes 54(2):482–491
Liadis N et al (2005) Caspase-3-dependent β-cell apoptosis in the initiation of autoimmune diabetes mellitus. Mol Cell Biol 25(9):3620–3629
Liadis N et al (2007) Distinct in vivo roles of caspase-8 in β-cells in physiological and diabetes models. Diabetes 56(9):2302–2311
Lim GE, Piske M, Johnson JD (2013) 14-3-3 proteins are essential signalling hubs for β cell survival. Diabetologia 56(4):825–837
Lin CY et al (2005) Activation of peroxisome proliferator-activated receptor-gamma by rosiglitazone protects human islet cells against human islet amyloid polypeptide toxicity by a phosphatidylinositol 3′-kinase-dependent pathway. J Clin Endocrinol Metab 90 (12):6678–6686
Lovell JF et al (2008) Membrane binding by tBid initiates an ordered series of events culminating in membrane permeabilization by Bax. Cell 135(6):1074–1084
Lu H et al (2008) The identification of potential factors associated with the development of type 2 diabetes: a quantitative proteomics approach. Mol Cell Proteomics 7(8):1434–1451
Luciani DS et al (2013) Bcl-2 and Bcl-xL suppress glucose signaling in pancreatic β-cells. Diabetes 62(1):170–182
Luo X, Kraus WL (2012) On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1. Genes Dev 26(5):417–432
Lupi R et al (2002) Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that β-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. Diabetes 51(5):1437–1442
Lyssenko V, Groop L (2009) Genome-wide association study for type 2 diabetes: clinical applications. Curr Opin Lipidol 20(2):87–91
Lyssenko V et al (2008) Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med 359(21):2220–2232
Maedler K, Donath MY (2004) β-cells in type 2 diabetes: a loss of function and mass. Horm Res 62 (Suppl 3):67–73
Maedler K et al (2004) Glucose- and interleukin-1β-induced β-cell apoptosis requires Ca^{2+} influx and extracellular signal-regulated kinase (ERK) 1/2 activation and is prevented by a sulfonylurea receptor 1/inwardly rectifying K^+ channel 6.2 (SUR/Kir6.2) selective potassium channel opener in human islets. Diabetes 53(7):1706–1713

Maedler K et al (2005) Sulfonylurea induced β-cell apoptosis in cultured human islets. J Clin Endocrinol Metab 90(1):501–506
Marshall C et al (2005) Evidence that an isoform of calpain-10 is a regulator of exocytosis in pancreatic β-cells. Mol Endocrinol 19(1):213–224
Martinez SC et al (2008) Inhibition of Foxo1 protects pancreatic islet β-cells against fatty acid and endoplasmic reticulum stress-induced apoptosis. Diabetes 57(4):846–859
Mathis D, Vence L, Benoist C (2001) β-cell death during progression to diabetes. Nature 414 (6865):792–798
McKenzie MD et al (2008) Proapoptotic BH3-only protein Bid is essential for death receptor-induced apoptosis of pancreatic β-cells. Diabetes 57(5):1284–1292
Mehran AE et al (2012) Hyperinsulinemia drives diet-induced obesity independently of brain insulin production. Cell Metab 16(6):723–737
Meier JJ et al (2008) β-cell replication is the primary mechanism subserving the postnatal expansion of β-cell mass in humans. Diabetes 57(6):1584–1594
Miao G et al (2006) Dynamic production of hypoxia-inducible factor-1α in early transplanted islets. Am J Transplant 6(11):2636–2643
Moore F et al (2009) PTPN2, a candidate gene for type 1 diabetes, modulates interferon-gamma-induced pancreatic β-cell apoptosis. Diabetes 58(6):1283–1291
Nogueira TC et al (2013) GLIS3, a susceptibility gene for type 1 and type 2 diabetes, modulates pancreatic β cell apoptosis via regulation of a splice variant of the BH3-only protein Bim. PLoS Genet 9(5):e1003532
Ohsugi M et al (2005) Reduced expression of the insulin receptor in mouse insulinoma (MIN6) cells reveals multiple roles of insulin signaling in gene expression, proliferation, insulin content, and secretion. J Biol Chem 280(6):4992–5003
Okada T et al (2007) Insulin receptors in β-cells are critical for islet compensatory growth response to insulin resistance. Proc Natl Acad Sci U S A 104(21):8977–8982
Otani K et al. (2004) Reduced β-cell mass and altered glucose sensing impair insulin-secretory function in βIRKO mice. Am J Physiol Endocrinol Metab 286(1):E41–9
Oyadomari S, Araki E, Mori M (2002) Endoplasmic reticulum stress-mediated apoptosis in pancreatic β-cells. Apoptosis 7(4):335–345
Ozcan U et al (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 306(5695):457–461
Ozcan U et al (2006) Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science 313(5790):1137–1140
Pearson GL et al (2014) Lysosomal acid lipase and lipophagy are constitutive negative regulators of glucose-stimulated insulin secretion from pancreatic β cells. Diabetologia 57(1):129–139
Potter KJ et al (2009) Amyloid inhibitors enhance survival of cultured human islets. Biochim Biophys Acta 1790(6):566–574
Prentki M, Nolan CJ (2006) Islet β cell failure in type 2 diabetes. J Clin Invest 116(7):1802–1812
Preston AM et al (2009) Reduced endoplasmic reticulum (ER)-to-Golgi protein trafficking contributes to ER stress in lipotoxic mouse β cells by promoting protein overload. Diabetologia 52(11):2369–2373
Pugliese A et al (1997) The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. Nat Genet 15(3):293–297
Rhodes CJ (2005) Type 2 diabetes-a matter of β-cell life and death? Science 307(5708):380–384
Riggs AC et al (2005) Mice conditionally lacking the Wolfram gene in pancreatic islet β cells exhibit diabetes as a result of enhanced endoplasmic reticulum stress and apoptosis. Diabetologia 48(11):2313–2321
Robertson RP et al (2004) β-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. Diabetes 53(Suppl 1):S119–S124
Santin I et al (2011) PTPN2, a candidate gene for type 1 diabetes, modulates pancreatic β-cell apoptosis via regulation of the BH3-only protein Bim. Diabetes 60(12):3279–3288

Shu L et al (2008) Transcription factor 7-like 2 regulates β-cell survival and function in human pancreatic islets. Diabetes 57(3):645–653
Song B et al (2008) Chop deletion reduces oxidative stress, improves β cell function, and promotes cell survival in multiple mouse models of diabetes. J Clin Invest 118(10):3378–3389
Steer SA et al (2006) Interleukin-1 stimulates β-cell necrosis and release of the immunological adjuvant HMGB1. PLoS Med 3(2):e17
Stoy J et al (2007) Insulin gene mutations as a cause of permanent neonatal diabetes. Proc Natl Acad Sci U S A 104(38):15040–15044
Szabat M et al (2012) Maintenance of β-cell maturity and plasticity in the adult pancreas: developmental biology concepts in adult physiology. Diabetes 61(6):1365–1371
Thomas HE et al (2009) β cell apoptosis in diabetes. Apoptosis 14(12):1389–1404
Trudeau JD et al (2000) Neonatal β-cell apoptosis: a trigger for autoimmune diabetes? Diabetes 49 (1):1–7
Ueki K et al (2006) Total insulin and IGF-I resistance in pancreatic β cells causes overt diabetes. Nat Genet 38(5):583–588
Vandenabeele P et al (2010) Molecular mechanisms of necroptosis: an ordered cellular explosion. Nat Rev Mol Cell Biol 11(10):700–714
Wang M et al (2013) Is dynamic autocrine insulin signaling possible? A mathematical model predicts picomolar concentrations of extracellular monomeric insulin within human pancreatic islets. PLoS ONE 8(6):e64860
Wobser H et al (2002) Dominant-negative suppression of HNF-1 α results in mitochondrial dysfunction, INS-1 cell apoptosis, and increased sensitivity to ceramide-, but not to high glucose-induced cell death. J Biol Chem 277(8):6413–6421
Yamada K et al (1999) Essential role of caspase-3 in apoptosis of mouse β-cells transfected with human Fas. Diabetes 48(3):478–483
Yang YH, Johnson JD (2013) Multi-parameter, single-cell, kinetic analysis reveals multiple modes of cell death in primary pancreatic β-cells. J Cell Sci 126(Pt 18):4286–95
Yang YH et al (2011) Paracrine signalling loops in adult human and mouse pancreatic islets: netrins modulate β cell apoptosis signalling via dependence receptors. Diabetologia 54 (4):828–842
Yang YH et al (2013) Intraislet SLIT-ROBO signaling is required for β-cell survival and potentiates insulin secretion. Proc Natl Acad Sci U S A 110(41):16480–16485
Zehetner J et al (2008) PVHL is a regulator of glucose metabolism and insulin secretion in pancreatic β cells. Genes Dev 22(22):3135–3146

Clinical Approaches to Preserving β-Cell Function in Diabetes

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_17,

Abstract

In type 2 diabetes (DM2) there is progressive deterioration of β-cell function and mass. It was found that islet function was about 50 % of normal at the time of diagnosis and there was a reduction of β-cell mass of about 60 % at necropsy (accelerated apoptosis). Among the interventions to preserve the β-cells, those that lead to short-term improvement of β-cell secretion are weight loss, metformin, sulfonylureas, and insulin. Long-term improvement was demonstrated with short-term insulin therapy of newly diagnosed DM2. Besides, long-term intensive insulin therapy plus metformin or triple oral therapy (metformin + glyburide + pioglitazone) for 3.5 years enabled β-cell function to be preserved for at least that period of time. Furthermore, long-term improvement was also shown with the isolated use of anti-apoptotic drugs such as glitazones, and the use of glucagon-like peptide-1 receptor agonists (GLP-1 mimetics), not inactivated by the enzyme dipeptidyl peptidase-4, and/or to inhibit that enzyme (GLP-1 enhancers). The incretin hormones are released from the gastrointestinal tract in response to nutrient ingestion to enhance glucose-dependent insulin secretion from the pancreas and overall maintenance of glucose homeostasis. Of the incretins, only GLP-1 mimetics or enhancers can be used for the treatment of DM2. Although incretin-based medications maintain β-cell function, there is no evidence that they increase β-cell mass.

Abbreviations

A1c = HbA1c	Glycated hemoglobin
aGLP-1	Active glucagon-like peptide-1
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BID	Twice a day
BMI	Body mass index
CV	Cardiovascular
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DM2	Type 2 diabetes mellitus
DPP-4	Dipeptidyl peptidase-4
ER	Endoplasmic reticulum
FA	Fatty acid
FFA	Free fatty acid
FPG	Fasting plasma glucose
GFR	Glomerular filtration rate
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GLP-1R	Glucagon-like peptide-1 receptor
GLP-2	Glucagon-like peptide-2
HOMA	homeostasis model assessment

HOMA-β or B	HOMA of β-cell function
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
OW	Once a week
PI/IRI ratio	Proinsulin to total immunoreactive insulin ratio
PPARγ	Peroxisome proliferator-activated receptor γ
PPG	Postprandial plasma glucose
ROS	Reactive oxygen species
SBP	Systolic blood pressure
tGLP-1	Total GLP-1

Introduction

Type 2 diabetes (DM2) is caused by insufficient insulin secretion usually in the context of resistance of the peripheral tissues to the action of the hormone and characterized by progressive deterioration of the β-cell function over time. The deterioration occurs regardless of therapy allocation, albeit conventional (mainly diet), insulin, sulfonylureas, or sensitizers such as glitazones and metformin (UK Prospective Diabetes Study (UKPDS) Group 1998; Holman 2006). DM2 subjects show both quantitative and qualitative disturbances in plasma insulin levels (loss of acute insulin response to glucose loss of the first phase; impaired insulin oscillations during the sustained second phase of glucose-induced insulin secretion, and defects in proinsulin processing at the β-cell level, resulting in an increase in the proinsulin to insulin ratio (Wajchenberg 2007)). Associated with reduced β-cell function, which was found to be about 50 % of the normal level at the time of diagnosis, independent of the degree of insulin resistance and probably commencing 10–12 years before diagnosis, as well as being aggravated by increasing fasting plasma glucose levels (Holman 1998), a reduction in the β-cell mass of about 60 % has been observed at necropsy. The underlying mechanism was found to be increased β-cell apoptosis, while new islet formation and β-cell replication (normalized to relative β-cell volume) remained normal or increased (Butler et al. 2003).

While there is consensus that hyperglycemia develops in the context of insulin resistance only if insulin secretion is insufficient, the question remains whether this insufficiency reflects functional abnormalities in each β-cell or too few appropriately functioning β-cells, usually referred to as a low β-cell mass (Henquin et al. 2008). As indicated by Rahier et al. (2008), sub-optimal β-cell function leads to a higher risk of developing DM2 if there is also a low β-cell mass while the slow decrease in β-cell mass with duration of diabetes could, at least in part, be a secondary phenomenon caused by exposure to a metabolically abnormal environment: glucolipotoxicity (Poitout and Robertson 2008).

Paradoxically, it has also been proposed that an important mechanism contributing to β-cell failure in DM2 is the ability to hypersecrete insulin

(Aston-Mourney et al. 2008). Hypersecretion, a characteristic in the early stages of the disease, is beneficial in maintaining normal glucose tolerance, which may also be an important factor in the progression of β-cell failure (Aston-Mourney et al. 2008). A state of hyperinsulinemia can be caused by increased insulin demand (insulin resistance), a genetic abnormality leading to hypersecretion (as in persistent hyperinsulinemic hypoglycemia of infancy) or the use of insulin secretory drugs (sulfonylureas such as glibenclamide). The increased demands for insulin production could overload the endoplasmic reticulum (ER), resulting in ER stress and inducing the unfolded protein response. Furthermore, apoptosis of the β cells has been shown to be a result of the activation of an ER stress response (Laybutt et al. 2007). Alternatively, the increased glycolytic flux required for increased insulin secretion could result in oxidative stress. In individuals with a genetic predisposition, the increased ER stress could lead to β cell failure and subsequent diabetes. The treatment of diabetes with insulin secretory drugs could further promote insulin hypersecretion, leading to worsening of β-cell function.

Besides glucotoxicity, lipotoxicity, and glucolipotoxicity, which are secondary phenomena that play a role in β-cell dysfunction, other factors could contribute to the progressive loss of β-cell function in DM2 (Wajchenberg 2007).

In conclusion, drawing on all the information available, it can be suggested that the link between reduced β-cell mass and impaired function could be due to an increased demand on residual β-cells per se, leading to changes in function (ER stress or other mechanisms), or related to the hyperglycemia resulting from decreased β-cell mass, driving the impairment in β-cell function. In vitro and in vivo studies in rodents (not in humans, as shown previously) have indicated that persistently high glucose levels play a central role among those factors (free fatty acids [FFA], lipoproteins, leptin, and cytokines), contributing to β-cell demise.

Understanding the mechanisms of β-cell death and thus decreased β-cell mass, at least in rodents, and impaired function has provided the basis of β-cell preservation, especially when one considers that the impaired β-cell function and possibly β-cell mass appear to be reversible to a certain degree, particularly during the early stages of the disease, where the threshold for reversibility of decreased β-cell mass has probably not been passed. Therefore, any therapeutic intervention aimed at preserving β-cell activity should improve function and prevent further reduction in mass.

It is known that humans have a much lower capacity for β-cell regeneration than rodents, which declines even further with aging. Data from studies with human autopsy and human donor pancreata suggest that, after the age of 15–20 years β cell growth by replication (probably the primary mechanism for regeneration) is minimal (Ritzel 2009). Accordingly, in a small series of 10 deceased patients (aged 17–74 years) who had received thymidine analogs 8 days to 4 years before death, Perl et al. (2010) found that human β-cell turnover is limited to the first three decades of life. Since most humans requiring antidiabetic therapy are over 20 it will require long-term studies to identify which treatment option might induce β-cell regeneration in the clinical setting.

Clinical Impact of Therapies Aimed at β-Cell Preservation

Short-Term Improvement of β-Cell Insulin Secretion

The current diabetes treatment options that lead to short-term improvement of β-cell secretion include weight loss and antidiabetic medications: oral insulin secretagogues and insulin:

Weight loss improves insulin secretion in obese DM2 patients (Gumbiner et al. 1990). Among the oral antidiabetic drugs, metformin improves glucose levels before and after meals without significant changes in insulin secretion and levels, indicating improved glucose sensitivity (Wu et al. 1990). The sulfonylureas and glinides are commonly used to stimulate insulin secretion in DM2 patients, enhancing β-cell responsiveness to glucose (Shapiro et al. 1989). Several studies have shown that treatment with sulfonylureas is not associated with any change in the decay curve of β-cell function with time (UK Prospective Diabetes Study 16 1995; Kahn et al. 2006). Moreover, these compounds have been shown to cause apoptosis and therefore loss of β-cell mass (Maedler et al. 2005). Finally, short-term intensive insulin therapy in patients with DM2 has been shown to improve endogenous β-cell function and insulin resistance (Garvey et al. 1985; Glaser et al. 1988). However, prolonged benefit has rarely been demonstrated, with virtually all patients becoming hyperglycemic again after a few weeks (Gormley et al. 1986). Until recently, it was unknown whether such outcomes pertained to new-onset DM2, although patients having failed with diet therapy can show a good response to a short period of intensive insulin therapy by continuous subcutaneous insulin infusion (CSII), as initially demonstrated by Ilkova et al. (1997).

Long-Term Improvement of β-Cell Insulin Secretion

Treatments that may lead to long-term improvement in β-cell insulin secretion include short- and long-term intensive insulin therapy of newly diagnosed DM2 and the use of oral insulin sensitizers: glitazones and incretin mimetics (GLP-1 mimetics and enhancers), which have shown clinical evidence of effects on human β-cell function, the latter drugs having demonstrated, at least in rodents, that they are associated with expansion of β-cell mass via stimulation of β-cell proliferation, promotion of islet cell neogenesis, and inhibition of β-cell apoptosis (Wajchenberg 2007; Xu et al. 1999; Li et al. 2003; Baggio and Drucker 2006).

Short-Term Intensive Insulin Therapy of Newly Diagnosed DM2

Insulin therapy is the most effective antidiabetic therapy and has a variety of effects that may protect against the progression of β-cell dysfunction as suggested by the clinical studies to be outlined later. First, correcting hyperglycemia with insulin may alleviate glucolipotoxicity. Preclinical studies also suggest that insulin has

anti-apoptotic effects via its action on IRS (insulin receptor substrate) proteins and may promote β-cell growth (Tseng et al. 2002). Numerous in vitro and clinical studies have also demonstrated that insulin therapy has potential anti-inflammatory benefits, independent of its ability to lower blood glucose levels (Dandona et al. 2007). Further investigation is needed to determine the clinical implications of the anti-inflammatory properties of insulin in the progression of DM2.

Optimal metabolic control, especially early intensive glycemic control, plays a role in the prevention of progressive β-cell dysfunction and possibly destruction of the β-cells with worsening of diabetes, as will be presented below (Retnakaran and Drucker 2008).

Table 1 shows that in the available studies, early implementation of a short course of intensive insulin therapy, either by continuous subcutaneous insulin infusion or multiple daily injections can induce sustained euglycemia, in patients with DM2 (Ryan et al. 2004; Li et al. 2004; Weng et al. 2008; Chen et al. 2008; Wen et al. 2009), while off any antidiabetic therapy. The remission of DM2 achieved in these studies persisted for 1 year after cessation of insulin therapy in about 46 % of patients. In the small series of patients treated for 1 year, after a short-term bout of intensive insulin therapy, accompanied by Chen et al. (2008), HbA1c levels were significantly lower in the insulin group than in the oral hypoglycemic agent(s) group at 6 months, and after 1 year the glycated hemoglobin level remained lower in the insulin group. Furthermore, Li and colleagues (2004), as well as Weng et al. (2008) and Chen et al. (2008), reported that patients who maintained euglycemia while off oral antidiabetic therapy for 1 year showed greater recovery of β-cell function than their counterparts. In a set of patients studied by Wen et al. out of 84 newly diagnosed DM2 patients post-treatment with continuous sub-cutaneous insulin infusion for 2 weeks, and followed for 2 years, remission was observed in 53 % of the subjects studied (Wen et al. 2009).

It was suggested that an improvement in β-cell function, especially restoration of the first-phase insulin secretion, might be responsible for the ability of intensive insulin therapy to induce sustained euglycemia. Furthermore, proinsulin decreased highly significantly as did the proinsulin to total immunoreactive insulin (PI/IRI) ratio, indicating an improvement in the quality of insulin secretion (Li et al. 2004; Weng et al. 2008).

It should be noticed that in all series of patients, except in that from Ryan et al. (2004), the mean BMI was within or slightly above the normal range, which is infrequent in western countries where the majority of the patients are obese at admission. It could be suggested, at least for the Asian patients, that they presented a different phenotype of the disease with predominant β-cell failure and much less insulin resistance.

Long-Term Intensive Insulin Therapy in Newly Diagnosed DM2

Intensive insulin therapy (plus metformin) at the time of diagnosis of DM2, treatment-naïve, with a mean BMI of 36 kg/m^2, was followed by random assignment to insulin + metformin or triple oral therapy, with metformin, glyburide, and

Table 1 Intensive insulin therapy in newly diagnosed type 2 diabetes

Author	n	Mean age (years)	Mean BMI (kg/m^2)	Baseline HbA_1c (%)	Therapy		Patients c/ englycemia c/ therapy (%)	Patients c/ englycemia	
					Type	Duration (days)		At 6 months (%)	At 12 month (%)
Ilkova et al. (1997)	13	50	26.9	11.0	CSII	14	92	69	N/A
Ryan et al. (2004)	16	52	30.8	11.8	MDI	14–21	88	N/A	44
Li et al. (2004)	138	49	25.0	10.9	CSII	14	91	67	47
Weng et al. (2008)	133	50	25.1	9.8	CSII	14–35	97	N/A	51
	118	51	24.4	9.7	MDI	14–35	95	N/A	45
	101	52	25.1	9.5	OHA	14–35	84	N/A	27
Chen et al. (2008)	22	59	27.7	11.7	MDI[a]	1 year	N/A	65[b]	55[b]
	8	56	26.6	11.3	OHA[a]	1 year	N/A	35[b]	32[b]
Wen et al. (2009)	84	48	25.3	9.9	CSII	14	N/A	74	62[b] (24 mo:53%)

Modified, with permission, from Retnakaran and Drucker (2008)
N/A not available, *CSII* continius subcutaneous insulin infusion, *MDI* multiple daily injections, *OHA* oral hypoglycemic agents
[a]After 10–14 days of intensive (MDI) therapy
[b]Patients (%) with HbA1c<6.5

pioglitazone, for 3.5 years, with preservation of both glycemic control and β-cell function, which was assessed by a mixed-meal challenge test at 6, 12, 18, 30, and 42 months, measuring C-peptide. β-cell function was preserved for at least 3.5 years with either therapy (Harrison et al. 2012).

Glitazones

Indirect Effects with Amelioration of Insulin Sensitivity

The glitazones are agonists of PPARγ, a nuclear receptor that regulates transcription genes involved in lipid and glucose metabolism. Although predominantly expressed in adipose tissue, PPARγ is present in other insulin-sensitive tissues, including the pancreatic islet cells (Dubois et al. 2001). The development of small, insulin-sensitive adipocytes, enhances glucose uptake and decreases hepatic glucose output, improving glycemic control as well as lowering plasma FFAs in DM2. Improving insulin sensitivity in the periphery may improve the glucose-sensing ability of β-cells and preserve β-cell function by reducing the demand on these cells. It has been postulated that the improvement in β-cell function, particularly the normalization of the asynchronous insulin secretion that characterizes β-cell failure, could be related to a reduction in glucolipotoxicity due to improved glycemic control and/or improved insulin sensitivity seen with glitazones. This could suggest an increased ability of the β-cell to sense and respond to glucose changes within the physiological range after glitazone treatment (Wajchenberg 2007).

Direct Effects via PPARγ Activation in Pancreatic Islands

Preclinical data in rodents have suggested that glitazones might decrease β-cell apoptosis, maintaining β-cell neogenesis and preventing islet amyloidosis. Various mechanisms of action have been proposed to explain these effects (Wajchenberg 2007).

In humans, as a class effect, glitazones consistently improve basal β-cell function, as measured by the HOMA model and observed during glitazone monotherapy and combination therapy. Further evidence of the beneficial effects on β-cells originates from other studies, in which treatment with glitazones alone or added to maximal doses of sulfonylurea and metformin or insulin, restored the first-phase insulin response to an intravenous glucose tolerance test (Ovalle and Bell 2004). In all studies, the beneficial effect of glitazones on β-cell function was independent of glucose control (as suggested by a similar reduction in HbA1c, with no improvement in β-cell function found in the insulin-treated group), indicating that glitazones can promote recovery of β-cell function independently of the amelioration of insulin sensitivity (Wajchenberg 2007).

Furthermore, extension studies with glitazones indicate that improvements in β-cell function are sustained over time in some individuals, both as monotherapy and

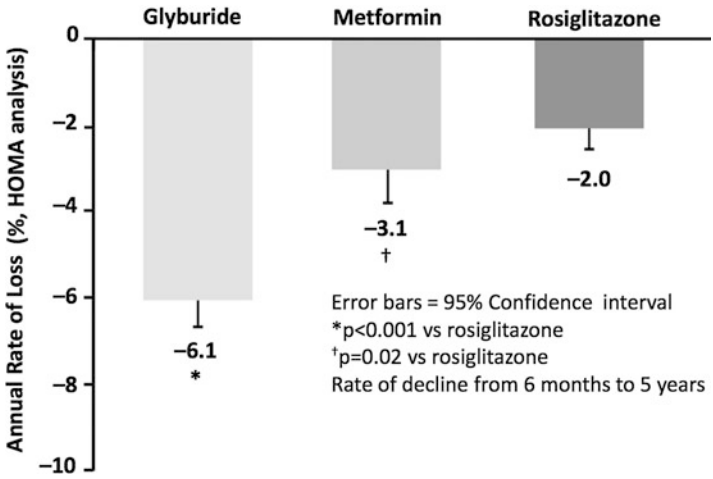

Fig. 1 ADOPT: Rosiglitazone reduces the rate of loss of β-cell function (Analysis includes only patients continuing on monotherapy (Adapted from Kahn et al. (2006))

in combination with metformin and/or sulfonylurea (Campbell 2004; Bell and Ovalle 2002). Another study evaluated the durability of the efficacy of rosiglitazone, metformin, and glyburide (glibenclamide) treatment for recently diagnosed DM2, in maintaining long-term glycemic control along with their effects on insulin sensitivity and β-cell function in 4,360 patients (Kahn et al. 2006). In this study, the cumulative incidence of monotherapy failure at 5 years was 15 % with rosiglitazone, 21 % with metformin, and 34 % with glyburide ($p < 0.001$; for both comparisons with rosiglitazone). During the first 6 months, levels of β-cell function (as evaluated by HOMA-β) increased more in the glyburide group than in either the rosiglitazone or the metformin groups. Thereafter, levels of β-cell function declined in all three groups. The annual rate of decline after 6 months was 6.1 % with glyburide, 3.1 % with metformin and 2 % with rosiglitazone ($p < 0.001$ vs. glyburide and $p = 0.02$ vs. metformin; Fig. 1) (Kahn et al. 2006). In conclusion, the study showed that the efficacy of glitazones, compared with other oral glucose-lowering medications, in maintaining long-term glycemic control in DM2.

Incretin Mimetics

Incretin hormones are released by the gastrointestinal tract in response to nutrient ingestion to enhance insulin secretion and aid in the maintenance of glucose homeostasis. The two major incretins are GLP-1 and GIP, which are released by enteroendocrine L cells located in the distal ileum and the colon, and by the K cells in the duodenum respectively (Drucker 2003). They provide the additional stimulus to insulin secretion during oral ingestion not provided by IV glucose infusion.

These incretins increase insulin secretion in a glucose-dependent manner through activation of their specific receptors in β-cells. In newly-diagnosed DM2 with relatively good glycemic control (HbA1c ~6.9 %), both GIP and GLP-1 secretion in response to glucose and mixed-meal challenges are the same or even increased compared with healthy subjects (Theodorakis et al. 2006; Vollmer et al. 2008). However, in long-standing DM2 with poor glycemic control (HbA1c ~8–9 %) the GLP-1 response is decreased whereas GIP secretion is unchanged (Chia and Egan 2008). In addition, insulin response to exogenous GLP-1 is three to fivefold lower in DM2. However, acute GLP-1 administration is able to increase insulin secretion to normal levels and to lower plasma glucose effectively (Kjems et al. 2003). In contrast, exogenous GIP, even at supraphysiological doses, has markedly reduced insulinotropic action with little or no glucose-lowering effects in DM2 (Nauck et al. 1993).

Thus, deterioration of glucose homeostasis can develop in the absence of any impairment in GLP-1 levels. This could suggest that the defects in GLP-1 concentrations previously described in patients with long-standing DM2 are likely to be secondary to other hormonal and metabolic alterations, such as fasting hyperglucagonemia and body weight, which were negatively associated with GLP-1 levels, as assessed by the incremental areas under the curves, after oral glucose and meal ingestion (Vollmer et al. 2008). Conversely, there is a positive relationship between GLP-1 and increasing age and a negative association with higher BMI levels. However, these associations were stronger after oral glucose ingestion than after mixed meal ingestion. Accordingly, another study found that obesity and glucose tolerance each attenuate the incretin effect (i.e., the gain in β-cell function after oral glucose versus intravenous glucose) on β-cell function and GLP-1 response (Muscelli et al. 2008). In both studies it was concluded that GIP and GLP-1 appeared to be regulated by different factors and are independent of each other (Vollmer et al. 2008; Muscelli et al. 2008).

Therefore, therapeutic strategies for DM2 within the incretin field focused on the use of GLP-1, GLP-1 analogs (GLP-1 receptor [GLP-1R] agonists or GLP-1 mimetics) and GLP-1 enhancers, but not GIP.

GLP-1 at pharmacological doses also has other non-insulinotropic effects beneficial for treating DM2: suppression of glucagon secretion in the presence of hyperglycemia and euglycemia, but not hypoglycemia, leading to improved hepatic insulin resistance and glycemic control; slowing of gastric emptying and gut motility, causing delayed nutrition, absorption, and dampened postprandial glucose excursion; and increasing the duration of postprandial satiety, leading to lower food intake, weight loss, and improved insulin resistance (Drucker 2003). More importantly, acute GLP-1 infusion normalized fasting plasma glucose in patients with long-standing uncontrolled DM2 who were no longer responsive to sulfonylureas or metformin (Willms et al. 1998). One major drawback of GLP-1 treatment is its short half-life (1–2 min), since it is rapidly degraded by dipeptidyl peptidase-4 (DPP-4), which cleaves the N-terminal dipeptides (His 7-Ala 8) from GLP-1 (Rahier et al. 2008; Aston-Mourney et al. 2008; Ovalle and Bell 2004).

Modifications in the GLP-1 molecule to prevent degradation by DPP-4 have resulted in the development of long-acting GLP-1 receptor agonists (GLP-1 mimetics) for the management of DM2.

1. Exendin-4-based: exenatide is a synthetic version of exendin-4, which is a 39-amino-acid peptide produced in the salivary glands of the lizard "Gila monster" with 53 % homology to full-length GLP-1. It binds more avidly to GLP-1R than GLP-1 and exendin-4 is not a substrate for DPP-4 because it has Gly8 in place of Ala8 (Deacon 2007). Available are exenatide with a short-half life (~2–4 h) has to be given at least twice daily (exenatide BID) and exenatide long-acting release (LAR) given once-weekly (OW). The other exendin-4 based GLP-1 receptor agonist, lixisenatide, given twice daily, is in phase 3 clinical trials.
2. Human GLP-1-based: liraglutide is a long-acting GLP-1 analog has 97 % homology with GLP-1 and resists DPP-4 degradation by fatty acid acylation and albumin binding, with a half-life of 12–14 h, allowing for single daily-dose administration (Table 2) (Agerso et al. 2002). The other two human GLP-1 analogs, albiglutide and dulaglutide, are in phase 3 trials and are to be given once a week.

The acute effect of exogenous GLP-1 or GLP-1 R agonists on β-cells in rodent models of diabetes and in cultured β-cells is the stimulation of glucose-dependent insulin release, whereas the subacute effect is enhancing insulin biosynthesis and stimulation of insulin gene transcription. Their chronic action is stimulation of β-cell proliferation, induction of islet neogenesis from precursor ductal cells, and inhibition of β-cell apoptosis, thus promoting an expansion of the β-cell mass. This

Table 2 Incretin mimetics: exenatide BID versus liraglutide versus exenatide OW

	Exenatide BID	Liraglutide	Exenatide OW[a]
Administration	Injection	Injection	Injection
Half life [h]	≈2–4	≈12–14	2 weeks
Frequency of inj.	twice daily	once daily	once weekly
Dose per injection	5–10μg	up to 1.8mg	2mg weekly
DPP-4 subtrate?	no	no	no
Insulin secretion[b]	↑	↑	↑
Glucagon secretion[b]	↓	↓	↓
Fasting glucose	↓↓	↓↓↓	↓↓↓
Postprandial glucose	↓↓↓	↓↓	↓↓
↓HbA 1c	≈0.6–1.4	≈1.0–1.8	≈1.3–1.9
Gastric emptying	↓	(↓)	yes
Antibody production	yes (≈45 %, ↓ c/therapy)	no	yes (≈22 %, ↓ c/therapy)

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[a]Hydrolyzable polymer microspheres

[b]Glucose-dependent

was also demonstrated in human islets freshly isolated from three cadaveric donors treated with liraglutide (Wajchenberg 2007). These effects have major implications for the treatment of DM2 because they directly address one of the fundamental defects in DM2, i.e., β-cell failure.

Exenatide BID

Clinical trials in DM2 patients who have not achieved adequate glycemic control on metformin and/or sulfonylurea, metformin and/or TZD, as well as comparative trials with insulin glargine and biphasic insulin aspart (30/70), are available in the literature (Drucker and Nauck 2006; Amori et al. 2007). With exenatide BID, 10 μg twice daily as adjuvant therapy to oral hypoglycemic agents, a significant proportion of patients (32–62 %) achieved HbA1c of 7 % or less compared with placebo (7–13 %), glargine (48 %), and biphasic insulin aspart (24 %). HbA1c reductions of 0.8–1.1 % were sustained over 3 years when added to metformin and/or sulfonylurea, resulting in significant and sustained improvements in glycemic control: HbA1c (-1.0 ± 0.1 %; $p < 0.0001$) and FPG (-23.5 ± 3.8 mg/dl; $p < 0.0001$), and improvement in HOMA-β as a surrogate of β-cell function (Klonoff et al. 2008).

There were reports of pancreatitis during the exenatide BID development program and the postmarketing period, which were passed to the FDA. Out of 30 cases 90 % reported one or more possible contributory factors, including the concomitant use of medications that list pancreatitis among the reported adverse effects in product labeling, or confounding conditions, such as obesity, gallstones, severe hypertriglyceridemia, and alcohol use (De Vries 2009).

It should be mentioned that a retrospective cohort study with a large US healthcare claims database found that the cohort with DM2 were at a 2.8-fold greater risk of acute pancreatitis compared with the cohort without diabetes (Noel et al. 2009).

Liraglutide

In the 52-week monotherapy trial, liraglutide was investigated versus the sulfonylurea glimepiride (Liraglutide Effect and Action in Diabetes [LEAD-3] trial), in which participants in the liraglutide groups lost weight, independent of the presence of nausea, up to 2.45 kg by end of study, compared with the weight gain of about 1.12 kg on glimepiride. HOMA-IR and fasting plasma glucagon showed significant decreases with liraglutide, but mean increases with glimepiride. The proinsulin to insulin ratio and HOMA-B showed no significant differences between treatments (Garber et al. 2009).

When liraglutide was used in combination trials with other oral agents and insulin, significant improvements were also demonstrated in β-cell function, as measured by HOMA-B and the proinsulin to insulin ratio. As with the

monotherapy trial, liraglutide resulted in substantial weight loss in the combination trials – up to 2.8 kg after 24 weeks (LEAD-2) – and also demonstrated clinically meaningful reductions in systolic blood pressure (up to an average of 6.7 mmHg in LEAD-4). Again, liraglutide was associated with a low rate of hypoglycemic events (Marre et al. 2009; Nauck et al. 2009; Zinman et al. 2008; Russell-Jones et al. 2008).

Meta-Analyses of the LEAD (Liraglutide Effect and Action in Diabetes) Trials

- **Factors affecting glycemic control with liraglutide**
  1. Adding liraglutide to existing therapy was more effective in lowering HbA1c than substituting an existing therapy with liraglutide.
  2. Liraglutide induced clinically relevant reductions in HbA1c across the continuum of disease progression, but achieved the greatest reductions in patients with poorer initial glycemic control (higher HbA1c).
  3. Liraglutide reduced HbA1c independent of concomitant weight loss.
- **Systolic Blood Pressure**
  1. Liraglutide significantly and sustainably reduced systolic blood pressure (SBP), with greatest improvements observed with elevated SBP at baseline.
  2. Mean SBP was significantly reduced by up to 2.6 mmHg from baseline within 2 weeks of liraglutide treatment, before any weight loss had occurred, and reductions were sustained up to 26 weeks (duration of the study).
  3. Patients with the highest quartile of baseline SBP (>140 mmHg to 190 mmHg) displayed the greatest reduction in SBP from baseline, independent of concomitant treatment with antihypertensive medication.
- **Lipids and Cardiovascular markers**

  Liraglutide significantly improved lipids and cardiovascular (CV) risk factors from baseline after 26 weeks of treatment:
  1. Total cholesterol, LDL cholesterol, FFA, and triglycerides all decreased significantly from baseline with liraglutide treatment ($p < 0.01$ for all).
  2. Liraglutide treatment also significantly reduced brain natriuretic peptide and high-sensitivity C-reactive protein, both markers of CV risk.

In relation to the use of liraglutide and the development of pancreatitis, acute pancreatitis occurred at a rate of 1.6 cases per 1,000 subject-years of liraglutide exposure and 0.6 cases per 1,000 subject-years of glimepiride exposure; chronic pancreatitis occurred at a rate of 0.6 cases per 1,000 subject-years of liraglutide exposure. Of the patients recruited for the LEAD program, 24 had previous histories of acute (n = 17) or chronic (n = 7) pancreatitis. Pancreatitis did not recur in any of these patients during liraglutide treatment (Liraglutide scientific synopsis 2010).

Data from the liraglutide phase 2 and 3 clinical trials, plus open-label controlled extension periods involving 6,638 subjects, 4,257 of whom had been exposed to liraglutide, provided consistent results across trials and did not indicate a signal of increased CV event (death, myocardial infarct or stroke) associated with liraglutide treatment, from the retrospective major adverse cardiovascular event (MACE) analyses (Liraglutide scientific synopsis 2010).

Exenatide Once-Weekly

The difference between exenatide BID and once-weekly (OW) is related to the continuous availability of the drug over 24 h compared with exposure only at mealtime with exenatide BID and deterioration of control during the night and at lunch time. Therefore, greater reduction of HbA1c with exenatide OW compared with BID is an indication that continuous glycemic control with once-weekly doses, not only at mealtime, but whenever blood glucose levels are elevated, thus resulting in the powerful efficacy of OW medication (Bergenstal et al. 2010; Drucker et al. 2008; Blevins et al. 2011; Buse et al. 2010, 2013; Diamant et al. 2010, 2012; Russell-Jones et al. 2012).

The core design of exenatide OW clinical trials corresponded to that of the so-called DURATION (Diabetes Therapy Utilization: Researching Changes in A1c, Weight and Other Factors Through Intervention With Exenatide Once Weekly) trials as follows: patients with DM2 and HbA1c from 7.1 % to 11 %, received either exenatide OW or a comparative agent(s) for 24–30 weeks, with optional OW extension. The primary endpoint was the change in HbA1c from baseline to endpoint. Secondary endpoints were the change in body weight, blood pressure, cardiovascular risk factors, safety, and tolerability from baseline to endpoint (Bergenstal et al. 2010; Drucker et al. 2008; Blevins et al. 2011; Buse et al. 2010, 2013; Diamant et al. 2010, 2012; Russell-Jones et al. 2012).

Relative to CV risk factors, patients treated with exenatide OW for 52 weeks (Buse et al. 2010) experienced improvements in the following factors: clinically significant blood pressure improvements were observed in patients treated with exenatide OW from baseline, SBP −6.2 mmHg and DBP −2.8 mmHg, and in patients switching from exenatide BID to OW, SBP −3.8 mmHg and DBP −1.8 mmHg. The majority (84 %) of the 154 patients who had been using an antihypertensive medication at screening did not change their dose after completing 52 weeks. Improvements in serum lipid profiles were demonstrated in both treatment groups, with clinically significant reductions in total cholesterol (−9.6 mg/dl vs. −9 mg/dl), LDL cholesterol (−3.4 mg/dl vs. −2.8 mg/dl), HDL cholesterol (−0.7 mg/dl vs. −1.6 mg/dl), triglycerides (−15 % vs. −13 %), exenatide OW vs. exenatide BID respectively.

Regarding baseline-to-end changes in cardiometabolic parameters and β-cell functions after 84 weeks of treatment with exenatide OW, greater reductions were observed compared with insulin glargine for waist and hip circumference ($p < 0.001$) and SBP −4.2 mmHg vs. −0.8 after insulin ($p = 0.027$), but there was no difference in DBP (−1.5 mmHg after exenatide OW vs. −1.4 mmHg after glargine; $p = 0.879$). While the heart rate increased after exenatide OW +1.97 it decreased to 0.79 beats/min after insulin ($p = 0.0034$). No differences were found in total cholesterol or triglycerides. C-reactive protein, urinary albumin to creatinine ratio decreased similarly after both treatments. HOMA-β values improved to a greater extent in the exenatide OW group than in the glargine group (Diamant et al. 2012).

Exenatide OW Safety and Tolerability

Durability of Glycemic Control with GLP-1 Receptor Agonists

There are three clinical trials with open-label extension:

1. Exenatide BID extension for over 3 years when added to metformin and/or sulphonylurea, with sustained improvement in glycemic control: A1c –1.0 % and FPG –23.5 mg/dl (Klonoff et al. 2008).
2. Exenatide OW extension for 3 years. This study followed the 30-week controlled trial (DURATION-1) in which, as already indicated, a more robust glucose lowering effect occurred than with the BID formulation of exenatide. The controlled period of the trial was followed by an open-label period in which all patients either continued with exenatide OW treatment or switched from exenatide BID to OW for 52 weeks (Diamant et al. 2012). Approximately 66 % (n = 194) of the original 295 patients completed 3 years of treatment. Baseline mean: HbA1c 8.2 %, FPG 167 mg/dl; weight: 101 kg; therapy at screening: metformin (33 %), metformin + sulphonylurea (29 %), and metformin + TZD (9 %). Significant HbA1c improvement was observed with 3 years of treatment (−1.6 %), 57 % achieving HbA1c $\leq$ 7.0 %. Significant improvements were observed in mean FPG (−33 mg/dl) and mean weight (−2.3 kg). There was also an improvement in CV risk factors: SBP, LDL cholesterol, and triglycerides (MacConell et al. 2011).
3. Liraglutide for 2 years. In this study, participants were randomized to receive once-daily liraglutide 1.2 mg, liraglutide 1.8 mg or glimepiride 8 mg. For patients completing 2 years of therapy, HbA1c reductions were −0.6 % with glimepiride vs. −0.9 % with liraglutide 1.2 mg (difference: −0.37 %, p = 0.0376) and −1.1 % with liraglutide 1.8 mg (difference: −0.55 %, p = 0.0016). Liraglutide was more effective in reducing HbA1c, FPG, and weight. Rates of minor hypoglycemia were significantly lower with liraglutide 1.2 mg and 1.8 mg compared with glimepiride ($p < 0.0001$) (Garber et al. 2011).

Regarding the important question of whether GLP-1 and GLP-1 mimetics have an effect on β-cell mass in humans, even though they have favorable effects on β cell function, such as first-phase insulin secretion and homeostasis assessment, the β-cell index (HOMA β), as seen with chronic exenatide BID use up to 3 years (Klonoff et al. 2008), this improvement in function may be due to the restoration of glucose competence to β-cells and the insulinotropic glucose-lowering and weight-loss effects of exenatide, and perhaps not because of any direct effect of exenatide on β-cell mass, as previously indicated. At present there is no strong evidence that incretin mimetics and DPP-4 inhibitors can expand or at least maintain β-cell mass in humans, and as such be able to delay the progression of the disease (Salehi et al. 2008).

Another question yet to be elucidated is the mechanism by which GLP-1 and GLP-1 mimetics lower glucagon secretion from α-cells. The ability of GLP-1 and incretin mimetics to lower glucagon levels in DM2 patients, in whom they are high throughout the day, contributes to the overall glucose-lowering effect. By enhancing endogenous insulin secretion with suppression of glucagon secretion, a more

physiological insulin to glucagon ratio in the portal vein should be established, resulting in better suppression of hepatic glucose output. The mechanism(s) by which GLP-1 and GLP-1 mimetics lower glucagon secretion remain(s) unclear and discussion of this issue is beyond the scope of this publication. According to Dunning and Gerich (Dunning and Gerich 2007) in a review of published studies, the defect(s) in α-cell function that occur(s) in type 2 diabetes reflect(s) impaired glucose sensing. Because local insulin is a key regulator of glucagon secretion and defective β-cell glucose sensing in DM2 is indisputable, many, if not all, of the characteristic defects in the α-cell function may be secondary to β-cell dysfunction. It is interesting to note that attenuated and delayed glucagon suppression in DM2 occurs after oral ingestion of glucose, while isoglycemic intravenous administration of glucose results in normal suppression of glucagon, possibly because of the glucagonotropic action of GIP and GLP-1 after oral glucose. This phenomenon contributes both to the glucose intolerance and to the reduced incretin effect observed in DM2 patients (Knop et al. 2007; Meier et al. 2007).

Incretin Enhancers (DPP-4 Inhibitors)

Preclinical studies have demonstrated that DPP-4 inhibitors, which prevent the degradation of native GLP-1 by inhibiting the activity of the DPP-4 enzyme and thus increasing endogenous GLP-1 (and GIP) levels, may promote β-cell proliferation and neogenesis and inhibit apoptosis (Wajchenberg 2007). Thus, they have emerged as a therapeutic strategy for enhancing GLP-1 action in vivo. However, there are suggestions that mediators other than GLP-1 might contribute to the therapeutic effect of DPP-4 inhibition (Nauck and El-Ouaghlidi 2005). Alternatively, there are indications that GLP-1 may work indirectly through activation of the autonomic nervous system (Ahrén 2004).

DPP-4 inhibitors were developed to augment biologically active, endogenously secreted plasma GLP-1. In humans, sitagliptin, a DPP-4 inhibitor, both after a single dose and a once-daily dose for 10 days, resulted in an approximately twofold increase in active GLP-1 (aGLP-1) after meals. Besides, sitagliptin decreased total GLP-1 (tGLP-1) in the presence of increased aGLP-1 (Chia and Egan 2008). Whether the twofold increase in aGLP-1 is sufficient to explain the glucose-lowering effect with a reduction of HbA1c remains a matter of controversy.

If DPP-4 inhibitors lower blood glucose as a direct consequence of increased aGLP-1 levels, plasma insulin should also increase. However, fasting and postprandial plasma insulin and C-peptide levels did not differ before and after 10 days of DPP-4 inhibition in both healthy and DM2 subjects (Bergman et al. 2006; Ahrén et al. 2004). Indeed, infusions of GLP-1 that result in comparable plasma aGLP-1 attained by DPP-4 inhibition do not induce insulin secretion, but the same amount of insulin is secreted at a lower glucose level, or the insulinogenic index is improved.

DPP-4 inhibition results in lower postprandial plasma glucagon levels (Drucker and Nauck 2006). However, the reduced glucagon secretion is not evident in the fasting state when it would be most beneficial to decreasing nocturnal hepatic

glucose output. The postprandial glucagon suppressive effects of DPP-4 inhibitors, although significantly different from placebo, are weak and short-lived, while levels are much higher than in nondiabetic subjects, and therefore are unlikely to account for the full antihyperglycemic effect (Chia and Egan 2008).

Currently, four DPP-4 inhibitors have been approved for treatment of DM2: sitagliptin, saxagliptin, linagliptin, and vildagliptin. The latter is not available in the USA. Another DPP-4 inhibitor is undergoing clinical development, namely alogliptin.

Pharmacological Differences Among the DPP-4 Inhibitors

While all DPP-4 inhibitors share a common mechanism of action, there are clinically important differences within the group considering their structural heterogeneity, which may account for variations in the pharmacokinetic profile among the DPP-4 inhibitors (Gerich 2010).

The different DPP-4 inhibitors are distinctive in their metabolism (saxagliptin and vildagliptin are metabolized in the liver, whereas sitagliptin and linagliptin are not), their excretion (linagliptin is excreted mostly unchanged by the liver, in contrast to other DPP-4 inhibitors, which are mainly excreted via the kidneys) and the potential of cytochrome-mediated drug–drug interactions (observed only with saxagliptin). These differences may be clinically relevant in patients with renal or hepatic impairment (Scheen 2012).

Liver Impairment

Regarding the safety of the DPP-4 inhibitors the risk of acute pancreatitis with DPP-4 therapy remains controversial. A recently published study that analyzed a large administrative database in the USA from 2005 to 2008, of DM2 patients aged 18–64 years, identified 1,269 hospitalized cases with acute pancreatitis and 1,269 control subjects matched for age, category, sex, enrollment pattern, and diabetes complications. Cases were significantly more likely than controls to have hypertriglyceridemia, alcohol use, gallstones, tobacco abuse, obesity, and biliary and pancreatic cancer (2.8 % vs. 0 %) and any neoplasm (20.94 % vs. 18.05 %). The conclusion of this administrative database study of US adults with DM2, treatment with GLP-1-based therapies, sitagliptin and exenatide BID, was associated with increased odds of hospitalization for acute pancreatitis (Singh et al. 2013). However, the limitations of observational claims-based analyses cannot exclude the possibility of an increased risk of acute pancreatitis (Garg et al. 2010).

As indicated by Scheen (2012) "further investigation is needed and long-term careful postmarketing surveillance is mandatory. Indeed, various experimental data in animal model suggested that there are grounds for concern that GLP-1 class of drugs may induce asymptomatic pancreatitis and perhaps over time, in some individuals, induce pancreatic cancer (Butler et al. 2010)."

Butler et al. examining the pancreata from 20 age-matched organ donors with DM2 treated with incretin therapy (n = 8) for 1 year or more, 7 having received sitagliptin and 1 exenatide BID. The remaining 12 did not receive GLP-1 drugs. Pancreata were also obtained from 14 nondiabetic controls matched for age, sex, and BMI. The study revealed a ~40 % increased pancreatic mass in DM2 patients treated with incretin therapy, with both increased exocrine cell proliferation ($p < 0.0001$) and dysplasia (increased pancreatic intraepithelial neoplasia, $p < 0.01$). Pancreas in DM2 patients treated with incretin therapy presented α-cell hyperplasia and glucagon microadenomas (3/8) and a neuroendocrine tumor. β-cell mass was reduced by approximately 60 % in those with DM2, yet a sixfold increase was observed in incretin-treated subjects, although diabetes persisted. Endocrine cells co-staining for insulin and glucagon were increased in diabetics compared with nondiabetic controls ($p < 0.05$) and markedly further increased with incretin therapy ($p < 0.05$). The authors concluded that, in humans, incretin therapy resulted in a marked expansion of the exocrine and endocrine pancreatic compartments, the former being accompanied by increased proliferation and dysplasia, the latter by α-cell hyperplasia with the potential for evolution into neuroendocrine tumors (Butler et al. 2013). The US Food and Drug Administration (FDA) is evaluating the reported findings by Butler et al. (2013). "These findings were based on examination of a small number of pancreatic tissue specimens taken from patients after they died from unspecified causes. FDA has asked the researchers to provide the methodology used to collect and study these specimens and to provide the tissue samples so the Agency can further investigate potential pancreatic toxicity associated with the incretin mimetics. FDA will communicate its final conclusions and recommendations when its review is complete or when the Agency has additional information to report" (Drug Safety and Availability. FDA Drug Safety Communication 2013).

At this time, the FDA and The Endocrine Society advise that patients should continue to take their medicine as directed until they talk to their health care professional, and health care professionals should continue to follow the prescribing recommendations on the drug labels (FDA Drug Safety Communication 2013).

The European Medicines Agency´s Committee for Medicinal Products for Human Use (CHMP) has finalized a review of GLP-1-based diabetes therapies. The Committee concluded that presently available data do not confirm recent concerns over an increased risk of pancreatic adverse events with these medicines (European Medicines Agency (EMA) 2013).

DPP-4 Inhibitors and CV Protection

A potential benefit of incretin-based therapies is their effect on CVD. In a meta-analysis of 41 randomized controlled trials (RCTs), 32 published (9 unpublished), performed in type 2 diabetic patients with DPP-4 inhibitors, with a duration >12 weeks, at the time of publication, the risk of CV events and all-cause death was 0.76 (0.46–1.28) and 0.78 (0.40–1.51) respectively (Monami et al. 2010).

There is some plausibility based on the influences of GLP-1-based drugs on CV risk factors: cardioprotection (rodents); mimicking of cardiac pre- and post-conditioning (Rahmi et al. 2013); improved myocardial survival in ischemic heart disease; improved myocardial performance in non-ischemic heart failure; reduced systolic blood pressure by 2–5 mmHg, mechanistically explained improved endothelial function and vasodilation, enhanced natriuresis and fluid excretion; lipid profiles are modestly improved; there is weight loss (incretin mimetics) or weight neutrality (DPP-4 inhibitors) (Ussher and Drucker 2012).

Long-Term Clinical Efficiency

Studies in humans with DM2 showed improvement of islet-cell function, in fasting and post-prandial states, and these beneficial effects were sustained in studies of up to 2 years' duration. However, there is at present no evidence in humans to suggest that DPP-4 inhibition has durable effects on β-cell function after cessation of therapy, as previously indicated with GLP-1 analogs (Scheen 2012; Gomis et al. 2012). The duration of these trials was too short to draw any definite conclusions. There are no long-term controlled trials to evaluate the effects of DPP-4 inhibitors on β-cell function in humans and the durability of the glucose-lowering effect of gliptins, as opposed to the escape phenomenon observed with sulfonylureas. Perhaps the analysis of large ongoing clinical trials with CV outcomes will provide additional information regarding the durability of glucose control with gliptins.

Mechanism of Action of DPP-4 Inhibitors

The contributions of increased insulin secretion and inhibition of glucagon secretion in the glucose-lowering effects in both fasting and post-prandial states still need to be better explored (Scheen 2012). Unresolved issues, such as the effects of GLP-1 mimetics and DPP-4 inhibitors on β-cell mass in humans, the mechanism by which GLP-1 mimetics lower glucagon levels from α-cells, the modest increase in active GLP-1 levels as a possible sole modulator of glycemia using DPP-4 inhibitors, and exactly how DPP-4 inhibition leads to a decline in plasma glucose levels without an increase in insulin secretion, need to be further evaluated (Chia and Egan 2008).

The measurement of islet function and glucose utilization was performed with the DPP-4 inhibitor, vildagliptin, given to DM2 patients for 6 weeks versus the same period of time with placebo. Vildagliptin increased postprandial GLP-1 and GIP, after oral glucose, by three- and twofold respectively, reduced FPG and PPG significantly (both $p < 0.01$), and improved the glucose responsiveness of insulin secretion by 50 % ($p < 0.01$). Vildagliptin lowered postprandial glucagon by 16 % ($p < 0.01$). Examined using a hyperinsulinemic euglycemic clamp, insulin sensitivity and glucose clearance improved after the administration of the DPP-4

inhibitor ($p < 0.01$). This was due to an increase in the glucose oxidation rate at the expense of fat oxidation and was also associated with a decrease in fasting lipolysis. Decreasing fasting lipolysis over 6 weeks is predicted to decrease stored triglycerides in non-fat tissues and may explain the increased glucose oxidation during use of the clamp at the expense of lipid oxidation. Thus, it was demonstrated that vildagliptin improves islet function in DM2 and glucose metabolism in peripheral tissues (Azuma et al. 2008).

To assess the effect of a DPP4-inhibitor, sitagliptin, on β-cell function in patients with DM2, a C-peptide minimal model was applied to extensive blood sampling of a nine-point meal tolerance test performed at baseline and at the end of treatment for 18–24 weeks of sitagliptin 100 mg q.d. as an add-on to metformin therapy or as monotherapy. In this model-based analysis, sitagliptin improved β-cell function relative to placebo in both fasting and postprandial states in the patients with DM2. The disposition indices (DIs), which assess insulin secretion in the context of changes in insulin sensitivity, for all measures performed, were significantly ($p < 0.05$) increased with sitagliptin treatment compared with placebo. The AA concluded that in their model-based analysis, sitagliptin improved β-cell function relative to placebo in both fasting and postprandial states in patients with DM2 (Xu et al. 2008).

The effects of saxagliptin on β-cell function of DM2 were assessed at baseline and at week 12 using an intravenous hyperglycemic clamp (fasting state) and an intravenous–oral hyperglycemic clamp (postprandial state) following oral ingestion of 75 g glucose. DPP-4 inhibition improved β-cell function in both postprandial (increased insulin secretion by 18.5 % adjusted difference vs. placebo, $p = 0.04$, associated with increased plasma concentrations of GLP-1 and GIP) and fasting states (increased insulin secretion by 27.9 % adjusted difference vs. placebo, $p = 0.02$). Saxagliptin also improved the glucagon area under the curve in the postprandial state (adjusted difference –21.8 % vs. placebo, $p = 0.03$). The AA indicated that given the magnitude of insulin response in the fasting state, further study into the effect of DPP-4 inhibition on the β-cell is warranted (Henry et al. 2011).

To quantify the incretin effect by comparing insulin secretory responses with oral as well as isoglycemic intravenous glucose infusions in patients with DM2 with and without the administration of a DPP-4 inhibitor, Vardarli et al. (2011) assessed the incretin effect after treatment with the DPP-4 inhibitor vildagliptin or placebo in patients previously treated with metformin. Vildagliptin augmented insulin secretory responses both after oral glucose (accompanied by the release of incretin hormones: GLP-1 and GIP) and during the intravenous infusion of glucose (without a major incretin response). Thus, against expectations, according to the AA, the incretin effect is not enhanced by DPP-4 inhibitor treatment, mainly because of a "surprising" augmentation of insulin secretory responses, even with intravenous glucose infusions. Thus, slight variations in basal incretin levels may be more important than previously thought. Another possibility is that the DPP-4 inhibitor-induced change in the incretin-related environment of islets might persist overnight, augmenting insulin secretory

responses to intravenous glucose as well. Alternatively, as yet unidentified mediators of DPP-4 inhibition may have caused these effects (Vardarli et al. 2011).

Regarding the effect of alogliptin on pancreatic β-cell function, there are several studies of short duration (26 weeks) showing modest or nonsignificant increases in the proinsulin/insulin ratio and a trend toward increased homeostasis model assessment of β-cell function (HOMA-β) (DeFronzo et al. 2008; Pratley et al. 2009), but long-term studies will be needed to prove that alogliptin, like other DPP-4 inhibitors, preserves β-cell function.

Table 3 shows the differences between GLP-1 agonists and DPP-4 inhibitors (Wajchenberg 2007).

In conclusion, the DPP-4 inhibitors make an important contribution to the treatment of DM2, providing effective glucose control with a low risk for hypoglycemia, a neutral effect on body weight, and a general lack of gastrointestinal and other side effects differentiate DPP-4 inhibitors from some other classes of oral antidiabetic drugs. Experimental and particularly clinical studies suggest that DPP-4 inhibitors could preserve the progressive destruction of β-cells and the loss of insulin secretory capacity characteristic of DM2 (Gerich 2010).

As indicated by Nauck (personal communication), generally speaking, the optimism that incretin-based medications clinically improve β-cell function has fallen away, especially since it has been shown that in old rodents, GLP-1 and its derivatives do not increase β-cell replication and mass. Also, much of the "improved β-cell function" has been measured during treatment with GLP-1 receptor agonists, which acutely stimulate insulin secretion (which is not "improved β-cell function"); thus, some of the information is misleading.

Table 3 GLP-1 agonists versus DPP 4 inhibitors

	GLP-1 R	DPP-4 Inhibitors
Administration	Injection	Orally available
GLP-1 concentrations	Pharmacological	Physiological
Mechanism of actions	GLP-1	GLP-1 + GIP
Activation of portal glucose sensor	No	Yes
↑ Insulin secretion	+++	+
↓ Glucagon secretion	++	++
Gastric emptying	Inhibited	+/−
Weight loss	Yes	No
Expansion of β-cell mass in preclinical studies	Yes	Yes
Nausea and vomiting	Yes	No
Potential immunogenicity	Yes	No

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Cross-References

- ▶ Mechanisms of Pancreatic β-Cell Apoptosis in Diabetes and Its Therapies
- ▶ Physiological and Pathophysiological Control of Glucagon Secretion by Pancreatic α Cells
- ▶ The β-Cell in Human Type 2 Diabetes

References

Agerso H, Jensen LB, Elbrond B, Rolan P, Zdravkovic M (2002) The pharmacokinetic, pharmacodynamics, safety and tolerability of NN2211, a long-acting GLP-1 derivative, in healthy men. Diabetologia 45:195–202

Ahrén B (2004) Sensory nerves contribute to insulin secretion by glucagon-like peptide-1(GLP-1) in mice. Am J Physiol Regul Integr Comp Physiol 286:R269–R272

Ahrén B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A (2004) Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustain insulin levels, and reduces glucagon levels in type 2 diabetes. J Clin Endocrinol Metab 89:2078–2084

Amori RE, Lau J, Pittas AG (2007) Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis. JAMA 298:194–206

Aston-Mourney K, Proietto J, Morahan G, Andrikopoulos S (2008) Too much of a good thing: why it is bad to stimulate the β cell to secrete insulin. Diabetologia 51:540–545

Azuma K, Rádiková Z, Mancino J, Toledo FGS, Thomas E, Kangani C, Dalla Man C, Cobelli C, Holst JJ, Deacon CF, He Y, Ligueros-Saylan M, Serra D, Foley JF, Kelley DE (2008) Measurements of islet function and glucose metabolism with the dipeptidyl peptidase 4 inhibitor vildagliptin in patients with type 2 diabetes. J Clin Endocrinol Metab 93:459–464

Baggio LL, Drucker DJ (2006) Incretin hormones in the treatment of type 2 diabetes: therapeutic applications of DPP-IV inhibitors. Medscape Diabetes Endocrinol 8:1–5

Bell DSH, Ovalle F (2002) Long-term efficacy of triple oral therapy for type 2 diabetes mellitus. Endocr Pract 8:271–275

Bergenstal RM, Wyshm C, MacConell L, Malloy J, Walsh B, Yan P, Wilhelm K, Malone J, Porter LE, DURATION-2 Study Group (2010) Efficacy and Safety of exenatide once weekly versus sitagliptin or pioglitazone as an adjunct to metformin for treatment of type 2 diabetes (DURATION-2): a randomised trial. Lancet 376:431–439

Bergman AJ, Stevens C, Zhou Y, Yi B, Laethem M, De Smet M, Snyder K, Hilliard D, Tanaka W, Zeng W, Tanen M, Wang AQ, Chen L, Winchell G, Davies MJ, Ramael S, Wagner JA, Herman GA (2006) Pharmacokinetic and pharmacodynamic properties of multiple oral doses of sitagliptin, a dipeptidyl peptidase-IV inhibitor: a double-blind, randomized, placebo-controlled study in healthy male volunteers. Clin Ther 28:55–72

Blevins T, Pullman J, Malloy J, Yen P, Taylor K, Schulteis C, Trautmann M, Porter L (2011) DURATION-5: exenatide once weekly resulted in greater improvements in glycemic control compared with exenatide twice daily in patients with type 2 diabetes. J Clin Endocrinol Metab 96:1301–1310

Buse JB, Drucker DJ, Taylor KL, Kim T, Walsh B, Hu H, Wilhelm K, Trautmann M, Shen LZ, Porter LE, for the DURATION-1 Study Group (2010) DURATION-1: exenatide once weekly produces sustained glycemic control and weight loss over 52 weeks. Diabetes Care 33:1255–1261

Buse JB, Nauck M, Forst T, Sheu WH, Shenouda SK, Heilmann CR, Hoogwerf BJ, Gao A, Boardman MK, Fineman M, Porter L, Schernthaner G (2013) Exenatide once weekly versus liraglutide once daily in patients with type 2 diabetes (DURATION-6): a randomized, open-label study. Lancet 381:117–124

Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA (2003) Butler PC: β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 52:102–110

Butler PC, Matveyenko AV, Dry S, Bhushan A, Elashoff R (2010) Glucagon-like peptide-1 therapy and the exocrine pancreas: innocent bystander or friendly fire? Diabetologia 53:1–6

Butler AE, Campbell-Thompson M, Gurlo T, Dawson DW, Atkinson M, Butler PC (2013) Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. Diabetes 62:2595–2604

Campbell JW (2004) Long-term glycemic control with pioglitazone in patients with type 2 diabetes. Int J Clin Pract 58:192–200

Chen H-S, Wu TE, Jap TS, Hsiao LC, Lee SH, Lin HD (2008) Beneficial effects of insulin on glycemic control and β-cell function in newly diagnosed type 2 diabetes with severe hyperglycemia after short-term intensive insulin therapy. Diabetes Care 31:1927–1932

Chia CW, Egan JM (2008) Incretin-based therapies in type 2 diabetes mellitus. J Clin Endocrinol Metab 93:3703–3716

Dandona P, Chaudhuri A, Mohanty P, Ghanim H (2007) Anti-inflammatory effects of insulin. Curr Opin Clin Nutr Metab Care 10:511–517

De Vries JH (2009) Is pancreatitis an adverse drug effect of GLP-1 receptor agonists? In: Advances in Glucagon-like peptides for the treatment of type 2 diabetes, vol 3. pp 9–13. http://cme.medscape.com/viewprogram/19082_pnt

Deacon CF (2004) Circulation and degradation of GIP and GLP-1. Horm Metab Res 36:761–765

Deacon CF (2007) Incretin-based treatment of type 2 diabetes: glucagon-like-peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. Diabetes Obes Metab 9(Suppl 1):23–31

DeFronzo RA, Fleck PR, Wilson CA, Mekki Q, Alogliptin Study 010 Group (2008) Efficacy and safety on the dipeptidyl peptidase-4 inhibitor alogliptin in patients with type 2 diabetes and inadequate glycemic control: a randomized, double-blind, placebo-controlled study. Diabetes Care 31:2315–2317

Diamant M, Van Gaal L, Stranks S, Northrup J, Cao D, Taylor K, Trautmann M (2010) Once weekly exenatide compared with insulin glargine titrated to target in patients with type 2 diabetes (DURATION-3): an open-label randomized trial. Lancet 375:2234–2243

Diamant M, Van Gaal L, Stranks S, Guerci B, MacConell L, Heber H, Scism-Bacon J, Trautmann M (2012) Safety and efficacy of once-weekly exenatide compared with insulin glargine titrated to target in patients with type 2 diabetes over 84 weeks. Diabetes Care 35:683–689

Drucker DJ (2003) Enhancing incretin action for the treatment of type 2 diabetes. Diabetes Care 26:2928–2940

Drucker DJ, Nauck MA (2006) The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl-peptidase-4 inhibitors in type 2 diabetes. Lancet 368:1696–1705

Drucker DJ, Buse JB, Taylor K, Kendall DM, Trautmann M, Zhuang D, Porter L, DURATION-1 Study Group (2008) Exenatide once weekly versus twice daily for treatment of Type 2 diabetes: a randomised, open-label, non-inferiority study. Lancet 372:1240–1250

Drug Safety and Availability. FDA Drug Safety Communication (2013) FDA investigating reports of possible increased risk of pancreatitis and pre-cancerous findings of the pancreas from incretin mimetic drugs for type 2 diabetes, 14 March 2013

Dubois M, Pattou F, Kerr-Conte J, Gmyr V, Vanderwalle B, Desreumaux P, Auswers J, Schoonjans K, Lefebvre J (2001) Expression of peroxisome-proliferator-activated receptor γ (PPARγ) in normal human pancreatic islet cells. Diabetologia 43:1165–1169

Dunning BE, Gerich JE (2007) The role of α-cell dysregulation in fasting and postprandial hyperglycemia in type 2 diabetes and therapeutic implications. Endocr Rev 28:253–283

European Medicines Agency (EMA) (2013) Investigation into GLP-1 based diabetes therapies concluded. 26 July 2013

FDA Drug Safety Communication (2013) FDA investigating reports of possible increased risk of pancreatitis and pre-cancerous findings of the pancreas from incretin mimetic drugs for type 2 diabetes. Endocrine Daily Briefing, The Endocrine Society, 15 March 2013

Garber A, Henry R, Ratner R, Rodriguez-Pattzi H, Olvera-Alvarez I, Hale PM, Zdravkovic M, Bode B, Garcia-Hernandez PA, for the LEAD-3 (Mono) Study Group (2009) Liraglutide versus glimepiride monotherapy for type 2 diabetes (LEAD-3 Mono): a randomized, 52-week, phase III, double-blind, parallel-treatment trial. Lancet 373:473–481

Garber A, Henry RR, Ratner R, Hale P, Chang CT, Bode B, LEAD-3 (Mono) Study Group (2011) Liraglutide, a once-daily human glucagon-like peptide 1 analogue, provides sustained improvements in glycaemic control and weight for 2 years as monotherapy compared with glimepiride in patients with type 2 diabetes. Diabetes Obes Metab 13:348–356

Garg R, Chen W, Pendergrass M (2010) Acute pancreatitis in type 2 diabetes treated with exenatide or sitagliptin. A retrospective observational pharmacy claims analysis. Diabetes Care 33:2349–2354

Garvey WT, Olefsky JM, Griffin J, Hamman RF, Kolterman OG (1985) The effect of insulin treatment on insulin secretion and insulin action in type II diabetes mellitus. Diabetes 34:222–234

Gerich J (2010) DPP-4 inhibitors: what may be the clinical differentiators? Diabetes Res Clin Practice 90:131–140. Review article

Glaser B, Leibovich G, Nesher R, Hartling S, Binder C, Cerasi E (1988) Improved β-cell function after intensive insulin treatment in severe non-insulin-dependent diabetes. Acta Endocrinol (Copenh) 118:365–373

Gomis R, Owens OR, Taskinen MR, Del Prato S, Patel S, Pivovarova A, Schlosser A, Woerle HJ (2012) Long-term safety and efficacy of linagliptin as monotherapy or in combination with other glucose-lowering agents in 2121 subjects with type 2 diabetes: up to 2 years exposure in 24-week phase III trials followed by a 78-week open-label extension. Int J Clin Pract 66:731–740

Gormley MJ, Hadden DR, Woods R, Sheridan B, Andrews WJ (1986) One month's insulin treatment of type II diabetes: the early and medium-term effects following insulin withdrawal. Metabolism 35:1029–1036

Gumbiner B, Polonsky KS, Beltz WF, Griver K, Wallace P, Brechtel G, Henry RR (1990) Effects of weight loss and reduced hyperglycemia on the kinetics of insulin secretion in obese non-insulin dependent diabetes mellitus. J Clin Endocrinol Metab 70:1594–1602

Harrison LB, Adams-Huet B, Raskin P, Ligvay I (2012) β-cell function preservation after 3.5 years after 3.5 years of intensive diabetes therapy. Diabetes Care 35:1406–1412

Henquin J-C, Cerasi E, Efendic S, Steiner DF, Boitard C (2008) Pancreatic β-cell mass or β-cell function? Editorial. Diabetes Obes Metab 10(Suppl 4):1–4

Henry RR, Smith SR, Schwartz SL, Mudaliar SR, Deacon CF, Holst JJ, Duan RY, Chen RS, List JF (2011) Effects of saxagliptin on β-cell stimulation and insulin secretion in patients with type 2 diabetes. Diabetes Obes Metab 13:850–858

Holman RR (1998) Assessing the potential for α-glucosidase inhibitors in prediabetic states. Diabetes Res Clin Pract 40(Suppl):S21–S25

Holman RR (2006) Long-term efficacy of sulfonylureas: a United Kingdom prospective diabetes study perspective. Metabolism 55(Suppl 1):S2–S5

Ilkova H, Glaser B, Tunckale A, Bagriacik N, Cerasi E (1997) Induction of long-term glycemic control in newly diagnosed type 2 diabetic patients by transient intensive insulin treatment. Diabetes Care 20:1353–1356

Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill C, Zinman B, Viberti G, for the ADOPT (A Diabetes Outcome Progression Trial) Study Group (2006) Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. N Engl J Med 355:2427–2443

Kjems LL, Holst JJ, Volund A, Madsbad S (2003) The influence of GLP-1 on glucose-stimulated insulin secretion: effects on β-cell sensitivity in type 2 and nondiabetic subjects. Diabetes 52:380–386

Klonoff DC, Buse JB, Nielsen LI, Guan X, Bowlus CL, Holcombe JH, Wintle ME, Maggs DG (2008) Exenatide effects on diabetes, obesity, cardiovascular risk factors and hepatic

biomarkers in patients with type 2 diabetes treated for at least 3 years. Curr Med Res Opin 24:275–286

Knop FK, Visboll T, Madsbad S, Holst JJ, Krarup T (2007) Inappropriate suppression of glucagon during OGTT but not during isoglycemic i.v. glucose infusion contributes to the reduce incretin effect in type 2 diabetes mellitus. Diabetologia 50:797–805

Laybutt DR, Preston AM, Akerfeldt MC, Kench JG, Bush AK, Biankin AV, Biden TJ (2007) Endoplasmic reticulum stress contributes to β cell apoptosis in type 2 diabetes. Diabetologia 50:752–763

Li Y, Hansotia Y, Yusta B, Ris F, Halban PA, Drucker DJ (2003) Glucagon-like-peptide-1 receptor signaling modulates β-cell apoptosis. J Biol Chem 278:471–478

Li Y, Xu W, Liao Z, Yao B, Chen X, Huang Z, Hu G, Weng JP (2004) Induction of long-term glycemic control in newly diagnosed type diabetic patients is associated with improvement of β-cell function. Diabetes Care 27:2597–2602

Liraglutide scientific synopsis. Novo Nordisk A/S, june 2010

MacConell L, Walsh B, Li Y, Pencek R, Maggs D (2013) Exenatide once weekly: sustained improvement in glycemic control and weight loss through 3 years. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy 6: 31–41

Maedler K, Carr RD, Bosco D, Zuellig RA, Berney T, Donath MY (2005) Sulfonylurea induced β-cell apoptosis in cultured human islets. J Clin Endocrinol Metab 90:501–506

Marre M, Shaw J, Brandle M, Bebakar WM, Kamaruddin NA, Strand J, Zdravkovic M, Le-Thi TD, Colagiuri S, LEAD-1 SU Study Group (2009) Liraglutide, a once-daily human GLP-1 analogue, added to a sulphonylurea over 26 weeks produces greater improvements on glycaemic and weight control compared with adding rosiglitazone or placebo in subjects with Type 2 diabetes (LEAD-1 SU). Diabet Med 26:268–278

Meier JJ, Deacon CF, Schmidt WE, Holst JJ, Nauck MA (2007) Suppression of glucagon secretion is lower after oral glucose administration than during intravenous glucose administration in human subjects. Diabetologia 50:806–813

Monami M, Iacomelli I, Marchionni N, Mannucci E (2010) Dipeptydil peptidase-4 inhibitors in type 2 diabetes: a meta-analysis of randomized clinical trials. Nutr Metab Cardiovasc Dis 20:224–235

Muscelli E, Mari A, Casolaro A, Camastra S, Seghieri G, Gastaldelli A, Holst JJ, Ferrannini E (2008) Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. Diabetes 57:1340–1348

Nauck MA, El-Ouaghlidi A (2005) The therapeutic actions of DPP-IV inhibition are not mediated by glucagon-like peptide-1. Diabetologia 48:608–611

Nauck MA, Heimesaat MM, Orskow C, Holst JJ, Ebert R, Creutzfeldt W (1993) Preserved incretin activity of glucagon-like peptide 1[17-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type 2 diabetes mellitus. J Clin Invest 91:301–307

Nauck M, Frid A, Hermansen K, Shah NS, Tankova T, Mitha IH, Zdravkovic M, During M, Matthews DR, for the LEAD-2 Study Group (2009) Efficacy and safety comparison of Liraglutide, glimepiride, and placebo, all in combination with metformin, in type 2 diabetes. Diabetes Care 32:84–90

Noel RA, Braun DK, Patterson RE, Bloomgren G (2009) Increased risk of acute pancreatitis and biliary disease observed in patients with type 2 diabetes: a retrospective, cohort study. Diabetes Care 32:834–838

Ovalle F, Bell DSH (2004) Effect of rosiglitazone versus insulin on the pancreatic β-cell function of subjects with type 2 diabetes. Diabetes Care 27:2585–2589

Perl S, Kushner JA, Buchholz BA, Meeker AK, Stein GM, Hsieh M, Kirby M, Pechhold S, Liu EH, Harlan DM, Tisdale JF (2010) Significant human β-cell turnover is limited to the first three decades of life as determined by in vivo thymidine analog incorporation and radiocarbon dating. J Clin Endocrinol Metab 95:E234–E239

Poitout V, Robertson RP (2008) Glucolipotoxicity: fuel excess and β-Cell dysfunction. Endocr Rev 29:351–366

Pratley RE, Kipnes MS, Fleck PR, Wilson C, Mekki Q, Alogliptin Study 007 Group (2009) Efficacy and safety of the dipeptidyl peptidase-4 inhibitor alogliptin in patients with type2 diabetes inadequately controlled by glyburide monotherapy. Diabetes Obes Metab 11:167–176

Rahier J, Guiot Y, Goebbels RM, Sempoux C, Henquin JC (2008) Pancreatic β-cell mass in European subjects with type 2 diabetes. Diabetes Obes Metab 10(Suppl 4):32–42

Rahmi RM, Uchida AH, Rezende PC, Lima EG, Garzillo CL, Favarato D, Strunz CMC, Takiuti M, Girardi P, Hueb W, Kalil Filho R, Ramires JAF (2013) Effect of hypoglycemic agents on ischemic preconditioning in patients with type 2 diabetes and symptomatic coronary artery disease. Diabetes Care 36:1654–1659

Retnakaran R, Drucker DJ (2008) Intensive insulin therapy in newly diagnosed type 2 diabetes. Lancet 371:1725–1726

Ritzel RA (2009) Therapeutic approaches based on β-cell mass preservation and/or regeneration. Front Biosci 14:1835–1850

Russell-Jones D, Vaag A, Schmitz O, Sethi BK, Lalic N, Antic S, Zdravkovic M, Ravn GM, Simo R (2008) Significantly better glycemic control and weight reduction with liraglutide, a once-daily human GLP-1 analog, compared with insulin glargine: all as add-on to metformin and a sulfonylurea in type 2 diabetes. Diabetes 57(Suppl 1):A 159

Russell-Jones D, Cuddihy RM, Hanefeld M, Kumar A, Gonzalez JG, Chan M, Wolka AM, Boardman MK, on behalf of the DURATION-4 Study Group (2012) Efficacy and safety of exenatide once weekly versus metformin, pioglitozone and sitagliptin used as monotherapy in drug-naive patients with Type 2 diabetes (DURATION-4): a 26-weekly double-blind study. Diabetes Care 35:252–258

Ryan EA, Imes S, Wallace C (2004) Short-term intensive insulin therapy in newly diagnosed type 2 diabetes. Diabetes Care 27:1028–1032

Salehi M, Aulinger BA, D'Alessio DA (2008) Targeting β-cell mass in type 2 diabetes: promise and limitations of new drugs based on incretins. Endocr Rev 29:357–379

Scheen AJ (2012) A review of gliptins in 2011. Expert Opin Pharmacother 13:81–99

Shapiro ET, Van Cauter E, Tillil H, Given BD, Hirsch L, Beebe C, Rubenstein AH, Polonsky KS (1989) Glyburide enhances the responsiveness of the β-cell to glucose but does not correct the abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 69:571–576

Singh S, Chang HY, Richards TM, Weiner JP, Clark JM, Segal JB (2013) Glucagon-like peptide-1 based therapies and risk of hospitalization for acute pancreatitis in type 2 diabetes mellitus: a population-based matched cases-control study. JAMA Intern Med 173:534–539

Theodorakis MJ, Carlson O, Michopoulos S, Doyle ME, Juhaszova M, Petraki K, Egan JM (2006) Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP. Am J Physiol Endocrinol Metab 290:E550–E559

Tseng YH, Ueki K, Kriauciunas KM, Kahn CR (2002) Differential roles of insulin receptor substrates in the anti-apoptotic function of insulin-like growth factor-1 and insulin. J Biol Chem 277:31601–31611

UK Prospective Diabetes Study (UKPDS) Group (1998) Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 352:837–853

UK Prospective Diabetes Study 16 (1995) Overview of 6 years' therapy of type II diabetes: a progressive disease. Diabetes 44:1249–1258

Ussher JR, Drucker DJ (2012) Cardiovascular biology of the incretin system. Endocr Rev 33:187–215

Vardarli I, Nauck MA, Köthe LD, Deacon CE, Holst JJ, Schweizer A, Foley JE (2011) Inhibition of DPP-4 with vildagliptin improved insulin secretion in response to oral as well as "isoglycemic" intravenous glucose without numerically changing the incretin effect in patients with type 2 diabetes. J Clin Endocrinol Metab 96:945–954

Vollmer K, Holst JJ, Baller B, Elrichmann M, Nauck MA, Schmidt WE, Meyer JJ (2008) Predictors of incretin concentrations in subjects with normal, impaired, and diabetic glucose tolerance. Diabetes 57:678–687

Wajchenberg BL (2007) β-cell failure in diabetes and preservation by clinical treatment. Endocr Rev 28:187–218

Wen XU, Li Y-B, Deng W-P, Hao Y-T, Weng J-P (2009) Remission of hyperglycemia following intensive insulin therapy in newly diagnosed type 2 diabetic patients: a long-term follow-up study. Chin Med J 122:2554–2559

Weng JP, Li Y, Xu W, Shi L, Zhang Q, Xhu D, Hu Y, Zhou Z, Yan X, Tian H, Ran X, Luo Z, Xian J, Yan L, Li F, Zeng L, Chen Y, Yang L, Yan S, Liu J, Li M, Fu Z, Cheng H (2008) Effect of intensive insulin therapy on β-cell function and glycaemic control in patients with newly diagnosed type 2 diabetes: a multicentre randomised parallel-group trial. Lancet 371:1753–1760

Willms B, Idowu K, Holst JJ, Creutzfeldt W, Nauck MA (1998) Overnight GLP-1 normalizes fasting but not daytime plasma glucose levels in NIDDM patients. Exp Clin Endocrinol Diabetes 106:103–107

Wu MS, Johnston P, Sheu WH, Hollenbeck CB, Jeng CY, Goldfine ID, Chen YD, Reaven GM (1990) Effect of metformin on carbohydrate and lipoprotein metabolism in NIDDM patients. Diabetes Care 13:1–8

Xu G, Stoffers DA, Habener JF, Bonner-Weir S (1999) Exendin-4 stimulates both β-cell replication and neogenesis, resulting in increased β-cell mass and improved glucose tolerance in diabetic rats. Diabetes 48:2270–2276

Xu L, Man CD, Charbonnel B, Meninger G, Davies MJ, Williams-Herman D, Cobelli C, Stein PP (2008) Effect of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on β-cell function in patients with type 2 diabetes. Diabetes Obes Metab 10:1212–1220

Zinman B, Gerich J, Buse J, Lewin A, Schwartz SL, Raskin P, Hale PM, Zdravkovic M, Blonde L (2008) Effect of the GLP-1 analog liraglutide on glycemic control and weight reduction in patients on metformin and rosiglitazone: a randomized double-blind placebo-controlled trial. Diabetologia 51(Suppl 1):A 898

Role of NADPH Oxidase in β Cell Dysfunction

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_46,

Abstract

Dysfunction of pancreatic β cells and loss of β cell mass is a major factor in the development of diabetes. Currently there is no cure for diabetes, and available therapies do not focus on halting or reversing the loss of β cell function. New strategies to preserve β cells in diabetes are needed. Conferring protection to the β cells against the effects of sustained intracellular reactive oxygen species (ROS) presents a novel approach to preserve β cell mass. Important contributors to increases in intercellular ROS in β cells are nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzymes. Discussed in this review are the roles of NADPH oxidases in the β cell, their contribution to β cell dysfunction, and new emerging selective inhibitors of NADPH oxidase.

Keywords

β cell • Diabetes • Inhibitors • Islet • NADPH oxidase • NOX • Proinflammatory cytokines • ROS

Introduction

An underlying feature of diabetes is a loss of functional β cell capacity. The therapeutic potential to cure diabetes by restoring functional β cell mass has been best illustrated in islet transplantation studies. These proof-of-principle efforts reversed insulin dependence. As a therapeutic strategy, islet transplantation is not currently viable (Taylor-Fishwick et al. 2008). However, these studies validate efforts to seek approaches to preserve and protect β cell mass in diabetes. Fundamental to this is a better appreciation of key events that drive β cell failure. Sensitivity of β cells to reactive oxygen species and oxidative stress is well recognized (Lenzen 2008). Relative to other cells, pancreatic β cells are vulnerable to sustained elevation in intracellular reactive species (Lenzen 2008). This is due, in part, to the low activity of free-radical detoxifying enzymes such as catalase, superoxide dismutase, and glutathione peroxidase in β cells (Grankvist et al. 1981; Lenzen et al. 1996; Tiedge et al. 1997; Modak et al. 2007). Islets also exhibit poor ability to rectify oxidative damage to DNA (Modak et al. 2007). Consequently, β cells are easily overwhelmed by elevated reactive oxygen species (ROS) and enter a state of oxidative stress (Lenzen 2008). Under oxidative stress conditions, ROS in addition to oxidizing proteins, lipids, and DNA also activate stress-sensitive second messengers such as p38-mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK), and protein kinase C (PKC) (Koya and King 1998; Purves et al. 2001). For example, a consequence of JNK activation is translocation of the homeodomain transcription factor *pdx-1* from the nucleus to the cytoplasm. *Pdx-1* is a key transactivator of the insulin gene in the nucleus (Ohneda et al. 2000). An outcome of cytoplasmic translocation of *pdx-1* is defective insulin expression that contributes to β cell dysfunction. As *pdx-1* transactivates its own expression, the impact of a cytoplasmic relocation for *pdx-1* is amplified

(Kawamori et al. 2003). It seems a paradox then that while β cells are susceptible to sustained elevations in ROS, transient increases in ROS are required for function and the generation of intracellular signaling (Goldstein et al. 2005; Pi et al. 2007; Newsholme et al. 2009). The source of intracellular ROS in β cells is a fertile area of research and reflects recognition for the key role elevated ROS can play in β cell pathophysiology. Intracellular events such as mitochondrial stress and/or endoplasmic reticulum stress do contribute to elevated intracellular ROS in the β cells (reviewed in Newsholme et al. (2009), Volchuk and Ron (2010)). Peroxisome metabolism of fatty acids elevates cellular ROS, and this ROS elevation has been linked to β cell lipotoxicity and dysfunction (Gehrmann et al. 2010; Elsner et al. 2011; Fransen et al. 2012). More recently, recognition has been made for an important contribution of the ROS-generating NADPH oxidase enzyme family in β cell pathophysiology. Reviewed in this chapter are the roles that NADPH oxidases have in the β cell with an emphasis on contributions to β cell dysfunction. Initially, the family of NADPH oxidase enzymes is introduced with a description of common features for this family in terms of structure, function, and expression. Specific features of each member of the family are considered relative to structure, function, expression, and regulation. The contribution of NADPH oxidase enzymes to β cell function and dysfunction is considered by describing the members and subunits that are expressed and functional in β cells. The activity of these NADPH oxidase enzymes is regulated by stimuli known to be elevated in diabetes. The consequence of stimulating NADPH oxidase activity is relevant for both β cell physiology and β cell pathophysiology. The role of NADPH oxidases in insulin secretion, β cell signaling, and induced β cell dysfunction is explored. Newly recognized are the contributions of intracellular ROS to discrete activation of second messengers. Thus, action of NADPH oxidase in terms of influencing the β cell ranges from subtle control to frank cell destruction. Lastly, major advances in the development of new selective inhibitors of NADPH oxidases are reviewed. These inhibitors represent candidate agents to advance the field and propose new therapeutic strategies to preserve and protect β cell mass in diabetes.

NADPH Oxidase Family

NADPH oxidases are multi-protein complexes that generate reactive oxygen species, including superoxide ($O_2^{\bullet -}$) and hydroxyl radical ($^{\bullet}OH$). Characteristically, the family shares a common basic structure but differ by their membrane localization and protein components (illustrated in Fig. 1).

There are seven identified members of the NADPH oxidase family. In humans, all seven enzymes are expressed. NADPH oxidases contain the following characteristic elements: (1) a COOH terminus, (2) six conserved transmembrane domains, (3) a flavin adenine dinucleotide (FAD)-binding site, (4) a NADPH-binding site at the COOH terminus, and (5) generally two heme-binding histidines in the third transmembrane domain and two in the fifth transmembrane domain, although the location of these sites vary (Rotrosen et al. 1992; Cross et al. 1995; Finegold

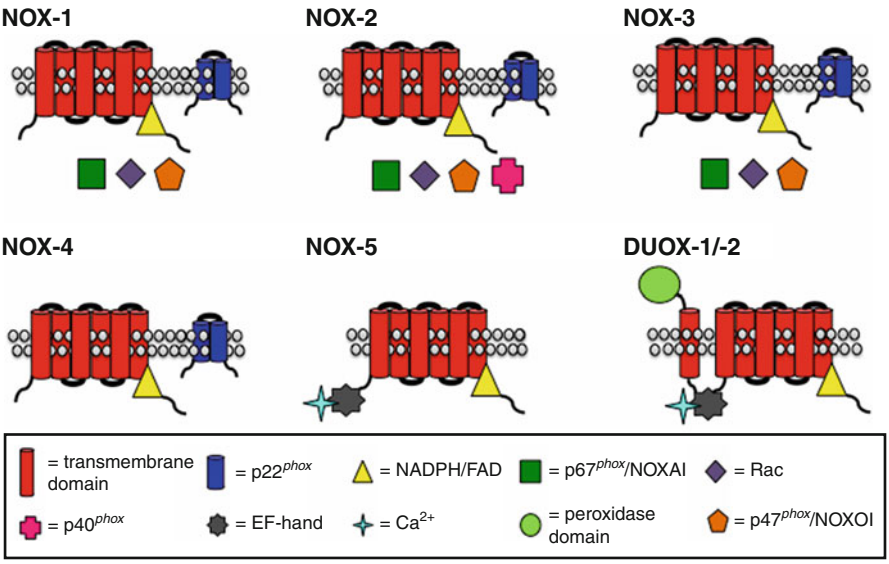

Fig. 1 NADPH oxidase (*NOX*) family members and their structures. Each NOX member contains six conserved transmembrane domains and a NADPH/FAD site at the COOH terminus. Additional structures needed for an active complex for some of the NOXs include $p22^{phox}$, $p67^{phox}$/NOXA1, $p47^{phox}$, Rac, and $p40^{phox}$. NOX-5 and DUOX-1/-2 have an EF-hand where Ca^{2+} binds for activation. DUOX-1/-2 have an extra-transmembrane domain with an extracellular peroxidase domain

et al. 1996; Biberstine-Kinkade et al. 2001; Lambeth et al. 2007). Some family members have additional domains, such as EF hands, or an additional NH_2-terminal transmembrane domain. As a class, NADPH oxidases are members of the flavocytochrome family. Functional enzymes require association with protein subunits to form complexes. Lambeth et al. reviewed subunit and domain interaction in NADPH oxidase enzymes (Lambeth et al. 2007).

Functionally, NADPH oxidase complexes traverse membranes and convert oxygen to a reactive radical via electron transfer across plasma or organelle membranes. NADPH serves as the single electron donor. NADPH donates a single electron to FAD which is located in the cytoplasmic end of the NOX enzyme (Nisimoto et al. 1999). The electron is passed between heme groups before reacting with oxygen to create superoxide (Finegold et al. 1996). Superoxide can then be converted to hydrogen peroxide (H_2O_2), a principle stable product.

NADPH oxidase family members are found primarily in eukaryotic cells of animals, plants, and fungi. Members of the NADPH oxidase family are implicated in numerous biological functions in animals. For example, phagocyte NADPH oxidase plays a critical role in host immune defense by activating the respiratory burst associated with neutrophil-mediated innate immunity (Rada et al. 2008). NADPH oxidases function in thyroid hormone synthesis and help regulate inner ear function (Caillou et al. 2001; Paffenholz et al. 2004; Ris-Stalpers 2006). Maintenance of the vascular system, hypertensive tone, and vascular wall function

are influenced by NADPH oxidase activity (reviewed in Manea (2010)). Indeed, reflective of the central influence of ROS in many biological processes, NADPH oxidases are associated with physiological or pathophysiological processes of most mammalian organ systems (reviewed (Bedard and Krause 2007; Bedard et al. 2007; Lambeth et al. 2007)). Additionally, an elevation of intracellular ROS and association of NADPH oxidase activity are implicated in numerous diseases involving these mammalian organ systems. In plants, NADPH oxidase plays a role in plant defenses and development (reviewed in Marino et al. (2012)) (Torres and Dangl 2005; Gapper and Dolan 2006; Sagi and Fluhr 2006). NADPH oxidase regulates root growth by cell expansion (Foreman et al. 2003). Fungi express several different isoforms of NOXs (reviewed in Tudzynski et al. (2012)). Filamentous fungi express two subfamilies of NADPH oxidase that are similar to mammalian phagocyte NADPH oxidase. Homologous subunits to other mammalian NADPH oxidases have also been reported in fungi (Aguirre et al. 2005). The NADPH oxidase subfamilies present in fungi play a role in growth and development including germination (Lara-Ortiz et al. 2003; Malagnac et al. 2004; Cano-Dominguez et al. 2008). Evidence to suggest that fungal NADPH oxidases may play a role in pathogenesis of cellulose degradation has also been reported (Brun et al. 2009).

NADPH Oxidase Family Members

Phagocyte NADPH oxidase, named for its expression in phagocytes, especially neutrophils, was the first identified NADPH oxidase and plays a critical role in the respiratory burst response associated with innate immune defense (Babior et al. 1973, 2002). Subsequent gene discoveries have identified a family of genes/proteins that are distinct enzymes but homologous to the catalytic core subunit of phagocyte NADPH oxidase. This family of NADPH oxidases is termed NOX/DUOX with the nomenclature of the seven members being termed NOX-1, NOX-2, NOX-3, NOX-4, NOX-5, DUOX-1, and DUOX-2. Under this nomenclature the core catalytic subunit of phagocyte NADPH oxidase (previously termed $gp91^{phox}$) is called NOX-2. A brief overview of each family member is provided starting with the archetypal phagocyte NADPH oxidase (NOX-2) before consideration of the role of NADPH oxidase in β cells (Table 1).

NOX-2

The structure of the functional NOX-2 enzyme is made up of five protein subunits that are located in the plasma membrane or translocated to the plasma membrane from the cytosol. Based upon the original name, phagocyte NADPH oxidase, the naming convention of subunits is gpX^{phox}, where gpX indicates glycoprotein of "X" molecular weight. Activation of phagocyte NADPH oxidase (NOX-2) occurs through a complex series of protein interactions. The core catalytic component of NADPH oxidase, $gp91^{phox}$, is stabilized in the membrane by $p22^{phox}$. The adaptor

Table 1 The members of the NADPH oxidase family have distinct structures, functions, locations of expression, and specific regulators. NOX-1 and NOX-2 have roles in β cell function with respect to insulin signaling and secretion

Complex	Structure	Function	Expression	Regulation	β cell role
NOX-1	gp91^{phox} NOXO1 NOXA1 p22^{phox} Rac	Immune modulator Cell proliferation	Colon epithelium Pancreas Vascular smooth muscle	AngII Interferon PMA EGF 12-LO	Increases intracellular ROS Uncouples GSIS Induces apoptosis
NOX-2	gp91^{phox} p47^{phox} p67^{phox} p22^{phox} p40^{phox} Rac	Immune defense	Phagocytes Pancreas B lymphocytes Neurons Cardiomyocytes	AngII Endothelin-1 Growth factors Cytokines LPS	Increases intracellular ROS Uncouples GSIS Induces apoptosis
NOX-3	p22^{phox} NOXO1 NOXA1 Rac p47^{phox}	Balance Gravity	Inner ear Fetal kidney Fetal spleen Skull bone Brain		
NOX-4	p22^{phox} Rac	Cell migration Apoptosis Cell survival Cell differentiation Insulin signaling	Kidney Pancreas Vascular endothelial cells Osteoclasts	Constitutively active TGF-β Insulin IGF-1	

NOX-5	EF-hand domain	Cell proliferation Cell migration Angiogenesis Cancer growth Sperm maturation Pregnancy Fetal development	Lymphoid tissue Testis Spleen Endothelium Uterus Placenta Fetal tissue	Calcium Thrombin Platelet-derived growth factor AngII Endothelin-1
DUOX-1	EF-hand domain Peroxidase domain	Thyroid hormone synthesis Immune defense Inflammation	Thyroid Respiratory tract Prostate	Calcium Forskolin IL-4 IL-13
DUOX-2	EF-hand domain Peroxidase domain	Thyroid hormone synthesis Heme peroxidase Immune defense Inflammation Antibacterial response	Thyroid Respiratory tract	Calcium Interferon gamma Flagellin Phorbol esters

NOX: Nicotinamide adenine dinucleotide phosphate-oxidase enzyme; DUOX: Dual oxidase; NOXO1: NOX Organizer 1; gp: glycoprotein; NOXA1: NOX Activator 1; AngII: Angiotensin II; PMA: Phorbol 12-myristate 13-acetate; EGF: Epidermal growth factor; 12-LO: 12-Lipoxygenase; LPS: Lipopolysaccharide; TGF-β: Transforming growth factor-β; IGF-1: Insulin-like growth factor 1; IL-4: Interleukin-4; IL-13: Interleukin-13; ROS: Reactive oxygen species; GSIS: Glucose-stimulated-insulin-secretion

protein $p47^{phox}$ is activated by phosphorylation. Recruitment of $p47^{phox}$ facilitates addition to the complex of $p40^{phox}$, $p67^{phox}$, and Rac (small GTP-binding protein). The latter two appear to regulate catalysis (Abo et al. 1991; Ando et al. 1992; Wientjes et al. 1993; Heyworth et al. 1994; Hordijk 2006; Orient et al. 2007; Guichard et al. 2008).

NOX-2 in phagocytes functions in the host defense by providing a respiratory burst response that serves to kill bacteria enclosed within the phagosome through production of hypochlorous acid. NOX-2 also activates inflammatory and immune responses (Rada et al. 2008). Genetic defects in NOX-2 activity arising from mutations in the genes encoding $gp91^{phox}$, $p22^{phox}$, $p47^{phox}$, and $p67^{phox}$ are associated with chronic granulomatous disease (Heyworth et al. 2003). Generation of ROS by NOX-2 has also been shown to activate pathways involved in angiogenesis (as reviewed in Ushio-Fukai (2006)).

In addition to phagocytes, NOX-2 expression has been reported in pancreas, B lymphocytes, neurons, cardiomyocytes, endothelium, skeletal muscle, hepatocytes, smooth muscle, and hematopoietic stem cells (Bedard and Krause 2007).

NOX-2 is regulated by angiotensin II (AngII), endothelin-1, growth factors such as vascular endothelial growth factor (VEGF), cytokines such as tumor necrosis factor α (TNF-α), interferon gamma (IFN-γ), lipopolysaccharide, mechanical forces, and hyperlipidemia (Cassatella et al. 1990; Dworakowski et al. 2008).

NOX-1

The first homolog of the NOX-2 catalytic subunit ($p91^{phox}$) to be described was NOX-1. Similar to functional NOX-2, the functional NOX-1 enzyme is a multiprotein complex. The subunits that bind NOX-1 are NOXO1 (NOX organizer 1) and NOXA1 (NOX activator 1), which while being distinct proteins are homologs of $p47^{phox}$ and $p67^{phox}$, respectively (Takeya et al. 2003; Uchizono et al. 2006). For a functional complex, active NOX-1 requires the NOX-1 catalytic core bound to the membrane subunit $p22^{phox}$, NOXO1, and NOXA1 along with the GTPase Rac subunit. NOXO1 is predominantly associated with membrane-bound $p22^{phox}$ (Sumimoto 2008). NOXA1 and Rac translocate to the membrane, in a phosphorylation-dependent process, and are required for NOX-1 activation (Miyano et al. 2006). Transfection experiments have shown that NOX-1 can use the $p47^{phox}$ and $p67^{phox}$ subunits of NOX-2, raising the possibility of a dynamic interaction amongst NOX isotypes where subunits are interchangeable between the different NOX family members (Banfi et al. 2003).

NOX-1 activity is associated in immune defense in inflammatory bowel disease and cancer (Rokutan et al. 2006, 2008). Studies have linked NOX-1 activity to colon cancer by affecting cell proliferation of colon carcinoma cell lines and controlling cell migration of colon adenocarcinoma cells (de Carvalho et al. 2008; Sadok et al. 2008). NOX-1 also functions in the vasculature by regulating smooth muscle growth, migration (cell movement), and blood pressure (Cave et al. 2006; Gavazzi et al. 2006; Garrido and Griendling 2009). NOX-1

helps regulate neuronal differentiation by negatively affecting excessive neurite outgrowth and influences pain sensitivity during inflammation (Ibi et al. 2006, 2008).

NOX-1 is expressed in the colon epithelium and at lower levels in the pancreas, vascular smooth muscle, endothelium, uterus, placenta, prostate, osteoclasts, and retinal pericytes (Nisimoto et al. 2008). Patterns of expression for NOX-1 are related to species. For example, the expression of NOX-1 is found in rodent stomach but not in human stomach (Kawahara et al. 2005; Kusumoto et al. 2005; Rokutan et al. 2008).

Regulators of NOX-1 include AngII, IFN-γ, protein kinase C (PKC) activation (PMA: 4β-phorbol 12-myristate 13-acetate), and epidermal growth factor receptor ligation (Suh et al. 1999; Lassegue et al. 2001; Wingler et al. 2001; Katsuyama et al. 2002; Seshiah et al. 2002; Touyz et al. 2002; Geiszt et al. 2003a; Takeya et al. 2003; Fan et al. 2005). In the liver cell line FaO, NOX-1 regulation involves an autoregulatory feedforward loop involving second messenger activation of Src kinase and extracellular-signal-regulated kinase (ERK) (Fan et al. 2005; Adachi et al. 2008; Sancho and Fabregat 2010).

NOX-3

Activity of NOX-3 is dependent on $p22^{phox}$. Additional cytosolic subunits NOXO1, NOXA1, $p47^{phox}$, or Rac are not needed for basal NOX-3 activity. The activity of NOX-3 is however significantly increased following association with cytosolic subunits (Banfi et al. 2004a; Cheng et al. 2004; Ueyama et al. 2006).

A major site for NOX-3 expression is the inner ear where its function is to assist biogenesis of otoconia and regulation of balance and gravity (Paffenholz et al. 2004). Expression of NOX-3 has additionally been described in fetal kidney, fetal spleen, skull bone, brain, and lung endothelial cells where it is associated with the development of emphysema (Banfi et al. 2004a; Zhang et al. 2006; Bedard and Krause 2007).

NOX-4

NOX-4 activity requires association with the $p22^{phox}$ subunit, but unlike NOX-2, other subunits are not essential (Martyn et al. 2006). It is unresolved whether the Rac subunit is required for NOX-4 activity (Gorin et al. 2003). Unlike the other NOX family members that initially produce superoxide, NOX-4 produces H_2O_2 (Martyn et al. 2006; Serrander et al. 2007).

NOX-4 function has been associated with cell migration, apoptosis, cell survival, cell differentiation, insulin signaling, cell migration, the unfolded protein response, and differentiation (Mahadev et al. 2004; Pedruzzi et al. 2004; Vaquero et al. 2004; Cucoranu et al. 2005; Li et al. 2006; Meng et al. 2008; Pendyala et al. 2009; Santos et al. 2009). It is a major source of oxidative stress in the failing heart (Kuroda et al. 2010).

NOX-4 is expressed mainly in the kidney but is also detected in vascular endothelial cells, osteoclasts, endothelium, smooth muscle, hematopoietic stem cells, fibroblasts, keratinocytes, melanoma cells, neurons, pancreas, and adipocytes (Geiszt et al. 2000; Cheng et al. 2001; Shiose et al. 2001; Ago et al. 2004; Bedard and Krause 2007).

NOX-4 is constitutively active and levels of mRNA directly correlate with enzyme function (Serrander et al. 2007). The activity of NOX-4 can be upregulated by tumor growth factor β (TGF-β) in cardiac fibroblasts, pulmonary artery smooth muscle cells, and lungs (Cucoranu et al. 2005; Sturrock et al. 2006, 2007). Insulin activates NOX-4 in adipocytes, and it can be activated by insulin-like growth factor-1 (IGF-1) in vascular smooth muscle cells (VSMCs) (Mahadev et al. 2004; Meng et al. 2008; Schroder et al. 2009).

NOX-5

Distinct to the other NOX enzymes, activation of NOX-5 is calcium dependent. At its amino terminal, NOX-5 has a calmodulin-like domain that has four calcium-binding EF-hand domains (Lambeth 2007). NOX-5 does not require $p22^{phox}$ or other phox subunits for activity. Several splice variant forms of NOX-5 exist (Banfi et al. 2001, 2004b; BelAiba et al. 2007; Pandey et al. 2012).

NOX-5 activity has functional relevance in endothelial cell proliferation, migration, and angiogenesis (BelAiba et al. 2007; Jay et al. 2008; Schulz and Munzel 2008). NOX-5 activity is linked to cancer, including prostate cancer growth, esophageal adenocarcinoma, breast cancer, and hairy cell leukemia (Brar et al. 2003; Kamiguti et al. 2005; Fu et al. 2006; Kumar et al. 2008; Juhasz et al. 2009). NOX-5 exerts a role in fetal development and sperm maturation (reviewed Bedard et al. (2012)).

NOX-5 is expressed mainly in the lymphoid tissue, testis, and spleen (Banfi et al. 2001; Cheng et al. 2001). Expression of NOX-5 in endothelium, smooth muscle, pancreas, placenta, ovary, uterus, stomach, certain prostate cancers, and various fetal tissues has been reported (Banfi et al. 2001; Cheng et al. 2001; Bedard and Krause 2007; BelAiba et al. 2007).

Calcium, thrombin, platelet-derived growth factor (PDGF), AngII, and endothelin-1 are factors reported to regulate NOX-5 (Montezano et al. 2010, 2011). IFN-γ has also been shown to activate NOX-5 in smooth muscle cells possibly through the release of intracellular calcium stores (Manea et al. 2012).

DUOX-1 and DUOX-2

DUOX-1 and DUOX-2 do not require subunits to form an active enzyme complex. Like NOX-5, calcium is required to activate to each DUOX enzyme (Selemidis et al. 2008; Rigutto et al. 2009). Structurally, DUOX enzymes differ to NOX enzymes by having an additional transmembrane domain at the amino terminal

and an extracellular peroxidase domain (De Deken et al. 2000). DUOX-1/DUOX-2 locate to the plasma membrane from the ER with the help of maturation factors DUOXA1 and DUOXA2 (Grasberger and Refetoff 2006; Morand et al. 2009).

DUOX-1 and DUOX-2 are highly expressed in the thyroid and directly produce H_2O_2 that is used during thyroid hormone T4 synthesis (Dupuy et al. 1989, 1999; De Deken et al. 2000; Caillou et al. 2001; Ris-Stalpers 2006). While current knowledge suggests the major role for DUOX is in the synthesis of thyroid hormone, expression of DUOX has been described in other sites including the respiratory tract, prostate, testis, pancreas, colon, and heart (Edens et al. 2001; Geiszt et al. 2003b; Harper et al. 2005; Allaoui et al. 2009; Gattas et al. 2009).

In addition to elevation in calcium, activation of DUOXs can be regulated by forskolin, interleukin IL-4, IL-13, IFN-γ, phorbol esters, and insulin (Morand et al. 2003; Harper et al. 2005, 2006; Rigutto et al. 2009).

NADPH Oxidases and β Cells

Select NOX family members are expressed in pancreatic β cells. NOX-1, NOX-2, NOX-4, NOXO1 (homolog of $p47^{phox}$), NOXA1 (homolog of $p67^{phox}$), and $p40^{phox}$ have been described in a variety of pancreatic and islet studies, including isolated rat β cells (Oliveira et al. 2003; Nakayama et al. 2005; Lupi et al. 2006; Shao et al. 2006; Uchizono et al. 2006; Rebelato et al. 2012). Expression of NOX-5, DUOX-1 and DUOX-2, as determined by RT-PCR, has additionally been described in the pancreas, though the functional relevance of their expression has yet to be determined (Cheng et al. 2001; Edens et al. 2001). Historically, expression of NADPH oxidase family members in β cells has been associated with regulation of glucose-stimulated insulin secretion (Morgan et al. 2007; Pi et al. 2007; Morgan et al. 2009). More recently, activity of NOX enzymes has been linked to β cell dysfunction. β cell damage likely arises from generation of intracellular ROS stimulating redox signaling pathways and, more chronically, oxidative stress. In the following sections, the roles of the specific NOXs expressed in the β cell are discussed.

NOX and Glucose-Stimulated Insulin Secretion

Several key observations have linked NADPH oxidase activity with regulation of insulin secretion. The product of NADPH oxidase activity, generation of H_2O_2, is required for insulin secretion (Pi et al. 2007). Elevated glucose leads to an increase in H_2O_2 generation, thus linking NADPH oxidase activity to regulation of insulin secretion (Morgan et al. 2007, 2009). Inhibition of NADPH oxidase by the general inhibitor, diphenyleneiodonium (DPI), led to a decrease in H_2O_2 production and also impaired insulin secretion (Imoto et al. 2008). Antisense-mediated decrease in the expression of $p47^{phox}$, an important subunit for the active NOX enzyme, reduced glucose-stimulated insulin secretion (Morgan et al. 2009). Activation and

translocation of $p47^{phox}$ is required for an active NADPH complex. Translocation of proteins to membranes or subunits is supported since inhibitors of protein prenylation or protein farnesyltransferase significantly decrease NOX-2-induced ROS generation and decrease glucose-stimulated insulin secretion (Syed et al. 2011b; Matti et al. 2012). These results suggest NOX activity is necessary for the transient increase in ROS that is needed for glucose-stimulated insulin secretion.

Which NOX family isotypes are involved in insulin secretion and what exactly their role is remains an unanswered question. A major limitation to resolving this question has been the lack of isoform-specific inhibitors of the NOX enzymes.

NOX and β Cell Dysfunction

Associated with a diabetic state is an increase in the serum levels of proinflammatory cytokines, free-fatty acids (FFA), and glucose. These serum mediators have been shown to elevate the expression and activity of NADPH oxidases (Morgan et al. 2007). Additionally, deposition of fibrillar human islet amyloid polypeptide (IAPP) in the β cell line RIN5mF cells increases NADPH oxidase activity and intracellular lipid peroxidation (Janciauskiene and Ahren 2000). Accumulation of amyloid in islets is a pathogenic state associated with type 2 diabetes (Marzban and Verchere 2004). In a small sample group, we showed that NOX-1 expression is elevated in islets from human type 2 diabetic donors (Weaver et al. 2012). Animal models of type 2 diabetes have also reported an increase in NOXs. For example, the role of NOX-2 in β cell dysfunction has been explored in the Zucker diabetic fatty rat (Syed et al. 2011a). The Zucker diabetic fatty (ZDF) rat is a model of type 2 diabetes where rats become obese and develop hyperinsulinemia, hyperglycemia, and β cell dysfunction. Examination of islets isolated from ZDF rats showed an increase in intracellular ROS levels that corresponded with elevated expression of the NOX enzyme subunits $p47^{phox}$, $gp91^{phox}$, and Rac1 (Syed et al. 2011a). Exposure of islets from Wistar rats to the free-fatty acid palmitate resulted in elevation of $p47^{phox}$ protein and an increase in the mRNA levels of $p22^{phox}$, $gp91^{phox}$, $p47^{phox}$, proinsulin, and the G protein-coupled protein receptor 40, a signaling receptor that activates the phospholipase C signaling pathway (Graciano et al. 2011). With global inhibition of NADPH oxidase activity, β cells are protected from the effects of cytokine or FFA treatment (Michalska et al. 2010).

Advanced glycation end products (AGE) contribute to oxidative stress and the development of diabetes (Kaneto et al. 1996; Hofmann et al. 2002; Peppa et al. 2003; Cai et al. 2008; Zhao et al. 2009; Coughlan et al. 2011). AGEs form when carbohydrates, such as glucose, react nonenzymatically (e.g., glycation and oxidation) with amino groups. Binding of AGE to its receptor, RAGE (receptor for advanced glycation end products), generates ROS leading to oxidative stress in β cells. Evidence suggests that AGE may be increasing ROS generation through NADPH oxidase. In isolated rat islets, NADPH-dependent superoxide generation

in homogenates increased after treatment with high glucose plus glycolaldehyde (Costal et al. 2013); addition of the NADPH oxidase inhibitor DPI decreased superoxide production. VAS2870, a NADPH oxidase inhibitor, also decreased intracellular superoxide production in islets treated with glucose plus glycolaldehyde (Costal et al. 2013). The increase in superoxide preceded apoptosis. Pancreatic β cell lines including INS-1, MIN6, and BTC-6 cells and isolated primary rat islets treated with AGE showed an increase in ROS generation that was followed by apoptosis (Lim et al. 2008). INS-1 β cells exposed to varying concentrations of AGE showed a time-dependent increase in intracellular ROS and apoptosis that was dependent upon NADPH oxidase (Lin et al. 2012). Since superoxide production in vascular smooth muscle cells exposed to AGE has been linked to an increase in the transcription of NOX-1, a similar pathway linking AGE and NADPH oxidase-1 in β cells may occur (San Martin et al. 2007).

NOX-2 and β Cell Function

(a) *NOX-2 and Regulation of Insulin Secretion*

Of the NOX family members expressed in β cells, NOX-2 has most closely been associated with regulation of insulin secretion. In an attempt to address critical roles of NOX enzymes in insulin secretion and discern isotype function, Li et al. performed studies using knockout mice, an approach that uses genetic gene depletion. Knockout mice that were genetically depleted of the core catalytic unit of NOX-1, NOX-2, or NOX-4 were evaluated to determine the role of each NADPH oxidase member in physiological insulin secretion. In an unanticipated outcome, none of the knockout mice showed a decrease in insulin secretion when challenged for glucose-stimulated insulin secretion (Li et al. 2012). This raises the question of whether the NADPH oxidase family is necessary for physiological glucose-stimulated insulin secretion. These results differ from a previous report in isolated rat islets. When treated with the general NADPH oxidase inhibitor, diphenyleneiodonium (DPI) chloride, a decrease in glucose-stimulated insulin secretion was observed (Uchizono et al. 2006). The discrepancy between the outcomes of the two studies has been explained by the nonspecific inhibitory action of DPI. As a nonspecific inhibitor of flavoenzymes, DPI inhibits numerous flavoproteins in addition to NOX enzymes. However, glucose-stimulated insulin secretion was reduced in isolated rat islets following depletion of $p47^{phox}$ using antisense oligonucleotide (Morgan et al. 2009). This effect was not seen with control (scrambled) oligonucleotide. The $p47^{phox}$ subunit is a required element for activity in certain NOX isoforms. This experimental approach is not subject to the arguments of nonspecificity attributed to the use of the chemical inhibitor of flavoenzymes, DPI. Depletion of $p47^{phox}$ was associated with a reduction in intracellular ROS (H_2O_2) production supporting a functional decrease in NOX activity. The results for inhibition of glucose-stimulated insulin secretion reported by Morgan et al. using depletion of $p47^{phox}$ were matched with their evaluation of DPI. The role of NOX enzymes in physiological insulin secretion may not yet be resolved. Clarity will be better achieved with discovery of selective

isoform-specific inhibitors of NOX enzymes. An additional consideration is the possibility of the cross-use of isotype subunits between the NOX enzymes ensuring homeostatic regulation. It is possible that adaptation in genetic deletion (transgenic knockout) studies may occur or be overcome by functional compensation. This was addressed in the studies by Li et al., which looked in each NOX-knockout mouse for a homeostatic upregulation in remaining NOX enzymes. None was reported nor was physiological insulin secretion affected following a transient knockdown of NOX-2 (Li et al. 2012). Li et al. reported that NOX-2 played a role in insulin secretion through cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) signaling. In isolated wild-type islets, an increase in NOX-2 activity and generation of intracellular ROS results in negative modulation of the insulin secretory response and reduction in adenylate cyclase/cAMP/PKA signaling. Overall, these results show a link between NOX-2 and insulin regulation, though the mechanism may be more complex than first assumed.

(b) *NOX-2 and Regulation of ROS Generation*

Importantly, the β cell has to traverse a delicate balance in ROS generation. Acutely, a transient increase in ROS is a physiological requirement for insulin secretion. However, chronic sustained ROS generation negatively regulates insulin secretion and promotes β cell dysfunction (Morgan et al. 2007; Pi et al. 2007; Morgan et al. 2009). The activity of NADPH oxidase, whose primary enzymatic function is the production of ROS, is a logical candidate for a sustained chronic increase in intracellular ROS in the β cell. In addition to direct activation of NOX, mitochondrial activity has been related to NOX-2 activity in β cells (Syed et al. 2011a, b; Matti et al. 2012). Glucose or specific mitochondrial fuels (monomethyl succinate and α-ketoisocaproate) lead to an increase in NOX-2 activity and generation of ROS. In both the isolated rat islets and the homogeneous INS-1 β cell line, stimulation of NOX-2 by hyperglycemia and hyperlipidemia results in ROS generation and activation of the JNK1/2 signaling pathway. Activation of the JNK pathway precedes mitochondrial dysfunction and an increase in caspase-3 activity (Syed et al. 2011a). NOX enzymes can be upregulated in response to pathogenic stimuli, including proinflammatory cytokines, elevated FFAs, and high glucose. These serum factors are recognized as promoting β cell dysfunction, presumably by sustained activation of NAPDH oxidase and elevation of ROS.

(c) *NOX-2 and β Cell Dysfunction/Survival*

In NOX-2-deficient mice, protection to β cell destruction associated with streptozotocin (STZ) exposure was observed (Xiang et al. 2010). NOX-2 expression in the β cell line INS 832/13 is increased in response to hyperglycemic conditions, a condition known to cause β cell dysfunction (Mohammed and Kowluru 2013). Upon exposure of β cells to FFA or low-density lipoprotein, NOX-2 activity was linked to β cell dysfunction (Yuan et al. 2010b; Jiao et al. 2012). Decreased glucose-stimulated insulin secretion and apoptosis occur in pancreatic NIT-1 cells following exposure to very low-density lipoprotein (VLDL). This β cell dysfunction is co-associated with an increase in NOX-2

generated ROS and a decrease in expression and secretion of insulin (Jiao et al. 2012). NIT-1 cells showed only expression of NOX-2 and its subunits, and no expression of any of the other NADPH oxidase family members (Yuan et al. 2010a, b). In contrast, when NIT-1 cells were treated with VLDL plus siRNA-NOX-2, β cell function was preserved. Similar results were found when NIT-1 cells were treated with palmitate or oleate (Yuan et al. 2010b). FFAs induced β cell dysfunction and increased apoptosis through elevated ROS generation that arises from an increase in NOX-2 activity. When siRNA-NOX-2 was used to knock down NOX-2 protein, FFA-treated cells responded like control (untreated) cells with preserved β cell function and negligible apoptosis.

NOX-1 and β Cell Function

(a) *NOX-1 and Inflammation*

Our own studies have explored the regulation of NOX enzyme expression in β cells following stimulation with inflammatory cytokines. The cocktail combination of inflammatory cytokines used (TNF-α, IL-1β, IFN-γ) is widely reported to induce β cell dysfunction. We have shown that acute treatment of primary human islets, mouse islets, or homogeneous murine β cell lines with this inflammatory cytokine cocktail (termed PICs) induces gene expression, leads to a loss of glucose-stimulated insulin secretion, and induces apoptosis (Weaver et al. 2012; Weaver and Taylor-Fishwick 2013). In these model systems, expression of NOX-1 is selectively upregulated relative to other NOX isotypes, following stimulation with PICs (Weaver et al. 2012). The upregulation of NOX-1 co-associates with elevated intracellular ROS, loss of glucose-stimulated insulin secretion, and induction of β cell apoptosis (Weaver et al. 2012; Weaver and Taylor-Fishwick 2013). Significantly, inhibition of NOX-1 protected β cells from the damaging effects resulting from PIC stimulation, suggesting NOX-1 may be an important target for β cell preservation in diabetes (Weaver and Taylor-Fishwick 2013).

(b) *NOX-1 and 12-Lipoxygenase*

In terms of intracellular regulators of NOX-1 expression, the lipid-metabolizing enzyme, 12-lipoxygenase (12-LO), has been shown to induce NOX-1 expression in β cells (Weaver et al. 2012). 12-LO and one of its major bioactive lipid products, 12-HETE, are key mediators of β cell dysfunction and inflammation (as detailed in the chapter "▶ Inflammatory pathways linked to β cell demise in diabetes" by Imai et al.). The 12-LO pathway is associated with inflammation and activation of the transcription factor STAT4 (signal transducer and activator of transcription 4) and cytokines IL-12 and IFN-γ. The significance of the 12-LO pathway for diabetes has been shown in several mouse models. Mice with a deletion in 12-LO are resistant to diabetes induced by low-dose streptozotocin (Bleich et al. 1999). The non-obese diabetic (NOD) mouse model of type 1 diabetes was also protected from

spontaneous diabetes development when 12-LO was deleted (McDuffie et al. 2008). When β cells were treated with 12-HETE, a product of 12-LO activity, or proinflammatory cytokines, there was an induction of NOX-1 expression (Weaver et al. 2012). Conversely, selective inhibition of 12-LO activity by selective small molecules (Kenyon et al. 2011) reduced proinflammatory cytokine-induced NOX-1 expression (Weaver et al. 2012). These data integrate inflammation with induction of 12-LO activity and NOX-1 expression in a pathway regulating β cell dysfunction.

(c) *NOX-1 and Feedforward Regulation in β Cells*

Our studies have additionally provided evidence for a feedforward regulation of NOX-1 in β cells. An autoregulatory feedback loop amplifies NOX-1 upregulation in β cells. This work parallels similar observations in a liver cell line (Sancho and Fabregat 2010). In the β cell, where relatively limited defense mechanisms exist to counter a sustained increase in ROS, oxidative stress arising from feedforward regulation of NOX-1 could be a significant event. Identifying and inhibiting such regulation could prove important in developing new strategies for preservation and protection of functional β cell mass in diabetes. Our studies demonstrated that induced expression of NOX-1 by PIC stimulation of β cells was abrogated with inhibitors of NADPH oxidase activity. Assuming NOX activity is subsequent to induced gene expression, the simplest explanation of the data is that NOX activity upregulates NOX-1 gene expression. The resultant elevation of intracellular ROS was implicated. General antioxidants, which neutralize cellular ROS, inhibited NOX-1 expression induced by PIC stimulation. In contrast, pro-oxidants that directly elevate cellular ROS in the absence of other stimuli induced NOX-1 expression. Redox-sensitive signaling pathways (discussed below) were shown to mediate this feedforward regulation of NOX-1 in β cells. It will be interesting to evaluate if feedforward regulation of NADPH oxidase in β cells is a phenomenon restricted to NOX-1 or has a more broad relevance to other NOX enzymes functional in β cells. Selective inhibition of NOX-1 or key redox signaling events arising from NOX-1 activity in β cells may offer therapeutic opportunities.

In summary, NADPH oxidases play important roles in regulation of β cell biology. This is in terms of both regulation of physiological insulin secretion and mediation of β cell pathophysiology. The latter may result in β cell dysfunction arising from uncontrolled oxidative stress or more discrete modulation of function mediated by redox signaling initiated events. In terms of diabetes, NADPH oxidase activity is stimulated by several diabetes-associated stimuli.

NOX-4 and Insulin Signaling

NOX- 4 activity has additionally been implicated in the regulation of insulin signaling (Mahadev et al. 2004). NOX-4 is expressed in insulin-sensitive adipose cells (Mahadev et al. 2004). It has been previously shown that 3T3-L1 adipocytes

produce H_2O_2 in response to insulin (Krieger-Brauer and Kather 1995). Dominant negative deletion constructs (missing either the NADPH-binding domain or the FAD/NADPH domains of NOX-4) were expressed in differentiated 3T3-L1 adipocyte cells. These cells expressing a deregulated/mutated NOX-4 showed a decrease in the generation of H_2O_2 when stimulated with insulin and a decrease in tyrosine phosphorylation of both the insulin receptor and insulin receptor substrate-1 (IRS-1) (Mahadev et al. 2004). Intracellular events associated with activation of the insulin signaling pathway, such as activation of ERK1/2 and glucose uptake, were also inhibited in the cells expressing the NOX-4 mutants. These studies highlight a link between NOX-4, ROS generation, and changes in insulin signaling (Mahadev et al. 2004).

Redox Signaling

A consequence of NADPH oxidase activation is production of ROS. Sustained increase in ROS results in oxidative stress that can be destructive to cells, resulting in dysfunction and cell death. Transient increases in ROS are, however, part of normal physiological processes. There is increasing recognition that intracellular signaling pathways are sensitive to changes in redox levels (Goldstein et al. 2005). Thus, in addition to global oxidative stress, NADPH oxidase activity is likely to also play an important role in regulation of discrete signaling pathways, kinase activation in particular. Upregulation of ROS, especially superoxide and hydrogen peroxide, can lead to activation of specific signaling pathways. Several signaling pathways are regulated by changes in intracellular ROS (reviewed in Goldstein et al. (2005), Mittler et al. (2011)). As NADPH oxidase is upregulated, there is an increase in ROS generation. For example, overexpression of NOX-1 in NIH 3T3 cells resulted in elevation in intracellular ROS and activation of signaling kinases JNK and ERK1/2 (Go et al. 2004). Adjustments to intracellular ROS levels likely lead to conformational changes in kinases and access to phosphorylation sites. Modification of kinase phosphorylation can result in either activation or inhibition of subsequent signals in a specific pathway. Known signaling pathways regulated by cellular redox state include kinases in the mitogen-activated protein kinase (MAPK) family, ERK, JNK, p38 kinase, and the Src-kinase family (Giannoni et al. 2005). Some transcription factors, such as NF-κB, AP-1, Nrf2, and c-Jun, are also sensitive to redox signaling. Elevated ROS produced from NADPH oxidase activity also inactivate phosphatases, including protein-tyrosine phosphatase 1B (PTP1B) (Mahadev et al. 2004).

NOX-1 and Second Messenger Src-Kinase Signaling

The expression of NOX-1 in the INS-1 β cell line is regulated through a feedforward loop in which NOX-mediated ROS generation affects second messengers resulting in a signal to upregulate NOX-1 protein expression. The second

messengers involved in this pathway include activation of Src kinase (Weaver and Taylor-Fishwick 2013). When INS-1 cells were treated with proinflammatory cytokines, elevation in intracellular ROS and NADPH oxidase activity led to an increase in NOX-1 expression. This feedforward regulation was blocked by the selective Src-kinase inhibitor, PP2. Importantly, PP3, the structural chemical analog of PP2 that is inactive for Src-kinase inhibition, did not block the upregulation of NOX-1. Signaling pathways associated with NOX-1 activation include p38MAPK, Akt, and Src kinase (Gianni et al. 2008; Sancho and Fabregat 2010). Both p38MAPK and Akt are downstream mediators of NOX-1-activated ROS elevation in vascular smooth muscle cells (Lassegue et al. 2001).

NOX-2 and AngII Signaling

In addition to NOX-2 activation of cAMP/PKA in β cells, stimulation of NOX-2 by AngII also mediates JNK and Janus kinase (JAK)/STAT activation (Alves et al. 2012; Li et al. 2012). Alves et al. found that when rat islets were stimulated with AngII, NOX-2 was activated and ROS was generated. This elevation in intracellular ROS led to phosphorylation of JAK/STAT and JNK proteins (Alves et al. 2012). Islets isolated from the type 2 diabetic-like animal model, ZDF rat, have increased expression and phosphorylation of the NADPH oxidase $p47^{phox}$ subunit. They also have increased expression of the $gp91^{phox}$ subunit and increased activation of Rac (Syed et al. 2011a). Increase in NOX subunit expression and phosphorylation was correlated with activation of JNK1/2 and a decrease in activation of ERK1/2 in isolated ZDF islets. This study therefore linked the increase in NOX-2 subunits with second messenger activation and β cell dysfunction (Syed et al. 2011a). Supportive data was additionally presented in the INS-1 rat β cell line. Upon treatment with high glucose or the FFA palmitate, JNK1/2 phosphorylation increased, ERK1/2 phosphorylation decreased, and caspase-3 was active (Syed et al. 2011a). Both high glucose and palmitate are effective activators of NADPH oxidase in β cells. Studies in human islets have provided similar results. Islets isolated from human type 2 diabetic donors show an increase in Rac expression, JNK1/2 activation, and caspase-3 degradation, results that were analogous to the ZDF model. Exposure of human islets to high glucose activated Rac (Syed et al. 2011a). Collectively, these data indicate that exposure of β cells to stimuli (high glucose, cytokines, FFAs) results in an NADPH oxidase-mediated activation of second messengers. Activation of these second messengers is associated with β cell dysfunction.

NOX-4 and Renal Signaling

Redox signaling involving NOX-4 activity is an important contributor to renal dysfunction. High glucose leads to an upregulation of NOX-4 and an associated increase in ROS generation in the type 2 diabetic-like mouse model db/db. Elevated

ROS results in phosphorylation of p38MAPK and increased expression of TGF-β1/2 and fibronectin (Sedeek et al. 2010). NOX-4 generated ROS in adipose cells inhibits PTP1B by blocking its catalytic activity and affecting insulin signaling (Mahadev et al. 2004).

Redox Signaling and Activators of Transcription

In terms of signaling mediators, several transcription factors are associated with NAPDH oxidase activation. Both NOX-1 and NOX-2 were shown to activate NF-κB in vascular smooth muscle cells and MCF-7 cells (human mammary epithelial cells). Generation of H_2O_2 by endosomal NOX-2 facilitates formation of an active TNF receptor 1 (TNFR1) complex which is required for NF-κB activation. These are redox-dependent events (Li et al. 2009). Similarly, in vascular smooth muscle cells, ligation of receptors for IL-1β or TNF-α triggers receptor-ligand internalization into an endosomal compartment containing NOX-1. The elevation in ROS that is mediated by NOX-1 results in NF-κB activity (Miller et al. 2007). AngII activates the transcription factors NF-κB and AP-1 in arterial smooth muscle cells. This redox signaling-mediated event is dependent on NOX-1 activity and leads to cell migration and proliferation (Valente et al. 2012).

Adenoviral expression of NOX-4 results in *GATA-4* gene transcription in pluripotent progenitor cells (Murray et al. 2013). Signaling is mediated via ROS generation and activation of c-Jun. NOX-4 is implicated in the regulation of Smad2/3 and differentiation of cardiac fibroblasts into myofibroblasts. TGF-β activates the transcription factors Smad2/3 (Cucoranu et al. 2005). The knockdown of NOX-4 with siRNA blocked TGF-β-stimulated Smad2/3, highlighting the key role of NOX-4 in this signaling pathway. These studies, described in cardiomyocytes, may have relevance to β cells. TGF-β signaling activates Smad3 in β cells, which regulates insulin gene transcription (Lin et al. 2009). Smad3-deficient mice developed moderate hyperinsulinemia and mild hypoglycemia (Lin et al. 2009).

The contribution of redox signaling to β cell function has likely been underappreciated. NADPH oxidase family members NOX-1, NOX-2, and NOX-4 play significant roles in elevating intracellular ROS and therefore regulating redox signaling events in the β cell. NOX enzymes clearly regulate signaling outcomes in non-β cell systems. Whether this influence on cell regulation by NADPH oxidase isotypes is cell type-specific or translates also to regulation of the β cell will be revealed with further study. The activation of specific kinases, phosphatases, and transcription factors can be dependent upon changes in intracellular ROS. Induction of these pathways has influence on major physiological and pathophysiological responses by the β cell. Mapping redox signaling responses in the β cell will help characterize the relative importance and therapeutic potential of redox signaling events in terms of β cell survival and preservation.

NOX Inhibition

Early identified inhibitors of NADPH oxidase activity have helped in defining the important contribution of NADPH oxidase enzymes to biological processes and disease. The resulting identification of pathophysiology associated with NADPH oxidase and elevated NADPH oxidase products has stimulated the search for improved inhibitors. In addition to enhanced efficacy, the focus has been to identify inhibitors that are selective to different NOX isoforms, thereby establishing the relative contribution of each NOX enzyme to disease processes and facilitating development of a therapeutic strategy to control, treat, or reverse the disease.

Historically accepted inhibitors of NADPH oxidase include apocynin and diphenyleneiodonium (DPI). Apocynin was first identified in the 1800s from plant root and was recognized and widely used as a NADPH oxidase inhibitor from the mid-1900s. Apocynin (4′-hydroxy-3′-methoxyacetophenone) also known as acetovanillone is a naturally occurring methoxy-substituted catechol. It is not considered selective for NOX subunits. Apocynin is a pro-drug, being converted to an active form by peroxidase. Marketed as an inhibitor of NADPH oxidase with limited adverse effects *in vivo*, apocynin is reported to exhibit off-target effects (Lafeber et al. 1999). Significantly, peroxide-deficient cells are sensitive to apocynin inhibition, and ROS production in non-phagocytes has been associated with apocynin treatment (Vejrazka et al. 2005). Apocynin demonstrated antioxidant effects in endothelial and vascular smooth muscle cells (Heumuller et al. 2008). Reported mechanisms of action for apocynin include a block in the membrane translocation of $p67^{phox}$ and $p47^{phox}$, sequestration of H_2O_2, and interaction of an apocynin radical with NOX thiol groups (Stolk et al. 1994; Johnson et al. 2002; Ximenes et al. 2007). Diphenyleneiodonium (DPI) is an inhibitor of flavoprotein dehydrogenases and has been extensively described in the research literature as an inhibitor of NADPH oxidase activity. DPI inhibits electron transporters in flavoenzymes. Thus, in addition to inhibition of NADPH oxidase, DPI also inhibits other flavin-dependent enzymes including nitric oxide synthase, xanthine oxidase, NADPH dehydrogenase, glucose phosphate dehydrogenase, mitochondrial complex I, and cytochrome *P*-450 reductase. Other nonspecific NOX inhibitors have been reported including AEBSF (pefabloc, 4-(2-aminoethyl)-benzenesulfonyl fluoride), which is an irreversible serine protease inhibitor and blocks complex assembly by inhibiting binding of $p47^{phox}$ (Diatchuk et al. 1997).

Discovery of endogenous peptide inhibitors of NOX revealed candidate peptide sequences that could inhibit enzyme complex assembly by blocking targetable interacting domains (Kleinberg et al. 1990; Nauseef et al. 1993; DeLeo et al. 1995; Uhlinger et al. 1995; Shi et al. 1996). Exploitation of peptide inhibition has prompted efforts to design peptides with selectivity to the NOX enzyme isoforms. Peptide construct gp91ds-tat combines a peptide sequence from the NOX-2 B-loop that interacts with the $p47^{phox}$ subunit and a nine amino acid ds-tat sequence. While designed to be specific, concern of inhibition of other NOX enzymes has been raised. This was driven by the sequence homology between NOX-2 and, to a lesser extent, NOX-4 (Jackson et al. 2010). Reconstitution studies

that express enzyme components in an endogenously devoid cell however support specificity for NOX-2 (Csanyi et al. 2011). This 18 amino acid peptide (which has later been termed NOX-2ds-tat) inhibited angiotensin II-mediated ROS in vascular smooth muscle cells and attenuated vascular superoxide and systolic blood pressure in mice (Rey et al. 2001; Yang et al. 2005). The concept of peptide-based inhibition of complex assembly remains attractive due to the ability to target specific regions offering the potential for specificity and fewer off-target effects. Reviewed by Dahan and Pick is a detailed consideration of parameters to consider in peptide-based inhibition of NOX (Dahan and Pick 2012). Other peptide regions of NOX-2 have been targeted along with peptide regions in $p22^{phox}$, $p47^{phox}$, and accessory molecules (Rotrosen et al. 1990; Nauseef et al. 1993; Dahan et al. 2002). While offering the prospect of specificity, peptide-based therapeutics are limited by poor oral bioavailability, being readily inactivated in the gastrointestinal tract. It is possible that this limitation could be overcome by alternative delivery routes, including injection, patches, or inhalation, as has been explored for insulin administration. Further, advances in nanoparticle encapsulation and gene therapy/DNA therapeutics could help facilitate delivery of peptide-based strategies.

Current limitations in bioavailability of peptide approaches to selectively inhibit NOX enzymes paired with the emerging realization of the therapeutic potential of inhibitors targeted to NOX isotypes have renewed investigation of small molecular weight compound inhibitors. In recent years, significant investment in high-throughput screening of small molecule libraries has resulted in new emergent inhibitors with promising selectivity profiles. Initial evidenced inhibitors of neutrophil NADPH oxidase were VAS2870 and its second generation relative VAS3947. Both arose from high-throughput screens. These compounds display micromolar potency for NOX inhibition and have widely reported efficacy in assays dependent on NOX-induced ROS. Unlike earlier compounds, these molecules offer selectivity to NADPH oxidase and do not have activity against other flavoproteins, e.g., xanthine oxidase (ten Freyhaus et al. 2006; Wind et al. 2010). Although screened against neutrophil NADPH oxidase (NOX-2), the efficacy of these compounds in a variety of assays suggests they are selective pan-NOX inhibitors and lack isoform selectivity for individual NOX enzymes. Other high-throughput screening approaches have used NOX isoform-specific assays. These approaches have identified new compounds that preferentially inhibit NOX isoforms. Screening for ROS production in a cell-free assay of human NOX-4 identified a series of first-in-class pyrazolopyridine dione inhibitors with nanomolar potency (Laleu et al. 2010). Subsequent enhancement in chemical structure led to identification of GKT136901 and GKT137831, orally active potent inhibitors. The pyrazolopyridine dione inhibitors, while screened for NOX-4, also exhibit equipotent activity for NOX-1 inhibition. The lead compound has demonstrated a high degree of potency in several in vitro and in vivo assays (Laleu et al. 2010). Further, GKT136901 is 20-fold less potent at inhibiting NOX-2 and thus can be considered as a selective dual NOX-1/NOX-4 inhibitor. As an alternative approach, a cell-based high-throughput screen of NOX-1 expressing colon cancer cell line identified a chemical hit in a distinct chemical class. Studies of the structure-activity

relationship of the trifluoromethyl-phenothiazine hit led to characterization of ML171 (2-acetylphenothiazine), a nanomolar inhibitor of NOX-1. Moreover, ML171 displays isoform selectivity for NOX-1 inhibition, having a potency for NOX-1 inhibition 20-fold lower than NOX-2, NOX-3, and NOX-4. Inhibition of NOX-1 by ML171 is evidenced in human colon cancer cells that only express NOX-1 plus NOXO1 and NOXA1. Overexpression of NOX-1 reverses ML171 inhibition supporting a selective inhibitory profile for ML171 (Gianni et al. 2010).

Identification of NADPH oxidase and ROS efficacy in pathways promoting pathogenesis in the β cell fosters the potential utility of selective NOX inhibition as a strategy to preserve β cell mass in diabetes. The progress of a range of new selective inhibitors, either small molecule, peptide, or peptidomimetic, that target NOX isoforms greatly facilitates the discovery of the role of NADPH oxidases in islet biology and diabetes. GKT136901 has been implicated in mitigation of oxidative stress associated in diabetic nephropathy, stroke, and neurodegeneration (Schildknecht et al. 2013). Our own unpublished data adds support since selective NOX-1 inhibition with ML171 confers β cells with protection in an inflammatory environment. Clearly an underlying concern, even with highly efficacious and selective inhibitors, will be off-target effects since NOX enzymes are expressed and function in several tissues. The prospect of functional redundancy for physiological actions of NADPH oxidases, and a general theme of overactive NAPDH oxidases in pathological conditions, offers encouragement for the therapeutic potential of selective NOX inhibitors. Studies in vivo with newer NOX inhibitors have been well tolerated (Laleu et al. 2010). Evolution of these investigations is expected to identify the following: (1) new investigative tools, (2) a better understanding of β cell vulnerability, and (3) original therapeutic strategies that can be applied to diabetes (Fig. 2).

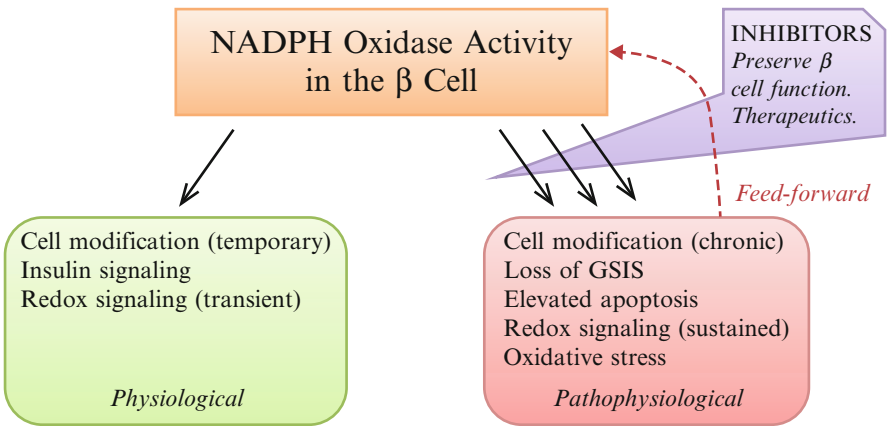

Fig. 2 NADPH oxidase (*NOX*) activity and β cell function. Physiologic responses involve transient NADPH oxidase activity and changes in ROS (*single arrow*). Chronic sustained NADPH oxidase activity in β cells (*multiple arrows*) is associated with pathophysiologic responses. This can be amplified with a feedforward regulation of NADPH oxidase activity. Inhibition of NOX could help protect islets and preserve β cell function

Conclusions

A major contributor to loss of functional β cell mass in diabetes is a sustained elevation in intracellular ROS. There are several sources of ROS in the β cell, including NADPH oxidase activity. In terms of β cell dysfunction, NADPH oxidases may have a greater role than previously appreciated. Upon chronic activation of NADPH oxidase activity and sustained elevation of intracellular ROS, β cell function and viability decreases. An increased appreciation for the role of NADPH oxidase activity in regulating β cell biology is driving the quest to understand the contribution of each NADPH oxidase isoform to β cell biology. The ability to selectively inhibit each isoform is a key goal. Selective inhibition of NOX enzymes may potentially reveal the factors and pathways needed to preserve a healthy β cell pool and offer new approaches in the treatment of diabetes.

Cross-References

- ▶ Current Approaches and Future Prospects for the Prevention of β-Cell Destruction in Autoimmune Diabetes
- ▶ Immunology of β-Cell Destruction
- ▶ Inflammatory Pathways Linked to β Cell Demise in Diabetes

References

Abo A, Pick E et al (1991) Activation of the NADPH oxidase involves the small GTP-binding protein p21rac1. Nature 353(6345):668–670

Adachi Y, Shibai Y et al (2008) Oncogenic Ras upregulates NADPH oxidase 1 gene expression through MEK-ERK-dependent phosphorylation of GATA-6. Oncogene 27(36):4921–4932

Ago T, Kitazono T et al (2004) Nox4 as the major catalytic component of an endothelial NAD(P)H oxidase. Circulation 109(2):227–233

Aguirre J, Rios-Momberg M et al (2005) Reactive oxygen species and development in microbial eukaryotes. Trends Microbiol 13(3):111–118

Allaoui A, Botteaux A et al (2009) Dual oxidases and hydrogen peroxide in a complex dialogue between host mucosae and bacteria. Trends Mol Med 15(12):571–579

Alves ES, Haidar AA et al (2012) Angiotensin II-induced JNK activation is mediated by NAD(P)H oxidase in isolated rat pancreatic islets. Regul Pept 175(1–3):1–6

Ando S, Kaibuchi K et al (1992) Post-translational processing of rac p21s is important both for their interaction with the GDP/GTP exchange proteins and for their activation of NADPH oxidase. J Biol Chem 267(36):25709–25713

Babior BM, Kipnes RS et al (1973) Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. J Clin Invest 52(3):741–744

Babior BM, Lambeth JD et al (2002) The neutrophil NADPH oxidase. Arch Biochem Biophys 397 (2):342–344

Banfi B, Molnar G et al (2001) A Ca^{2+}-activated NADPH oxidase in testis, spleen, and lymph nodes. J Biol Chem 276(40):37594–37601

Banfi B, Clark RA et al (2003) Two novel proteins activate superoxide generation by the NADPH oxidase NOX1. J Biol Chem 278(6):3510–3513

Banfi B, Malgrange B et al (2004a) NOX3, a superoxide-generating NADPH oxidase of the inner ear. J Biol Chem 279(44):46065–46072

Banfi B, Tirone F et al (2004b) Mechanism of Ca^{2+} activation of the NADPH oxidase 5 (NOX5). J Biol Chem 279(18):18583–18591

Bedard K, Krause KH (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 87(1):245–313

Bedard K, Lardy B et al (2007) NOX family NADPH oxidases: not just in mammals. Biochimie 89 (9):1107–1112

Bedard K, Jaquet V et al (2012) NOX5: from basic biology to signaling and disease. Free Radic Biol Med 52(4):725–734

BelAiba RS, Djordjevic T et al (2007) NOX5 variants are functionally active in endothelial cells. Free Radic Biol Med 42(4):446–459

Biberstine-Kinkade KJ, DeLeo FR et al (2001) Heme-ligating histidines in flavocytochrome b(558): identification of specific histidines in gp91(phox). J Biol Chem 276(33):31105–31112

Bleich D, Chen S et al (1999) Resistance to type 1 diabetes induction in 12-lipoxygenase knockout mice. J Clin Invest 103(10):1431–1436

Brar SS, Corbin Z et al (2003) NOX5 NAD(P)H oxidase regulates growth and apoptosis in DU 145 prostate cancer cells. Am J Physiol Cell Physiol 285(2):C353–C369

Brun S, Malagnac F et al (2009) Functions and regulation of the Nox family in the filamentous fungus Podospora anserina: a new role in cellulose degradation. Mol Microbiol 74(2):480–496

Cai W, He JC et al (2008) Oral glycotoxins determine the effects of calorie restriction on oxidant stress, age-related diseases, and lifespan. Am J Pathol 173(2):327–336

Caillou B, Dupuy C et al (2001) Expression of reduced nicotinamide adenine dinucleotide phosphate oxidase (ThoX, LNOX, Duox) genes and proteins in human thyroid tissues. J Clin Endocrinol Metab 86(7):3351–3358

Cano-Dominguez N, Alvarez-Delfin K et al (2008) NADPH oxidases NOX-1 and NOX-2 require the regulatory subunit NOR-1 to control cell differentiation and growth in Neurospora crassa. Eukaryot Cell 7(8):1352–1361

Cassatella MA, Bazzoni F et al (1990) Molecular basis of interferon-gamma and lipopolysaccharide enhancement of phagocyte respiratory burst capability. Studies on the gene expression of several NADPH oxidase components. J Biol Chem 265(33):20241–20246

Cave AC, Brewer AC et al (2006) NADPH oxidases in cardiovascular health and disease. Antioxid Redox Signal 8(5–6):691–728

Cheng G, Cao Z et al (2001) Homologs of gp91phox: cloning and tissue expression of Nox3, Nox4, and Nox5. Gene 269(1–2):131–140

Cheng G, Ritsick D et al (2004) Nox3 regulation by NOXO1, p47phox, and p67phox. J Biol Chem 279(33):34250–34255

Costal F, Oliveira E et al (2013) Dual effect of advanced glycation end products in pancreatic islet apoptosis. Diabetes Metab Res Rev 29(4):296–307

Coughlan MT, Yap FY et al (2011) Advanced glycation end products are direct modulators of β-cell function. Diabetes 60(10):2523–2532

Cross AR, Rae J et al (1995) Cytochrome b-245 of the neutrophil superoxide-generating system contains two nonidentical hemes. Potentiometric studies of a mutant form of gp91phox. J Biol Chem 270(29):17075–17077

Csanyi G, Cifuentes-Pagano E et al (2011) Nox2 B-loop peptide, Nox2ds, specifically inhibits the NADPH oxidase Nox2. Free Radic Biol Med 51(6):1116–1125

Cucoranu I, Clempus R et al (2005) NAD(P)H oxidase 4 mediates transforming growth factor-β1-induced differentiation of cardiac fibroblasts into myofibroblasts. Circ Res 97(9): 900–907

Dahan I, Pick E (2012) Strategies for identifying synthetic peptides to act as inhibitors of NADPH oxidases, or "all that you did and did not want to know about Nox inhibitory peptides". Cell Mol Life Sci 69(14):2283–2305

Dahan I, Issaeva I et al (2002) Mapping of functional domains in the p22(phox) subunit of flavocytochrome b(559) participating in the assembly of the NADPH oxidase complex by "peptide walking". J Biol Chem 277(10):8421–8432
de Carvalho DD, Sadok A et al (2008) Nox1 downstream of 12-lipoxygenase controls cell proliferation but not cell spreading of colon cancer cells. Int J Cancer 122(8):1757–1764
De Deken X, Wang D et al (2000) Cloning of two human thyroid cDNAs encoding new members of the NADPH oxidase family. J Biol Chem 275(30):23227–23233
DeLeo FR, Nauseef WM et al (1995) A domain of p47phox that interacts with human neutrophil flavocytochrome b558. J Biol Chem 270(44):26246–26251
Diatchuk V, Lotan O et al (1997) Inhibition of NADPH oxidase activation by 4-(2-aminoethyl)-benzenesulfonyl fluoride and related compounds. J Biol Chem 272(20):13292–13301
Dupuy C, Kaniewski J et al (1989) NADPH-dependent H2O2 generation catalyzed by thyroid plasma membranes. Studies with electron scavengers. Eur J Biochem 185(3):597–603
Dupuy C, Ohayon R et al (1999) Purification of a novel flavoprotein involved in the thyroid NADPH oxidase. Cloning of the porcine and human cdnas. J Biol Chem 274(52):37265–37269
Dworakowski R, Alom-Ruiz SP et al (2008) NADPH oxidase-derived reactive oxygen species in the regulation of endothelial phenotype. Pharmacol Rep 60(1):21–28
Edens WA, Sharling L et al (2001) Tyrosine cross-linking of extracellular matrix is catalyzed by Duox, a multidomain oxidase/peroxidase with homology to the phagocyte oxidase subunit gp91phox. J Cell Biol 154(4):879–891
Elsner M, Gehrmann W et al (2011) Peroxisome-generated hydrogen peroxide as important mediator of lipotoxicity in insulin-producing cells. Diabetes 60(1):200–208
Fan C, Katsuyama M et al (2005) Transactivation of the EGF receptor and a PI3 kinase-ATF-1 pathway is involved in the upregulation of NOX1, a catalytic subunit of NADPH oxidase. FEBS Lett 579(5):1301–1305
Finegold AA, Shatwell KP et al (1996) Intramembrane bis-heme motif for transmembrane electron transport conserved in a yeast iron reductase and the human NADPH oxidase. J Biol Chem 271 (49):31021–31024
Foreman J, Demidchik V et al (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 422(6930):442–446
Fransen M, Nordgren M et al (2012) Role of peroxisomes in ROS/RNS-metabolism: implications for human disease. Biochim Biophys Acta 1822(9):1363–1373
Fu X, Beer DG et al (2006) cAMP-response element-binding protein mediates acid-induced NADPH oxidase NOX5-S expression in Barrett esophageal adenocarcinoma cells. J Biol Chem 281(29):20368–20382
Gapper C, Dolan L (2006) Control of plant development by reactive oxygen species. Plant Physiol 141(2):341–345
Garrido AM, Griendling KK (2009) NADPH oxidases and angiotensin II receptor signaling. Mol Cell Endocrinol 302(2):148–158
Gattas MV, Forteza R et al (2009) Oxidative epithelial host defense is regulated by infectious and inflammatory stimuli. Free Radic Biol Med 47(10):1450–1458
Gavazzi G, Banfi B et al (2006) Decreased blood pressure in NOX1-deficient mice. FEBS Lett 580 (2):497–504
Gehrmann W, Elsner M et al (2010) Role of metabolically generated reactive oxygen species for lipotoxicity in pancreatic β-cells. Diabetes Obes Metab 12(Suppl 2):149–158
Geiszt M, Kopp JB et al (2000) Identification of renox, an NAD(P)H oxidase in kidney. Proc Natl Acad Sci U S A 97(14):8010–8014
Geiszt M, Lekstrom K et al (2003a) NAD(P)H oxidase 1, a product of differentiated colon epithelial cells, can partially replace glycoprotein 91phox in the regulated production of superoxide by phagocytes. J Immunol 171(1):299–306
Geiszt M, Witta J et al (2003b) Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defense. FASEB J 17(11):1502–1504

Gianni D, Bohl B et al (2008) The involvement of the tyrosine kinase c-Src in the regulation of reactive oxygen species generation mediated by NADPH oxidase-1. Mol Biol Cell 19 (7):2984–2994

Gianni D, Taulet N et al (2010) A novel and specific NADPH oxidase-1 (Nox1) small-molecule inhibitor blocks the formation of functional invadopodia in human colon cancer cells. ACS Chem Biol 5(10):981–993

Giannoni E, Buricchi F et al (2005) Intracellular reactive oxygen species activate Src tyrosine kinase during cell adhesion and anchorage-dependent cell growth. Mol Cell Biol 25(15):6391–6403

Go YM, Gipp JJ et al (2004) H2O2-dependent activation of GCLC-ARE4 reporter occurs by mitogen-activated protein kinase pathways without oxidation of cellular glutathione or thioredoxin-1. J Biol Chem 279(7):5837–5845

Goldstein BJ, Mahadev K et al (2005) Redox paradox: insulin action is facilitated by insulin-stimulated reactive oxygen species with multiple potential signaling targets. Diabetes 54 (2):311–321

Gorin Y, Ricono JM et al (2003) Nox4 mediates angiotensin II-induced activation of Akt/protein kinase B in mesangial cells. Am J Physiol Renal Physiol 285(2):F219–F229

Graciano MF, Santos LR et al (2011) NAD(P)H oxidase participates in the palmitate-induced superoxide production and insulin secretion by rat pancreatic islets. J Cell Physiol 226 (4):1110–1117

Grankvist K, Marklund SL et al (1981) CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase and glutathione peroxidase in pancreatic islets and other tissues in the mouse. Biochem J 199(2):393–398

Grasberger H, Refetoff S (2006) Identification of the maturation factor for dual oxidase. Evolution of an eukaryotic operon equivalent. J Biol Chem 281(27):18269–18272

Guichard C, Moreau R et al (2008) NOX family NADPH oxidases in liver and in pancreatic islets: a role in the metabolic syndrome and diabetes? Biochem Soc Trans 36 (Pt 5):920–929

Harper RW, Xu C et al (2005) Differential regulation of dual NADPH oxidases/peroxidases, Duox1 and Duox2, by Th1 and Th2 cytokines in respiratory tract epithelium. FEBS Lett 579 (21):4911–4917

Harper RW, Xu C et al (2006) Duox2 exhibits potent heme peroxidase activity in human respiratory tract epithelium. FEBS Lett 580(22):5150–5154

Heumuller S, Wind S et al (2008) Apocynin is not an inhibitor of vascular NADPH oxidases but an antioxidant. Hypertension 51(2):211–217

Heyworth PG, Bohl BP et al (1994) Rac translocates independently of the neutrophil NADPH oxidase components p47phox and p67phox. Evidence for its interaction with flavocytochrome b558. J Biol Chem 269(49):30749–30752

Heyworth PG, Cross AR et al (2003) Chronic granulomatous disease. Curr Opin Immunol 15 (5):578–584

Hofmann SM, Dong HJ et al (2002) Improved insulin sensitivity is associated with restricted intake of dietary glycoxidation products in the db/db mouse. Diabetes 51(7): 2082–2089

Hordijk PL (2006) Regulation of NADPH oxidases: the role of Rac proteins. Circ Res 98(4):453–462

Ibi M, Katsuyama M et al (2006) NOX1/NADPH oxidase negatively regulates nerve growth factor-induced neurite outgrowth. Free Radic Biol Med 40(10):1785–1795

Ibi M, Matsuno K et al (2008) Reactive oxygen species derived from NOX1/NADPH oxidase enhance inflammatory pain. J Neurosci 28(38):9486–9494

Imoto H, Sasaki N et al (2008) Impaired insulin secretion by diphenyleneiodium associated with perturbation of cytosolic Ca^{2+} dynamics in pancreatic β-cells. Endocrinology 149(11): 5391–5400

Jackson HM, Kawahara T et al (2010) Nox4 B-loop creates an interface between the transmembrane and dehydrogenase domains. J Biol Chem 285(14):10281–10290

Janciauskiene S, Ahren B (2000) Fibrillar islet amyloid polypeptide differentially affects oxidative mechanisms and lipoprotein uptake in correlation with cytotoxicity in two insulin-producing cell lines. Biochem Biophys Res Commun 267(2):619–625

Jay DB, Papaharalambus CA et al (2008) Nox5 mediates PDGF-induced proliferation in human aortic smooth muscle cells. Free Radic Biol Med 45(3):329–335

Jiao J, Dou L et al (2012) NADPH oxidase 2 plays a critical role in dysfunction and apoptosis of pancreatic β-cells induced by very low-density lipoprotein. Mol Cell Biochem 370(1–2): 103–113

Johnson DK, Schillinger KJ et al (2002) Inhibition of NADPH oxidase activation in endothelial cells by ortho-methoxy-substituted catechols. Endothelium 9(3):191–203

Juhasz A, Ge Y et al (2009) Expression of NADPH oxidase homologues and accessory genes in human cancer cell lines, tumours and adjacent normal tissues. Free Radic Res 43(6):523–532

Kamiguti AS, Serrander L et al (2005) Expression and activity of NOX5 in the circulating malignant B cells of hairy cell leukemia. J Immunol 175(12):8424–8430

Kaneto H, Fujii J et al (1996) Reducing sugars trigger oxidative modification and apoptosis in pancreatic β-cells by provoking oxidative stress through the glycation reaction. Biochem J 320 (Pt 3):855–863

Katsuyama M, Fan C et al (2002) NADPH oxidase is involved in prostaglandin F2α-induced hypertrophy of vascular smooth muscle cells: induction of NOX1 by PGF2α. J Biol Chem 277 (16):13438–13442

Kawahara T, Kohjima M et al (2005) Helicobacter pylori lipopolysaccharide activates Rac1 and transcription of NADPH oxidase Nox1 and its organizer NOXO1 in guinea pig gastric mucosal cells. Am J Physiol Cell Physiol 288(2):C450–C457

Kawamori D, Kajimoto Y et al (2003) Oxidative stress induces nucleo-cytoplasmic translocation of pancreatic transcription factor PDX-1 through activation of c-Jun NH(2)-terminal kinase. Diabetes 52(12):2896–2904

Kenyon V, Rai G et al (2011) Discovery of potent and selective inhibitors of human platelet-type 12- lipoxygenase. J Med Chem 54(15):5485–5497

Kleinberg ME, Malech HL et al (1990) The phagocyte 47-kilodalton cytosolic oxidase protein is an early reactant in activation of the respiratory burst. J Biol Chem 265 (26):15577–15583

Koya D, King GL (1998) Protein kinase C activation and the development of diabetic complications. Diabetes 47(6):859–866

Krieger-Brauer HI, Kather H (1995) Antagonistic effects of different members of the fibroblast and platelet-derived growth factor families on adipose conversion and NADPH-dependent H2O2 generation in 3 T3 L1-cells. Biochem J 307(Pt 2):549–556

Kumar B, Koul S et al (2008) Oxidative stress is inherent in prostate cancer cells and is required for aggressive phenotype. Cancer Res 68(6):1777–1785

Kuroda J, Ago T et al (2010) NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart. Proc Natl Acad Sci U S A 107(35):15565–15570

Kusumoto K, Kawahara T et al (2005) Ecabet sodium inhibits Helicobacter pylori lipopolysaccharide-induced activation of NADPH oxidase 1 or apoptosis of guinea pig gastric mucosal cells. Am J Physiol Gastrointest Liver Physiol 288(2):G300–G307

Lafeber FP, Beukelman CJ et al (1999) Apocynin, a plant-derived, cartilage-saving drug, might be useful in the treatment of rheumatoid arthritis. Rheumatology 38(11):1088–1093

Laleu B, Gaggini F et al (2010) First in class, potent, and orally bioavailable NADPH oxidase isoform 4 (Nox4) inhibitors for the treatment of idiopathic pulmonary fibrosis. J Med Chem 53 (21):7715–7730

Lambeth JD (2007) Nox enzymes, ROS, and chronic disease: an example of antagonistic pleiotropy. Free Radic Biol Med 43(3):332–347

Lambeth JD, Kawahara T et al (2007) Regulation of Nox and Duox enzymatic activity and expression. Free Radic Biol Med 43(3):319–331

Lara-Ortiz T, Riveros-Rosas H et al (2003) Reactive oxygen species generated by microbial NADPH oxidase NoxA regulate sexual development in *Aspergillus nidulans*. Mol Microbiol 50(4):1241–1255

Lassegue B, Sorescu D et al (2001) Novel gp91(phox) homologues in vascular smooth muscle cells : nox1 mediates angiotensin II-induced superoxide formation and redox-sensitive signaling pathways. Circ Res 88(9):888–894

Lenzen S (2008) Oxidative stress: the vulnerable β-cell. Biochem Soc Trans 36(Pt 3):343–347

Lenzen S, Drinkgern J et al (1996) Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. Free Radic Biol Med 20(3):463–466

Li J, Stouffs M et al (2006) The NADPH oxidase NOX4 drives cardiac differentiation: role in regulating cardiac transcription factors and MAP kinase activation. Mol Biol Cell 17 (9):3978–3988

Li Q, Spencer NY et al (2009) Endosomal Nox2 facilitates redox-dependent induction of NF-kappaB by TNF-α. Antioxid Redox Signal 11(6):1249–1263

Li N, Li B et al (2012) NADPH oxidase NOX2 defines a new antagonistic role for reactive oxygen species and cAMP/PKA in the regulation of insulin secretion. Diabetes 61(11):2842–2850

Lim M, Park L et al (2008) Induction of apoptosis of β cells of the pancreas by advanced glycation end-products, important mediators of chronic complications of diabetes mellitus. Ann N Y Acad Sci 1150:311–315

Lin HM, Lee JH et al (2009) Transforming growth factor-β/Smad3 signaling regulates insulin gene transcription and pancreatic islet β-cell function. J Biol Chem 284(18):12246–12257

Lin N, Zhang H et al (2012) Advanced glycation end-products induce injury to pancreatic β cells through oxidative stress. Diabetes Metab 38(3):250–257

Lupi R, Del Guerra S et al (2006) The direct effects of the angiotensin-converting enzyme inhibitors, zofenoprilat and enalaprilat, on isolated human pancreatic islets. Eur J Endocrinol 154(2):355–361

Mahadev K, Motoshima H et al (2004) The NAD(P)H oxidase homolog Nox4 modulates insulin-stimulated generation of H2O2 and plays an integral role in insulin signal transduction. Mol Cell Biol 24(5):1844–1854

Malagnac F, Lalucque H et al (2004) Two NADPH oxidase isoforms are required for sexual reproduction and ascospore germination in the filamentous fungus Podospora anserina. Fungal Genet Biol 41(11):982–997

Manea A (2010) NADPH oxidase-derived reactive oxygen species: involvement in vascular physiology and pathology. Cell Tissue Res 342(3):325–339

Manea A, Manea SA et al (2012) Positive regulation of NADPH oxidase 5 by proinflammatory-related mechanisms in human aortic smooth muscle cells. Free Radic Biol Med 52(9):1497–1507

Marino D, Dunand C et al (2012) A burst of plant NADPH oxidases. Trends Plant Sci 17(1):9–15

Martyn KD, Frederick LM et al (2006) Functional analysis of Nox4 reveals unique characteristics compared to other NADPH oxidases. Cell Signal 18(1):69–82

Marzban L, Verchere CB (2004) The role of islet amyloid polypeptide in type 2 diabetes. Can J Diabetes 28(4):39–47

Matti A, Kyathanahalli C et al (2012) Protein farnesylation is requisite for mitochondrial fuel-induced insulin release: further evidence to link reactive oxygen species generation to insulin secretion in pancreatic β-cells. Islets 4(1):74–77

McDuffie M, Maybee NA et al (2008) Nonobese diabetic (NOD) mice congenic for a targeted deletion of 12/15-lipoxygenase are protected from autoimmune diabetes. Diabetes 57 (1):199–208

Meng D, Lv DD et al (2008) Insulin-like growth factor-I induces reactive oxygen species production and cell migration through Nox4 and Rac1 in vascular smooth muscle cells. Cardiovasc Res 80(2):299–308

Michalska M, Wolf G et al (2010) Effects of pharmacological inhibition of NADPH oxidase or iNOS on pro-inflammatory cytokine, palmitic acid or H2O2-induced mouse islet or clonal pancreatic β-cell dysfunction. Biosci Rep 30(6):445–453

Miller FJ Jr, Filali M et al (2007) Cytokine activation of nuclear factor kappa B in vascular smooth muscle cells requires signaling endosomes containing Nox1 and ClC-3. Circ Res 101(7):663–671
Mittler R, Vanderauwera S et al (2011) ROS signaling: the new wave? Trends Plant Sci 16(6):300–309
Miyano K, Ueno N et al (2006) Direct involvement of the small GTPase Rac in activation of the superoxide-producing NADPH oxidase Nox1. J Biol Chem 281(31):21857–21868
Modak MA, Datar SP et al (2007) Differential susceptibility of chick and mouse islets to streptozotocin and its co-relation with islet antioxidant status. J Comp Physiol B 177 (2):247–257
Mohammed AM, Kowluru A (2013) Activation of apocynin-sensitive NADPH oxidase (Nox2) activity in INS-1 832/13 cells under glucotoxic conditions. Islets 5(3):129–131
Montezano AC, Burger D et al (2010) Nicotinamide adenine dinucleotide phosphate reduced oxidase 5 (Nox5) regulation by angiotensin II and endothelin-1 is mediated via calcium/calmodulin-dependent, rac-1-independent pathways in human endothelial cells. Circ Res 106 (8):1363–1373
Montezano AC, Burger D et al (2011) Novel Nox homologues in the vasculature: focusing on Nox4 and Nox5. Clin Sci 120(4):131–141
Morand S, Dos Santos OF et al (2003) Identification of a truncated dual oxidase 2 (DUOX2) messenger ribonucleic acid (mRNA) in two rat thyroid cell lines. Insulin and forskolin regulation of DUOX2 mRNA levels in FRTL-5 cells and porcine thyrocytes. Endocrinology 144(2):567–574
Morand S, Ueyama T et al (2009) Duox maturation factors form cell surface complexes with Duox affecting the specificity of reactive oxygen species generation. FASEB J 23(4):1205–1218
Morgan D, Oliveira-Emilio HR et al (2007) Glucose, palmitate and pro-inflammatory cytokines modulate production and activity of a phagocyte-like NADPH oxidase in rat pancreatic islets and a clonal β cell line. Diabetologia 50(2):359–369
Morgan D, Rebelato E et al (2009) Association of NAD(P)H oxidase with glucose-induced insulin secretion by pancreatic β-cells. Endocrinology 150(5):2197–2201
Murray TV, Smyrnias I et al (2013) NADPH oxidase 4 regulates cardiomyocyte differentiation via redox activation of c-Jun protein and the cis-regulation of GATA-4 gene transcription. J Biol Chem 288(22):15745–15759
Nakayama M, Inoguchi T et al (2005) Increased expression of NAD(P)H oxidase in islets of animal models of Type 2 diabetes and its improvement by an AT1 receptor antagonist. Biochem Biophys Res Commun 332(4):927–933
Nauseef WM, McCormick S et al (1993) Functional domain in an arginine-rich carboxyl-terminal region of p47phox. J Biol Chem 268(31):23646–23651
Newsholme P, Morgan D et al (2009) Insights into the critical role of NADPH oxidase(s) in the normal and dysregulated pancreatic β cell. Diabetologia 52(12):2489–2498
Nisimoto Y, Motalebi S et al (1999) The p67(phox) activation domain regulates electron flow from NADPH to flavin in flavocytochrome b(558). J Biol Chem 274(33):22999–23005
Nisimoto Y, Tsubouchi R et al (2008) Activation of NADPH oxidase 1 in tumour colon epithelial cells. Biochem J 415(1):57–65
Ohneda K, Mirmira RG et al (2000) The homeodomain of PDX-1 mediates multiple protein-protein interactions in the formation of a transcriptional activation complex on the insulin promoter. Mol Cell Biol 20(3):900–911
Oliveira HR, Verlengia R et al (2003) Pancreatic β-cells express phagocyte-like NAD(P)H oxidase. Diabetes 52(6):1457–1463
Orient A, Donko A et al (2007) Novel sources of reactive oxygen species in the human body. Nephrol Dial Transplant 22(5):1281–1288
Paffenholz R, Bergstrom RA et al (2004) Vestibular defects in head-tilt mice result from mutations in Nox3, encoding an NADPH oxidase. Genes Dev 18(5):486–491
Pandey D, Patel A et al (2012) Expression and functional significance of NADPH oxidase 5 (Nox5) and its splice variants in human blood vessels. Am J Physiol Heart Circ Physiol 302(10):H1919–H1928

Pedruzzi E, Guichard C et al (2004) NAD(P)H oxidase Nox-4 mediates 7-ketocholesterol-induced endoplasmic reticulum stress and apoptosis in human aortic smooth muscle cells. Mol Cell Biol 24(24):10703–10717

Pendyala S, Gorshkova IA et al (2009) Role of Nox4 and Nox2 in hyperoxia-induced reactive oxygen species generation and migration of human lung endothelial cells. Antioxid Redox Signal 11(4):747–764

Peppa M, He C et al (2003) Fetal or neonatal low-glycotoxin environment prevents autoimmune diabetes in NOD mice. Diabetes 52(6):1441–1448

Pi J, Bai Y et al (2007) Reactive oxygen species as a signal in glucose-stimulated insulin secretion. Diabetes 56(7):1783–1791

Purves T, Middlemas A et al (2001) A role for mitogen-activated protein kinases in the etiology of diabetic neuropathy. FASEB J 15(13):2508–2514

Rada B, Hably C et al (2008) Role of Nox2 in elimination of microorganisms. Semin Immunopathol 30(3):237–253

Rebelato E, Mares-Guia TR et al (2012) Expression of NADPH oxidase in human pancreatic islets. Life Sci 91(7–8):244–249

Rey FE, Cifuentes ME et al (2001) Novel competitive inhibitor of NAD(P)H oxidase assembly attenuates vascular O(2)(−) and systolic blood pressure in mice. Circ Res 89(5):408–414

Rigutto S, Hoste C et al (2009) Activation of dual oxidases Duox1 and Duox2: differential regulation mediated by camp-dependent protein kinase and protein kinase C-dependent phosphorylation. J Biol Chem 284(11):6725–6734

Ris-Stalpers C (2006) Physiology and pathophysiology of the DUOXes. Antioxid Redox Signal 8 (9–10):1563–1572

Rokutan K, Kawahara T et al (2006) NADPH oxidases in the gastrointestinal tract: a potential role of Nox1 in innate immune response and carcinogenesis. Antioxid Redox Signal 8(9–10):1573–1582

Rokutan K, Kawahara T et al (2008) Nox enzymes and oxidative stress in the immunopathology of the gastrointestinal tract. Semin Immunopathol 30(3):315–327

Rotrosen D, Kleinberg ME et al (1990) Evidence for a functional cytoplasmic domain of phagocyte oxidase cytochrome b558. J Biol Chem 265(15):8745–8750

Rotrosen D, Yeung CL et al (1992) Cytochrome b558: the flavin-binding component of the phagocyte NADPH oxidase. Science 256(5062):1459–1462

Sadok A, Bourgarel-Rey V et al (2008) Nox1-dependent superoxide production controls colon adenocarcinoma cell migration. Biochim Biophys Acta 1783(1):23–33

Sagi M, Fluhr R (2006) Production of reactive oxygen species by plant NADPH oxidases. Plant Physiol 141(2):336–340

San Martin A, Foncea R et al (2007) Nox1-based NADPH oxidase-derived superoxide is required for VSMC activation by advanced glycation end-products. Free Radic Biol Med 42(11):1671–1679

Sancho P, Fabregat I (2010) NADPH oxidase NOX1 controls autocrine growth of liver tumor cells through up-regulation of the epidermal growth factor receptor pathway. J Biol Chem 285 (32):24815–24824

Santos CX, Tanaka LY et al (2009) Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase. Antioxid Redox Signal 11(10):2409–2427

Schildknecht S, Weber A et al (2013) The NOX1/4 inhibitor GKT136901 as selective and direct scavenger of peroxynitrite. Curr Med Chem 21:365–376

Schroder K, Wandzioch K et al (2009) Nox4 acts as a switch between differentiation and proliferation in preadipocytes. Arterioscler Thromb Vasc Biol 29(2):239–245

Schulz E, Munzel T (2008) NOX5, a new "radical" player in human atherosclerosis? J Am Coll Cardiol 52(22):1810–1812

Sedeek M, Callera G et al (2010) Critical role of Nox4-based NADPH oxidase in glucose-induced oxidative stress in the kidney: implications in type 2 diabetic nephropathy. Am J Physiol Renal Physiol 299(6):F1348–F1358

Selemidis S, Sobey CG et al (2008) NADPH oxidases in the vasculature: molecular features, roles in disease and pharmacological inhibition. Pharmacol Ther 120(3):254–291
Serrander L, Cartier L et al (2007) NOX4 activity is determined by mRNA levels and reveals a unique pattern of ROS generation. Biochem J 406(1):105–114
Seshiah PN, Weber DS et al (2002) Angiotensin II stimulation of NAD(P)H oxidase activity: upstream mediators. Circ Res 91(5):406–413
Shao J, Iwashita N et al (2006) Beneficial effects of candesartan, an angiotensin II type 1 receptor blocker, on β-cell function and morphology in db/db mice. Biochem Biophys Res Commun 344(4):1224–1233
Shi J, Ross CR et al (1996) PR-39, a proline-rich antibacterial peptide that inhibits phagocyte NADPH oxidase activity by binding to Src homology 3 domains of p47 phox. Proc Natl Acad Sci U S A 93(12):6014–6018
Shiose A, Kuroda J et al (2001) A novel superoxide-producing NAD(P)H oxidase in kidney. J Biol Chem 276(2):1417–1423
Stolk J, Hiltermann TJ et al (1994) Characteristics of the inhibition of NADPH oxidase activation in neutrophils by apocynin, a methoxy-substituted catechol. Am J Respir Cell Mol Biol 11 (1):95–102
Sturrock A, Cahill B et al (2006) Transforming growth factor-β1 induces Nox4 NAD(P)H oxidase and reactive oxygen species-dependent proliferation in human pulmonary artery smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 290(4):L661–L673
Sturrock A, Hueckstead TP et al (2007) Nox4 mediates TGF-β1-induced retinoblastoma protein phosphorylation, proliferation, and hypertrophy in human airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 292(6):L1543–L1555
Suh YA, Arnold RS et al (1999) Cell transformation by the superoxide-generating oxidase Mox1. Nature 401(6748):79–82
Sumimoto H (2008) Structure, regulation and evolution of Nox-family NADPH oxidases that produce reactive oxygen species. FEBS J 275(13):3249–3277
Syed I, Kyathanahalli CN et al (2011a) Increased phagocyte-like NADPH oxidase and ROS generation in type 2 diabetic ZDF rat and human islets: role of Rac1-JNK1/2 signaling pathway in mitochondrial dysregulation in the diabetic islet. Diabetes 60(11):2843–2852
Syed I, Kyathanahalli CN et al (2011b) Phagocyte-like NADPH oxidase generates ROS in INS 832/13 cells and rat islets: role of protein prenylation. Am J Physiol Regul Integr Comp Physiol 300(3):R756–R762
Takeya R, Ueno N et al (2003) Novel human homologues of p47phox and p67phox participate in activation of superoxide-producing NADPH oxidases. J Biol Chem 278(27):25234–25246
Taylor-Fishwick DA, Pittenger GL et al (2008) Transplantation and beyond. Drug Dev Res 69:165–176
ten Freyhaus H, Huntgeburth M et al (2006) Novel Nox inhibitor VAS2870 attenuates PDGF-dependent smooth muscle cell chemotaxis, but not proliferation. Cardiovasc Res 71(2):331–341
Tiedge M, Lortz S et al (1997) Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes 46(11):1733–1742
Torres MA, Dangl JL (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. Curr Opin Plant Biol 8(4):397–403
Touyz RM, Chen X et al (2002) Expression of a functionally active gp91phox-containing neutrophil-type NAD(P)H oxidase in smooth muscle cells from human resistance arteries: regulation by angiotensin II. Circ Res 90(11):1205–1213
Tudzynski P, Heller J et al (2012) Reactive oxygen species generation in fungal development and pathogenesis. Curr Opin Microbiol 15(6):653–659
Uchizono Y, Takeya R et al (2006) Expression of isoforms of NADPH oxidase components in rat pancreatic islets. Life Sci 80(2):133–139
Ueyama T, Geiszt M et al (2006) Involvement of Rac1 in activation of multicomponent Nox1- and Nox3-based NADPH oxidases. Mol Cell Biol 26(6):2160–2174

Uhlinger DJ, Tyagi SR et al (1995) On the mechanism of inhibition of the neutrophil respiratory burst oxidase by a peptide from the C-terminus of the large subunit of cytochrome b558. Biochemistry 34(2):524–527

Ushio-Fukai M (2006) Redox signaling in angiogenesis: role of NADPH oxidase. Cardiovasc Res 71(2):226–235

Valente AJ, Yoshida T et al (2012) Angiotensin II enhances AT1-Nox1 binding and stimulates arterial smooth muscle cell migration and proliferation through AT1, Nox1, and interleukin-18. Am J Physiol Heart Circ Physiol 303(3):H282–H296

Vaquero EC, Edderkaoui M et al (2004) Reactive oxygen species produced by NAD(P)H oxidase inhibit apoptosis in pancreatic cancer cells. J Biol Chem 279(33):34643–34654

Vejrazka M, Micek R et al (2005) Apocynin inhibits NADPH oxidase in phagocytes but stimulates ROS production in non-phagocytic cells. Biochim Biophys Acta 1722(2):143–147

Volchuk A, Ron D (2010) The endoplasmic reticulum stress response in the pancreatic β-cell. Diabetes Obes Metab 12(Suppl 2):48–57

Weaver JR, Taylor-Fishwick DA (2013) Regulation of NOX-1 expression in β cells: a positive feedback loop involving the Src-kinase signaling pathway. Mol Cell Endocrinol 369 (1–2):35–41

Weaver JR, Holman TR et al (2012) Integration of pro-inflammatory cytokines, 12-lipoxygenase and NOX-1 in pancreatic islet β cell dysfunction. Mol Cell Endocrinol 358(1):88–95

Wientjes FB, Hsuan JJ et al (1993) p40phox, a third cytosolic component of the activation complex of the NADPH oxidase to contain src homology 3 domains. Biochem J 296(Pt 3):557–561

Wind S, Beuerlein K et al (2010) Comparative pharmacology of chemically distinct NADPH oxidase inhibitors. Br J Pharmacol 161(4):885–898

Wingler K, Wunsch S et al (2001) Upregulation of the vascular NAD(P)H-oxidase isoforms Nox1 and Nox4 by the renin-angiotensin system in vitro and in vivo. Free Radic Biol Med 31 (11):1456–1464

Xiang FL, Lu X et al (2010) NOX2 deficiency protects against streptozotocin-induced β-cell destruction and development of diabetes in mice. Diabetes 59(10):2603–2611

Ximenes VF, Kanegae MP et al (2007) The oxidation of apocynin catalyzed by myeloperoxidase: proposal for NADPH oxidase inhibition. Arch Biochem Biophys 457(2):134–141

Yang M, Foster E et al (2005) Insulin-stimulated NAD(P)H oxidase activity increases migration of cultured vascular smooth muscle cells. Am J Hypertens 18(10):1329–1334

Yuan H, Lu Y et al (2010a) Suppression of NADPH oxidase 2 substantially restores glucose-induced dysfunction of pancreatic NIT-1 cells. FEBS J 277(24):5061–5071

Yuan H, Zhang X et al (2010b) NADPH oxidase 2-derived reactive oxygen species mediate FFAs-induced dysfunction and apoptosis of β-cells via JNK, p38 MAPK and p53 pathways. PLoS One 5(12):e15726

Zhang X, Shan P et al (2006) Toll-like receptor 4 deficiency causes pulmonary emphysema. J Clin Invest 116(11):3050–3059

Zhao Z, Zhao C et al (2009) Advanced glycation end products inhibit glucose-stimulated insulin secretion through nitric oxide-dependent inhibition of cytochrome c oxidase and adenosine triphosphate synthesis. Endocrinology 150(6):2569–2576

The Contribution of Reg Family Proteins to Cell Growth and Survival in Pancreatic Islets

35

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Contents

Abstract

In 2008, we have reviewed Reg family proteins which have been found and characterized in several systems including cell growth and regeneration in the pancreas. Since then the research scope has expanded significantly to the (patho-)physiology of the liver, intestine, immunity, and cancer. More importantly, in communicating our research findings, we feel the need of further classification in the family of seven independent genes and among key species. A more

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_47,

uniformed terminology should help us to understand their isoform-specific functions and/or mode of activation.

Keywords
INGAP • Reg1, Reg2, Reg3, Reg4 • Orthology • EXTL3/EXTR1 • Regeneration • Transdifferentiation

Abbreviations

HIP	Gene expressed in hepatocellular carcinoma-intestine-pancreas
PAP	Pancreatitis-associated protein
PSP	Pancreatic stone protein
PTP	Pancreatic thread protein
RELP	Regenerating protein-like protein

Introduction

The mammalian pancreas is composed of three main cell types: the exocrine acini, endocrine islets, and ducts. The endocrine islets constitute about 5 % of the volume and consist of α, β, δ, ε, and PP cells that produce glucagon, insulin, somatostatin, ghrelin, and pancreatic polypeptide, respectively (Gu et al. 2003; Harbeck et al. 1996). Insulin and glucagon are two hormones with opposing roles working together to maintain the balance of glucose storage and utilization (Moses et al. 1996). Somatostatin and pancreatic polypeptide exert inhibitory effects on both pancreatic endocrine and exocrine secretions (D'Ercole 1999; Bonner-Weir 2000a). Ghrelin regulates insulin secretion and expression of genes essential for β-cell biology, promotes β-cell proliferation and survival, and inhibits β-cell apoptosis (Bonner-Weir 2000b). The exocrine cells that are organized into acini constitute about 85 % of the pancreas. They secrete digestive enzymes, such as amylase, elastase, trypsinogen, into the pancreatic ducts, a branched network of tubules formed by epithelial duct cells. These enzymes, along with bicarbonate and other electrolytes secreted by ductal cells, constitute the pancreas juice and are drained into the duodenum through the main duct (Gu et al. 2003). Also, increasing interest has been placed on the interactions between the exocrine and endocrine portions of pancreas in the structures and functions.

In both normal and pathophysiological states, β-cell mass is determined by changes in the rate of replication and neogenesis, individual cell volume, and cell death rate (Dheen et al. 1997). High rate β-cell replication has been observed in the embryos of late gestation and in newborns. A similar increase in the activity of cellular apoptosis also occurs during the newborn and postpartum period in mother. The dynamic changes in replication and apoptosis may contribute to the remodeling of β-cell mass during these periods (Dheen et al. 1997; Zhou et al. 2000; Cheng et al. 2000; Mauras et al. 2000). The rates of replication and apoptosis of β-cells are both reduced significantly beyond 3 months of life and remain low except in response to physiological/pathological changes. Low-rate β-cell replication lasts

throughout the lifespan, which is closely correlated to the body weight increment. In young ages, increases of both β-cell size and number contribute to the increase of β-cell mass, but in old animals the increase of β-cell size is mainly responsible for the increase in mass (Mauras et al. 2000). For in-depth discussion on apoptosis, please refer to the chapter entitled "► Mechanisms of Pancreatic β-Cell Apoptosis in Diabetes and Its Therapies."

A common pathology of diabetes is the loss of functional β-cells. Pancreatic islet regeneration is now an attractive alternative for diabetes cell therapy. There are three major efforts to achieve this goal:

1. To promote the replication and reverse the destruction of existing β-cells. Increasing interest has been focused on the initiation of growth and the role of proliferation factors in pancreatic islet, such as insulin-like growth factor (IGF)-I, hepatocyte growth factor (HGF), and glucagon-like peptide (GLP)-1 (Suarez-Pinzon et al. 2008).
2. To directly differentiate stem cells or pancreatic progenitor cells into β-cells. It is generally accepted that pancreatic ducts and ductular progenitor cells can differentiate to β-cells, as evidenced by the observation of islet budding from ductal structures during embryogenesis or postnatal growth (Scharfmann et al. 1989). Although replication is the major source of β-cell renewal, around 30 % of new β-cells can arise from neogenesis from non-β-cell precursors in adult rats (Bonner-Weir et al. 2004). A differentiation process that converts human embryonic stem cells (ESCs) to endocrine cells has been developed by using transcriptional factors at different stages (D'Amour et al. 2006).
3. To transdifferentiat from other endogenous pancreatic cells. Cells in the periphery of islets in the neonatal pancreas strongly express ductal markers CK19 and CK20 and may serve as islet progenitors (Bouwens et al. 1994). Shortly after birth, CK19 expression expands to the whole pancreas, which is turned off in differentiated islet cells (Billestrup and Nielsen 1991). This transient expression of CK19 suggests that new islets may arise from ductal tissues. Neogenesis of β-cells was also observed in response to various stresses, including 90 % pancreatectomy (Terazono et al. 1988) and partial obstruction of the pancreas (Sieradzki et al. 1988).

Recent studies also showed that adult ductal and acinar cells could be dedifferentiated into a progenitor state and then re-differentiated into β-like cells using a series of transcriptional factors, including Pdx1, neurogenin (Ngn)3, and Maf A (Liu et al. 2009). Transfection of Ngn3 into pancreatic ductal cells can also induce islet neogenesis (Xu et al. 2008). What should be pointed out is that there is a distinction between the terms islet regeneration and neogenesis. "Islet regeneration describes an increase in β-cell mass in general, regardless of the mechanism, while islet neogenesis refers specifically to an increase in β-cell mass via the transdifferentiation of adult pancreatic stem cells, putatively found in the ductal epithelium or acinar tissue, into functioning, physiologically regulated tissue" (Pittenger et al. 2009a). For additional consideration, please refer to the chapter entitled "► Stem Cells in Pancreatic Islets."

A number of growth factors have been reported to promote β-cell expansion in animal models, including IGF-I (George et al. 2002; Smith et al. 1991), gastrin

(Hansson et al. 1996; Hansson and Thoren 1995), transforming growth factor (TGF)-α (Song et al. 1999; Wang et al. 1993; Sandgren et al. 1990), GLP-1 (Pospisilik et al. 2003), exendin-4 (Gedulin et al. 2005; Xu et al. 1999; Tourrel et al. 2001; Xu et al. 2006), and Reg family proteins Reg1 (Terazono et al. 1990, 1988) and INGAP (Reg3δ) (Rosenberg et al. 2004). Transgenic mice with β-cell-specific overexpression of IGF-I displayed increased β-cell mass in parallel with a higher rate of neogenesis and β-cell replication, hence better recovery from the hyperglycemia and hypoinsulinemia induced by streptozotocin, compared to control animals (George et al. 2002). In the pancreatic regeneration model stimulated by duct ligation, gastrin expression was strongly induced in the ligated part at both mRNA and protein levels, shortly after surgery (Wang et al. 1997). Gastrin alone, or in combination with epidermal growth factor (EGF), increased the expression of Pdx1 and insulin in isolated CK19-positive human ductal cells (Hansson et al. 1996). Overexpression of TGF-α upregulates the Pdx1-expressing epithelium characterized by the expression of Pax6 and initiates islet neogenesis (Song et al. 1999). Mice that overexpress both gastrin and TGF-α showed significant increase in islet cell mass, suggesting a synergistic effect of the two factors on stimulating islet cell growth (Wang et al. 1993). Exendin-4, an agonist of GLP-1 with a longer half-life, facilitates β-cell neogenesis in rat and human pancreatic ducts (Xu et al. 2006). GLP-1 or exendin-4 treatment increased pancreatic insulin content and β-cell mass and decreased basal plasma glucose in streptozotocin-treated neonatal rats in both short and long terms (Tourrel et al. 2001). This chapter will focus on the contribution of Reg family proteins to cell growth and survival in pancreatic islets.

An Overview of the Regenerating Gene Family

Reg and Reg-related genes constitute a family within the C-type lectin superfamily (Lasserre et al. 1994; Chakraborty et al. 1995; Hartupee et al. 2001). In the last two decades, over 29 Reg genes have been discovered in several different species (Table 1). These secretory proteins share structural and functional properties associated with tissue injury, inflammation, diabetes, carcinogenesis, and cell proliferation or differentiation in the pancreas, liver, neurons, and gastrointestinal tract (Dusetti et al. 1994; Christa et al. 1996; Hill et al. 1999; He et al. 2010; Nishimune et al. 2000). Ever since Reg1 was discovered, special attention has been paid to the therapeutic potential of Reg proteins for the regeneration of pancreatic islets and treatment of diabetes (Okamoto 1999).

Classification of Reg Proteins Based on Protein Sequence

In the mouse, seven unique Reg genes have been discovered, all of which are located on chromosome 6C except Reg4. Consequently, five Reg genes in rat and

Table 1 ***Members of the Reg family proteins in the mouse, rat, and human.*** Literatures have used a few other names, such as HIP, gene expressed in hepatocellular carcinoma-intestine-pancreas; PAP, pancreatitis-associated protein; PSP, pancreatic stone protein; PTP, pancreatic thread protein; and RELP, regenerating protein-like protein. Reg stands for regenerating islet derived. The data is mostly based on NCBI collections. Based on the degree of sequence identity, mouse Reg1 seems to correspond to two human genes, Reg1A and Reg1B; and Reg3α and Reg3β to two unique rat genes and two human genes, respectively. When identical proteins were repeatedly submitted to Genbank, NCBI, or UniProtKB/Swiss-Prot, "=" is used to list identical protein IDs and orthologies in a single cell

Mouse genes	Orthology	Transcript	Polypeptide
Reg1	Reg, PTP, PSP, lithostathine	NM_009042	NP_033068 =P43137
	Rat Reg1/1α (Bimmler et al. 1999; Rouquier et al. 1991)	NM_012641	NP_036773 (Terazono et al. 1988; Li et al. 2013a) =P10758
	Human Reg1A, PSP, PTP, lithostathine	NM_002909	NP_002900 (Fujishiro et al. 2012; Rouimi et al. 1988) =P05451
	Human Reg1B, RegL (Bartoli et al. 1993), PSP2	NM_006507	NP_006498.1 =P48304.1
Reg2	PTP2, PSP2, lithostathine 2	NM_009043	NP_033069.1 (Luo et al. 2013; Unno et al. 1993) =Q08731
Reg3α	PAP2, PAP II, Reg IIIα	NM_011259	NP_035389 (Narushima et al. 1997; Lai et al. 2012) =O09037
	Rat Reg3α (REG 3A), Rat Reg III	NM_001145846 NM_172077.2	NP_001139318 =NP_742074.2 (Li et al. 2013a; Frigerio et al. 1993a)
	Rat PAP II	L10229	AAA02980.1 =P35231.1 (Frigerio et al. 1993a; Suzuki et al. 1994)
	Human Reg3G, PAPIB, Reg III	NM_198448 =NM_001008387.2 AB161037 AY428734	NP_940850.1 (Lee et al. 2012; Nata et al. 2004) =Q6UW15.1 =NP_001008388.1 =BAD51394.1 =AAR88147.1
Reg3β	PAP, PAP1, PAP I, HIP, Reg IIIβ	NM_011036	NP_035166 (Luo et al. 2013; Itoh and Teraoka 1993) =P35230
	Rat Reg3β, PAP	NM_053289 M98049	NP_445741 (Li et al. 2013a; Iovanna et al. 1991) =P25031.1 =AAA16341.1 (Iovanna et al. 1993)
	Rat Reg-2, Reg2	S43715	AAB23103.1 (Kamimura et al. 1992; Lieu et al. 2006)

(*continued*)

Table 1 (continued)

Mouse genes	Orthology	Transcript	Polypeptide
	Human Reg3A, HIP, PAP, INGAP (Rafaeloff et al. 1997)	NM_002580.2 NM_138937.2 NM_138938.2 BC036776	NP_002571.1 (Lai et al. 2012; Lasserre et al. 1992) =Q06141.1 =NP_620354.1 =NP_620355.1 =AAH36776.1
		M84337.1	AAA36415.1 (Orelle et al. 1992)
Reg3γ	PAP3, PAP III, Reg IIIgamma	NM_011260	NP_035390.1 (Narushima et al. 1997; Choi et al. 2013) =O09049
	Rat Reg3γ, PAP III	NM_173097	NP_775120.1 (Frigerio et al. 1993b; Konishi et al. 2013) =P42854.1
Reg3δ	INGAP, Reg3d, RegIII delta	NM_013893	NP_038921 (Sasahara et al. 2000; Skarnes et al. 2011) =Q9QUS9
		NM_001161741.1	NP_001155213.1 (Skarnes et al. 2011)
	INGAP-related protein	AB028625.1	BAA92141.1 (Sasahara et al. 2000)
Reg4	RELP, RegIV	NM_026328	NP_080604.2 (Hu et al. 2011; Kamarainen et al. 2003) =Q9D8G5
	Rat Reg4	NM_001004096.1	NP_001004096 (Namikawa et al. 2005) =Q68AX7.1
	Human Reg4	NM_032044 =NM_001159352.1	NP_114433.1 (Hartupee et al. 2001; Ying et al. 2013) =Q9BYZ8.1 =NP_001152824.1

five in human have been identified. The molecular relationships based on sequence comparison and alignment are summarized in Table 1. Based on the sequence homology, and the phylogenetic analysis conducted with blast method in the data retrieved from NCBI, Reg proteins can be divided into four groups: Reg1, Reg2, Reg3, and Reg4 (Okamoto 1999). With the exception of Reg4, all of the other Reg family genes are structured into six exons separated by five introns spanning about 3 kb. The first exon encodes the 5′-UTR, and the second encodes the remainder of the 5′-UTR, the ATG start codon, and the initial protein coding sequence. Exons 3–6 encode the body of the proteins with the 3′-UTR located in the sixth exon (Narushima et al. 1997). In Reg3 subfamily proteins, there is a common 5-aa insertion in the C-terminal regions (Narushima et al. 1997). Based on the high degrees of domain/sequences identities, the gene family is probably derived from

the same ancestor gene by gene duplication events accumulated during evolution. Currently, Reg2 and Reg3δ/INGAP are only found in mice; more Reg3 isoforms should be discovered in rats and humans.

All members of the Reg family contain the typical C-type lectin-like domain (CTLD). They are subject to trypsin cleavage at the Arg-Ile bond located at 11th residue at the N-terminal, resulting in the formation of insoluble fibrils (Graf et al. 2006). The sensitive cleavage site is conserved in 18 Reg/Reg-related proteins from 6 different species (human, bovine, mouse, hamster, pig, and rat). Studies on rat Reg proteins (Reg1, Reg3α, Reg3β) showed that cleavage of the N-terminal undecapeptide produces peptides of 133–138 residues. Trypsin cleavage converts three of the soluble 16-kDa Reg proteins (Reg1, Reg3β, Reg3γ) into 14-kDa insoluble products that are completely resistant to trypsin and partially resistant to other proteases from pancreatic juice.

Reg3α is also processed into the 14-kDa form, but its product remained soluble and is only resistant to trypsin, but not to other proteases. How this cleavage affects the function of Reg proteins remains to be understood. It was found that trypsin-activated insoluble isoforms of Reg1, Reg3β, and Reg3γ polymerize into highly organized fibrillar structures with helical configurations (Graf et al. 2001). The C-terminal cleavage product of rat Reg1 spontaneously precipitates at a neutral pH (Schiesser et al. 2001). This insoluble form may play a key role in forming protein plugs in chronic pancreatitis. In more than one half of patients with pancreaticobiliary maljunction, Reg1, together with trypsinogen and activated trypsin, was detected in both the duct bile and the gallbladder bile, whereas none of the pancreatic enzymes or Reg1 was detected in the controls (Ochiai et al. 2004).

Several consensus transcriptional regulatory elements have been identified after examining the 5′-flanking sequences of Reg family genes. IL-6 response elements, mediating putative acute-phase responses, are located in the 5′-flanking region of all mouse Reg genes. Pan-1 motif sequences (CACCTG) are located in the promoter regions of mouse Reg3α and Reg3β, rat Reg1, and hamster INGAP genes. Pit-1 element, which mediates pituitary-specific transcription, is located in the promoter regions of mouse Reg3α, Reg3δ, and Reg3γ and rat Reg3β genes (Narushima et al. 1997). In addition to the IL-6 and Pit-1 response elements shared with other Reg3 genes, Reg3δ also contains consensus motifs for MyoD and Irf 1/Irf 2 binding sites in the promoter region, which suggests isoform-specific expression and response (Abe et al. 2000).

Expression Pattern During Development in Human and Rodents

The expression pattern of Reg genes during development may differ from each other depending upon the types of tissue and the age of development. The expressions of Reg1 and Reg2 genes have been investigated in mouse embryos at 8.5–12 days of the development, along with the expression of Ins1 and Ins2 genes. Reg1 mRNA became detectable at day E9, following the onset of Ins2 expression at day E8.5. Reg2 mRNA was not detectable until E12, when Ins1 transcription takes place (Perfetti et al. 1996a). This suggests that the two insulin

genes and the two Reg genes are induced and expressed differentially during early development. In the human fetus, Reg1A expression was observed only in the pancreas, in contrast to its widespread expression in adults (Bartoli et al. 1998). The level of human pancreatic Reg1A transcript is low before 16 weeks of gestation, at which time they increase dramatically and reach a similar level as in the adult by 20-week gestation (Mally et al. 1994). Despite its early expression, the Reg1A/Reg1 gene might not be involved in β- or acinar cell growth during human and rat fetal development due to a lack of coordination between Reg mRNA levels and insulin gene expression (Moriscot et al. 1996; Smith et al. 1994). Human Reg1B transcript is present not only in the pancreas but also in the colon and brain of the fetus (Bartoli et al. 1998). Interestingly, expression of Reg1A was higher than Reg1B in human fetal pancreata, but the reversed expression pattern is observed in adult pancreas where Reg1B is higher than Reg1A (Sanchez et al. 2001). Reg3A/PAP mRNA expression displayed a broad distribution in the human fetus, being observed in the pancreas, stomach, jejunum, and colon and to a much lower level in the pituitary gland. The expression of Reg3A/PAP transcript in these tissues lasts throughout the adult lifespan, being especially high in the jejunum (Bartoli et al. 1998). Reg3A/PAP protein was first detectable at 8 weeks in endocrine nests co-stained with chromogranin A. The expression of Reg3A/PAP reached a level comparable with of the adult pancreas at 10 weeks of fetal life, being detected only in the glucagon-producing cells. In the meantime, no expression of Reg3A/PAP protein was detected in the pancreatic ducts or acinar cells of the fetal pancreas (Hervieu et al. 2006). Different from Reg3A/PAP in human, mouse INGAP/Reg3δ is present in cells that co-expressed insulin or somatostatin, but not glucagon, in the developing pancreatic bud of the embryo. Surprisingly, the colocalization of glucagon and Reg3δ/INGAP only occurred in the mouse islet cells to a significant level after birth (Hamblet et al. 2008).

Postnatal expression of the Reg proteins has only been systemically analyzed in rodents. After birth, total Reg1 and Reg2 expression in the pancreas showed an age-dependent decline, being decreased by 45 % at 30-month-old vs. 1-month-old in mice. While Reg1 mRNA level in the pancreas decreased progressively with age, Reg2 mRNA levels did not decline significantly, indicating that Reg1 and Reg2 expressions in the pancreas have differential age-dependent regulation (Perfetti et al. 1996b). Reg1 was also detectable in the duodenum and pancreas of newborn rats and dramatically increased at 3 weeks of age. Reg3β mRNA was undetectable in neonatal rat and displayed a sudden increase in the ileum around the time of weaning. A decline of Reg1 and Reg3β expressions in the ileum was observed in older rats (Chakraborty et al. 1995).

Reg Protein Receptor(s)

The putative interactions of Reg proteins and Reg receptor(s) have not been sufficiently elucidated to date. The Reg1α receptor cDNA was isolated from rat

islets from a 2,760 bp open reading frame. The 919-amino-acid protein was suggested to be a type II transmembrane protein with a long extracellular domain (868 aa), a single transmembrane domain (residues 29–51), and a short N-terminal intracellular region (Kobayashi et al. 2000). The rat receptor is homologous to human EXTL3/EXTR1, a member of the EXT family, and can modulate NF-κB signaling upon stimulation by TNF-α (Nguyen et al. 2006). The mRNA of Rat Reg1α receptor was detected in normal pancreatic islets, regenerating islets, and insulinoma RINm5F cells. The receptor transcript was also expressed in a wide range of other tissues, including the liver, kidney, spleen, thymus, testis, adrenal gland, stomach, ileum, colon, pituitary gland, and brain, but not in the heart and jejunum (Kobayashi et al. 2000). Reg1α receptor-expressing RINm5F cells showed significant Reg1-dependent growth acceleration as indicated by BrdU incorporation. However, the expression of Reg1α receptor remained unchanged in regenerating islets as compared to normal ones, suggesting that both proliferation and apoptosis of pancreatic β-cells are primarily regulated by the expression of the Reg genes, but not the receptor (Kobayashi et al. 2000).

Beside the isolation of Reg1α receptor, the mechanism of Reg3δ/INGAP action on RINm5F cell proliferation was also explored (Petropavlovskaia et al. 2012). Both the full-length recombinant protein and bioactive peptide of INGAP (INGAP-P, a pentadecapeptide corresponding to amino acids 104–118) stimulated cell regeneration via binding to Gi protein-coupled receptor and by activating the Ras/Raf/Erk signaling pathway. Activation of ERK1/2 can be blocked by pertussis toxin, a reagent that can prevent the G proteins from interacting with corresponding receptors on the cell membrane. Further, PI3K/Akt pathway was also activated after INGAP administration. But to date, the sequence of this particular INGAP receptor has not been determined.

The Roles in Promoting Cell Replication and Preventing Apoptosis of Adult β-Cells

Reg1 [hReg1A and hReg1B]

Reg1/pancreatic stone protein (PSP) was first found in the study of pancreatitis, which was proposed to control the formation of calcium carbonate crystals in the pancreas (Multigner et al. 1983). However, in the diabetes field, the term Reg1 was used more often to describe its function on islet regeneration (Graf et al. 2006). The cDNA of Reg1 was first isolated in screening the regenerating islets-derived library from 90 % pancreatectomized rats in 1988 (Terazono et al. 1988). The Reg1 gene was found to be expressed in rat regenerating islets and induced by the administration of the poly (ADP-ribose) polymerase (PARP) inhibitor nicotinamide to depancreatized rats and also by the treatment with aurothioglucose, a drug that induced islet hyperplasia (Terazono et al. 1988). As human homologues of mouse Reg1, both Reg1A and Reg1B proteins consist of 166 amino acids and differ only by 22 (Moriizumi et al. 1994); their protein sequences share 87 % identities (Bartoli et al. 1993).

Under physiological condition, very low concentration of Reg1 protein is detected in the islets. Most of the protein are located in the acinar cells (Kimura et al. 1992) and increased significantly under inflammatory stress or other forms of pancreatic injury. Its mRNA level was significantly elevated in response to interleukin IL-6, interferon (IFN), or tumor necrosis factor (TNF)-α but decreased by dexamethasone (Zenilman et al. 1997). Expression of Reg1 mRNA in the pancreas was also increased by 12-fold after a 2-week 75 % high-protein diet (Rouquier et al. 1991). A consistent increase of Reg1 protein in the pancreatic juice was also detected within 2 weeks of an 82 % high-protein feeding (Bimmler et al. 1999). In addition, Reg1 is also normally expressed in the gastrointestinal tract, such as in the duodenum and jejunum, and gastric mucosa, with species variations (Terazono et al. 1988; Rouquier et al. 1991; Unno et al. 1993; Perfetti et al. 1996b; Watanabe et al. 1990). Reg1 expression is closely associated with pancreatic β-cell function. It is proposed to be a paracrine or autocrine factor in the proliferation and differentiation of cells in the digestive and endocrine systems (Acquatella-Tran Van Ba et al. 2012). In regenerating islets induced by the administration of PARP inhibitors to 90 % depancreatized rats, Reg1 was found to colocalize with insulin in secretory granules, suggesting that Reg1 is synthesized in and secreted from regenerating β-cells (Terazono et al. 1990). Reg1 mRNA levels were increased threefold within 2 days in the rat pancreas that received surgical wrapping, which correlated with ductular proliferation and emerging insulin staining within the ductular epithelia in the wrapped lobe. However, the induced Reg1 gene expression was localized to the exocrine tissue, suggesting that Reg1 may be involved in the maintenance of normal islet function through induction of new islet formation from precursors of ductal origins (Zenilman et al. 1996a).

Reg1 plays a role in the replication of β-cells. Using isolated rat islets, Reg1 protein stimulated β-cell replication by increasing [^{3}H] thymidine incorporation in a dose-dependent manner (Watanabe et al. 1994). Reg1 proteins isolated from human and bovine pancreas were mitogenic to both ARIP ductal and RIN β-cell lines in a dose-dependent manner but had no effect on AR42J acinar cells or isolated mature islets (Zenilman et al. 1996b). Isolated human and rat Reg1 proteins were also mitogenic to primary ductal cells and may modulate the expansion of the pancreatic ductal population during islet regeneration (Zenilman et al. 1998). It suggests that Reg1 can potentiate proliferation of ductal cells and islet β-cells, but not acinar cells. (More discussion related to islet isolation can be found in chapter entitled "► Isolation of Rodent Islets of Langerhans.") Administration of rat Reg1 protein to depancreatized rats ameliorated the surgical diabetes after 2 months, as evidenced by decreased blood glucose and preserved insulin-producing capacity (Watanabe et al. 1994). Diabetic NOD mice treated with recombinant human Reg1A protein showed increased β-cell mass and decreased mortality rate than untreated animals. This was interpreted as a result of Reg1A-induced maturation of β-cell precursors in NOD mice (Gross et al. 1998). The expression of Reg1 may be directly stimulated by gastrin, since the induced mRNA abundance was diminished by gastrin/cholecystokinin B antagonist (O'Hara et al. 2013; Ashcroft et al. 2004). In murine pancreatic

tumors with acinar-specific overexpression of the gastrin receptor (CCK2R), expressions of Reg1 and Reg3α proteins were strongly upregulated in duct-like cells in preneoplastic lesions, or in the periphery of tumors and adjacent acini.

Moreover, the CCK2R transgenic mice showed improved glucose tolerance, increased insulin secretion, and doubled insulin contents compared to control animals (Gigoux et al. 2008), which indirectly indicated Reg1 could promote pancreatic cell proliferation and improve glucose tolerance. The rate of [^{3}H] thymidine incorporation was low in cultured pancreatic islets from Reg1-deficient mice (Unno et al. 2002) but high in those from β-cell-specific Reg1-overexpressing (Ins-Reg) mice, indicating that Reg1 protein was secreted from the islets which stimulated DNA synthesis through an autocrine mechanism. The Reg1-deficient mice had significantly smaller β-cell mass than control animals following gold thioglucose treatment, a drug inducing hyperplasic islets, suggesting that Reg1 might be essential for the cell cycle progression in pancreatic β-cells. The NOD mice carrying the Reg1 transgene showed a delayed onset of diabetes, which coincided with a threefold increase in islet cell volume. These data further support the notion that Reg1 promotes the regeneration of β-cells, which, as a consequence, compensates for the β-cell loss and delays the onset of autoimmune diabetes (Unno et al. 2002). It is thus conceivable that Reg1A level in the serum of both T1D and T2D patients was significantly elevated (Astorri et al. 2010). However, controversies existed in an early study on transgenic mice overexpressing Reg1 protein in the islets, i.e., the mice became diabetic as a result of increased β-cell apoptosis, as well as the development of various tumors (Yamaoka et al. 2000), as we have assessed before (Liu et al. 2008). Whether Reg1 promote islet cell proliferation and/or differentiation from other types of pancreatic cells needs to be studied further.

The identification of a putative receptor for Reg1, EXTL3, supports its direct effect on islet proliferation. The mechanism of how Reg1 stimulates DNA synthesis in islet β-cells appeared to involve phosphoinositide 3-kinase (PI3K) and downstream targets of transcriptional factor ATF-2 and cyclin D1 (Takasawa et al. 2006). Consequently, in Reg1 knockout islets, the levels of phospho-ATF-2, cyclin D1 and phospho-retinoblastoma protein (pRb), and the rate of DNA synthesis were all decreased. Cyclin D1 is established to promote cell cycle progression by inactivating retinoblastoma protein through cyclin-dependent kinases (cdks) and stimulating cell proliferation. Alternatively, ERK1/2 pathway was activated during mitogenesis of ductal and β-cell lines triggered by Reg1 overexpression or treatment with recombinant protein (Wang et al. 2011). Using cDNA microarray, significant elevations of mitogen-activated protein kinase phosphatases (MKP-1) and cyclins were detected in Reg1-treated cells.

In addition, Reg1 effects exhibited a dose-dependent manner. Endogenously expressed Reg1 in high concentration may form a complex with EXTL3, bind to MKP-1, and inactivate JNK, leading to cell apoptosis or differentiation into other cells (Mueller et al. 2008), a pathway that seemed to exist in both ductal and β-cells. In this experiment, Reg1 protein displayed a dual action on cell proliferation under low-dose administration, while high-dose Reg1 or endogenous

overexpression induced cell apoptosis. In fact, it has been reported that high extracellular level of Reg1 over 100 nM could inhibit cell growth (Jung and Kim 2002). With overexpression of Reg1, more differentiated state was observed in rat insulinoma cells. It was thought that under low dose of Reg1, the protein could bind to its receptor and activate MAPK-cyclin D1 pathway. When overexpressed within cells or cultured in high concentration, Reg1 can inhibit growth by binding to MKP-1, leading to differentiation into other types of cells.

Reg1 expression is associated with pancreatic pathology. In patients with cystic fibrosis (CF), in addition to its normal localization in acinar cells, Reg1 immunostaining was induced in the duct-like cells of the tubular complexes and dilated duct cells co-stained with the ductal marker CK19 (Sanchez et al. 2004). In vitro, Reg1 inhibited proliferation and migration of pancreatic stellate cells and stimulated fibrolysis by increasing the ratio of matrix metalloproteinases (MMPs) to tissue inhibitors of matrix metalloproteinases (TIMPs). Hence, it might rescue pancreatitis by promoting the resolution of fibrosis (Li et al. 2010). On the other hand, autoimmunity to Reg1 may be associated with the development of diabetes. A significant increase in anti-Reg1 autoantibodies was found in both T1D and T2D patients compared with healthy subjects. Serum from diabetic patients with Reg1 autoantibodies demonstrated significantly attenuated BrdU incorporation induced by Reg1, while nondiabetic serum without the autoantibodies had little effect (Shervani et al. 2004). It supports that Reg1 protein can be used as a replacement therapy for diabetes.

The action of Reg1 is not restricted to the pancreas. Reg1 knockout mice had a greater number of severe lesions in the small intestines induced by indomethacin, a nonsteroidal anti-inflammatory drug. These intestinal injuries were rescued by the administration of Reg1 protein, indicating a physiological role for Reg1 in maintaining the intercellular integrity in the small intestine (Pittenger et al. 2009b). In addition, Reg1 regulates cell growth that is required for the maintenance of the villous structure of the small intestine (Ose et al. 2007). In rat regenerating liver, after 2-acetylaminofluorene administration and subject to 70 % partial hepatectomy (2-AAF/PH), Reg1 was significantly induced, with increasing formation of bile ductules (Wilding Crawford et al. 2008). It suggested that Reg1 is closely related to the cell regeneration in the liver through activation of the stem cell compartment. Hence, the level of Reg1 expression is closely associated with the regeneration of the small intestine and liver.

Reg2

In addition to the pancreas, Reg2 is normally expressed in the mouse liver, duodenum, small intestine, and colon (Unno et al. 1993; Perfetti et al. 1996b). There have been different opinions on the precise cellular source of Reg2 in the pancreas. Sanchez et al. reported that the expression of Reg2 mRNA and protein was restricted to the exocrine tissue regardless of the age and the presence of insulitis and/or diabetes (Sanchez et al. 2000); we only detected Reg2 immunostaining in the peri-islet acinar cells of normal mice (Luo et al. 2013; Spak et al. 2010) using a

specific antibody from R&D System. However, Gurr et al. demonstrated expression of Reg2 in the endocrine cells of NOD mice (Gurr et al. 2007).

There have been numerous reports on the induction of Reg2 gene as part of islet protection or regeneration. During islet regeneration, 5 days after 50 % pancreatectomy, Reg2, Reg3β, and Reg3*y* were the most abundantly induced (>10-fold) transcripts in the pancreas (Rankin and Kushner 2010). Exendin-4 and INGAP-P, which stimulate β-cell replication and/or neogenesis, increase insulin production and partially reverse insulitis in diabetic mice also increased Reg2 gene expression. These evidences suggest its role in islet survival and/or regeneration (Huszarik et al. 2010). In the NOD mice, Reg2 expression was increased more compared to Reg1, irrespective of sex or state of the diabetes, suggesting a possible difference in the physiological functions of the two proteins (Baeza et al. 1997). Following mycobacterial adjuvant treatment that made a partial recovery of β-cell mass, Reg2 expression was significantly increased, which correlated with an increase in the number of newly formed small islets and improved glucose tolerance in NOD and in streptozotocin-induced diabetic mice (Huszarik et al. 2010). Similar to Reg3β, Reg2 was suggested to be a β-cell-derived autoantigen in NOD mice since vaccination with the C-terminal fragment of Reg2 delayed the onset of T1D (Gurr et al. 2007). We have reported that Reg2 overexpression protected MIN6 insulinoma cells from streptozotocin-induced mitochondrial disruption and cell apoptosis, by attenuating the activation of caspase-3 and cleavage of PARP. These changes correlated with a persistent suppression of JNK phosphorylation by streptozotocin and a clear reversal by Reg2. These data demonstrate that Reg2 protects insulin-producing cells against streptozotocin-induced apoptosis by interfering with its cytotoxic signaling upstream of the intrinsic pro-apoptotic events by preventing its ability to inactivate JNK (Liu et al. 2010). Our recent data suggested Reg2, as well as Reg3β, can be activated by glucocorticoids and IL-6 in pancreatic acinar and islet cells and may serve a role in response to inflammation during pancreatitis (Luo et al. 2013). The direct effect of Reg2 on islet regeneration has not been tested.

Reg3α [hReg3G]

Different from other Reg family members, the Reg3 proteins (Reg3a, Reg3b, Reg3g, and Reg3δ) are characterized by the extra five amino acids close to the C-terminus in their primary structure. Reg3α belongs to a subfamily with pancreatitis-associated protein (PAP/PAP I/Reg3β/peptide 23/HIP), which was first discovered in pancreatic juice and homogenate of rat pancreatitis in 1984, but not normal pancreas, and differed from Reg1 (pancreatic stone protein, PTP) (Keim et al. 1984; Closa et al. 2007). Because of 74 % amino acid identity with Reg3β, rat Reg3α (PAP II) was first discovered in 1993 (Frigerio et al. 1993a); the human homology is Reg3G/RegIII/PAPIB which shares 66 % identity to mouse Reg3α in protein sequence, while shares 65 % identity to another mouse homology, Reg3γ (Lee et al. 2012; Nata et al. 2004; Lasserre et al. 1992; Laurine et al. 2005). Normally in rats it is hardly detectable but can be drastically induced after

pregnancy and pancreatitis, indicating its involvement in functional adaption (Frigerio et al. 1993a; Bimmler et al. 2004; Honda et al. 2002). In the endocrine pancreas, it was reported to be expressed by mouse islet α-cells (Gurr et al. 2007). Reg3α protein was also detected in the small intestine, the proximal colon, and the pancreatic primordium (Hervieu et al. 2006).

Some indirect evidences support its effects on promoting islet proliferation. Acinar-specific overexpression of gastrin/CCK2 receptor induced carcinogenesis and the level of Reg3α/RegIII protein, which is proposed to be involved in the adaptive and regenerative responses of the endocrine tissues (Gigoux et al. 2008). C-Myc is a potent driver of β-cell proliferation (Pelengaris et al. 2004); activation of Myc transcription factor in mouse islets significantly promoted cell cycle progression, with steady induction of the mRNAs of Reg2, Reg3α, Reg3β, and Reg3γ up to twofold within 24 h, supporting their role in cell cycle progression or islet cell transformation. In NOD mice, pancreatic Reg3α mRNA level (but not other isoforms) was increased 1.5-fold after onset of insulitis leading to T1D (GEO profile ID 15736194, 15738507). Among the changes brought by diabetes, hyperglycemia seemed to have specific effect on Reg3α. In primary rat islets, increasing glucose concentrations from 2 to 10 mM caused no change in its expression. However, further increase to 30 mM which is known to be detrimental or proliferative, significantly doubled Reg3α mRNA level (GEO profile ID 59898598). This response was isoform specific and did not occur to other Reg proteins.

Reg3α overexpression in vitro: in order to explore whether Reg3α can directly stimulate islet β-cell replication, similar to Reg1 (Takasawa et al. 2006), we overexpressed its cDNA in stably transfected MIN6 cells (Cui et al. 2009). Using real-time PCR and Western blots, Reg3α expression was barely detectable in vector-transfected cells; in contrast, the levels of its mRNA and protein in pcDNA-Reg3α-expressing clones were increased 10- and 6-fold, respectively. Western blots also revealed Reg3α protein being released into the culture medium, which is consistent with its detection in patients' serum (Astorri et al. 2010) and supports its endocrine action. In MTT cell viability assay, Reg3α-overexpression caused ~2-fold higher rate of growth vs. vector-transfected cells. In order to investigate possible intracellular mechanisms, we detected an average 1.8-fold increase in Akt phosphorylation and 2.2- and 2.5-fold increase in the levels of cyclin D1 and cdk-4 in these cells vs. vector-transfected cells. These effects were not revealed when Reg2 or Reg3β gene was transfected, indicating isoform specificity. It is well established that β-cell replication is associated with increased cyclin D1 and cdk-4 levels (Cozar-Castellano et al. 2004), deficiency in cdk-4 or cyclin D2 results in β-cell loss and diabetes (Rane et al. 1999; Georgia and Bhushan 2004), and both Reg1 and Reg3δ cause PI3K-mediated increases in cyclin D1 and cdk-4 levels (Takasawa et al. 2006; Jamal et al. 2005; Sherr 2001; Diehl et al. 1998; Rane and Reddy 2000). Our result thus suggests that Reg3α stimulate β-cell replication, by activating Akt kinase and increasing the levels of cyclin D1/cdk-4 (Cui et al. 2009). The identical effect of Rat Reg3α in proliferation has since been confirmed in human pancreatic carcinoma Panc-1 cells, with increased expression of the islet transcription factors NeuroD, Nkx6.1, and Pax6 (Choi et al. 2010).

Reg3β (hReg3A)

In 1986, another Reg protein was found in the rat and named pancreatitis-associated protein (PAP) due to its induction during experimental pancreatitis and correlation with the severity of pancreatitis (Keim and Loffler 1986). In fact, Peptide-23 gene was first detected in primary cultures of rat pituitary and was then proved to be identical with Reg3β (Chakraborty et al. 1995). Pancreatic expression of Reg3β was strong in glucagon-producing islet cells and acinar cells close to the islets, but quite low in other endocrine cells located in the center of islets or other cells located in their outer rim (Hervieu et al. 2006; Baeza et al. 2001). This suggests Reg3β may act as a paracrine modulator of β-cell function. Reg3β is also expressed in the ileum and to a lesser extent in the jejunum and duodenum (Waelput et al. 2000). Surprisingly, its homology in human is hReg3A/HIP, with 70 % identities. In a panel of 36 adult human tissues, human Reg3A was only expressed in the pancreas and small intestines, with 100-fold higher levels than others (profile ID 10124642, Gene Expression Omnibus (GEO), www.ncbi.nlm.nih.gov/geo).

Pathophysiological roles of Reg3β in diabetes have been proposed. Reg3β protein can be released into the culture medium from primary islets taken from a T1D patient, and this release was stimulated by IL-6, indicating that Reg3β is involved in a local inflammatory response in diabetic islets (Gurr et al. 2002). The pancreatic mRNA level of Reg3β in NOD mice was significantly higher than in control IOPS-OF1 mice (Baeza et al. 2001). And the protein was also expressed in the islets and ductal epithelium in pancreata of prediabetic and diabetic NOD mice, in contrast to its restricted expression in acinar cells and peri-islet cells in nondiabetic controls. The lymphocytes from islets infiltrates and pancreatic lymph nodes of 7-week-old NOD mice showed a strong proliferative response to Reg3β, suggesting a possible role as an autoantigen (Gurr et al. 2002).

In order to test whether it can promote islet cell growth or survival against experimental damage, we established a pancreatic islet-specific overexpression of Reg3β mouse model using rat insulin I promoter and evaluated the changes in normal islet function, gene expression profiles, and the response to streptozotocin-induced diabetes. Significant and specific overexpression of Reg3β was achieved in the pancreatic islets of RIP/Reg3β mice, which exhibited normal islet histology, β-cell mass, and insulin secretion in response to high glucose yet were slightly hyperglycemic and low in islet GLUT2 level. Upon streptozotocin treatment, in contrast to wild-type littermates that became hyperglycemic in 3 d and lost 15 % weight, RIP/Reg3β mice were significantly protected from hyperglycemia and weight loss. To identify specific targets affected by Reg3β overexpression, cDNA microarray on islet RNA isolated from the transgenic mice revealed that more than 45 genes were either up- or downregulated significantly. Among them, islet-protective osteopontin/SPP1 and acute responsive nuclear protein p8/NUPR1 were significantly induced. These results were further confirmed by real-time PCR, Western blots, and immunohistochemistry (Xiong et al. 2011). This suggests that, compared to the regenerating effects of other Reg proteins on islets, Reg3β is more likely an islet-protective factor in response to stress and inflammation.

As regards the effect of human Reg3A in pancreatic regeneration, it is still being debated whether the regeneration of pancreatic β-cells is achieved by self-replication (Meier et al. 2008; Teta et al. 2007; Dor et al. 2004), neogenesis, or both (Seaberg et al. 2004). Based on rodent experimental evidence, the adult pancreas may harbor a small progenitor population, perhaps resident among centroacinar or ductal cells, which can be activated by injury and inflammation and give rise to new islet cells (Reichert and Rustgi 2011). Transcription factors and extracellular regulators control this process of islet neogenesis. Direct evidence was demonstrated by a 5-d injection of a 15-aa peptide based on human Reg3A (Human proIslet Peptide: IGLHDPTQGTEPNGE). It increased the volume of small extra-islets, insulin-positive clusters 1.5-fold in NMRI mouse pancreas and showed a tendency of increased Ngn3 and Nkx6.1 expression in IHC (Kapur et al. 2012). This was the first report to indicate that human Reg3A peptide has the bioactivity in vivo to promote new islet formation by elevation of transcription factors. Earlier, a similar 16-aa peptide based on Reg3A (WIGLHDPTQGTEPNGE) prevented streptozotocin diabetes by increasing the islet cell mass in mice (Levetan et al. 2008, 2010).

Reg3β also seems to be a tumor promoter as its deficiency caused increased tumor cell apoptosis, decreased tumor growth, and impaired angiogenesis in pancreatic cancer (Gironella et al. 2013). Moreover, Reg3β is a crucial mitogenic and antiapoptotic factor for the liver as its knockout caused cellular apoptosis and impaired liver regeneration (Lieu et al. 2006) (mislabeled as Reg2 in this publication; see our editorial correspondence (Liu and Cui 2007)). The mechanisms of Reg3β-mediated antiapoptotic and anti-inflammatory effects were further explored. Reg3β knockout mice displayed increased apoptosis in acinar cells in pancreatitis, as shown by elevated levels of caspase-3 and cleaved PARP; pretreatment with Reg3β protein reversed those effects and protected the pancreas (Gironella et al. 2007). This knockout pancreas also showed more neutrophil infiltration and higher levels of inflammatory cytokines, including TNF-α, IL-6, and IL-1β; both parameters were significantly reduced when the mice were pretreated with Reg3β, further supporting an anti-inflammatory role (Gironella et al. 2007). Conversely, antisense knockdown and antibody neutralization of Reg3β worsened the symptoms of pancreatitis induced by sodium taurocholate in rats (Viterbo et al. 2009).

Thus, Reg3β is antiapoptotic by inhibiting caspase-3 and PARP activation and anti-inflammatory by controlling cytokine output. Transcription factors of NF-κB family are crucial in controlling the inflammatory responses and cell survival. Pancreatic-specific deletion of RelA/p65 and thus NF-*k*B signaling abolished Reg3β induction and resulted in more severe pancreatitis, suggesting that Reg3β protects the pancreatic acinar cells (Algul et al. 2007). So far other Reg proteins, including Reg2 and Reg3β on pancreatic islets, have not been evaluated using similar knockout approaches. The mechanism of Reg3β action in pancreatic acinar cells (which seems distinct from that of Reg1/INGAP) involves MAPK (Ferrés-Masó et al. 2009) and/or cytokine receptor-mediated activation of JAK and STAT family of transcription factors. In acinar cell line AR42J, Reg3β activated JAK and caused the phosphorylation and nuclear translocation of STAT3 and the induction

of suppressor of cytokine signaling 3 (SOCS3). Meanwhile, it was shown to induce JAK-dependent NF-*k*B inhibition, pointing to a cross talk between JAK/STAT and NF-*k*B signaling pathways (Folch-Puy et al. 2006).

Reg3δ (INGAP)

Reg3δ was first identified and purified in hamsters after partial obstruction of the pancreatic duct in 1997 (Rafaeloff et al. 1997). It was originally known as islet neogenesis-associated protein (INGAP) since it was identified as the local pancreatic factor that reversed streptozotocin-induced diabetes presumably by the induction of islet neogenesis. Normally, it is expressed in the stomach and duodenum and in the glucagon-producing islets and pancreatic ductal cells (Abe et al. 2000; Borelli et al. 2005; Taylor-Fishwick et al. 2008). The expression level of INGAP increased significantly in acinar cells of cellophane-wrapped pancreata, but not in pancreatic islet cells (Rafaeloff et al. 1997). Pancreatic transcription factors, such as Pdx1, Ngn3, NeuroD, and Isl-1, can directly activate the INGAP promoter individually or in combination (Hamblet et al. 2008).

To demonstrate a direct effect, the administration of 15-amino-acid INGAP-P to nondiabetic and streptozotocin-induced diabetic mice or rats caused increased islet cell number and mass and new islet formation (small foci of islet-like cells budding from intralobular and terminal ductules), thus capable of hyperglycemia reversal in diabetic animals (Rosenberg et al. 2004; Lipsett et al. 2007a). Intramuscular injection of INGAP stimulated islet neogenesis in healthy dogs (Pittenger et al. 2007). Petropavlovskaia M et al. then explored the mechanisms of INGAP on the proliferation of RINm5F cells (Petropavlovskaia et al. 2012). Both the recombinant protein and INGAP-P stimulated cell regeneration via binding to Gi protein-coupled receptor and activating the Ras/Raf/Erk (Petropavlovskaia et al. 2012) and PI3K/Akt pathways. Ex vivo studies showed that INGAP-P peptide could enhance glucose- and amino acid-stimulated insulin secretion from both adult and neonatal rat islets without affecting the islet survival rate or the relative proportion of the islet cells. A significant increase in β-cell size was observed in the cultured islets in the presence of INGAP-P peptide compared to controls (Borelli et al. 2005). A microarray analysis of INGAP-P peptide-treated rat neonatal islets shows many genes that are upregulated, especially those related to islet metabolism, insulin secretion, β-cell mass, and islet neogenesis. They include hepatocyte nuclear factor 3β (Hnf3β), upstream stimulatory factor 1 (Usf1), K^+ channel proteins (Sur1 and Kir6.2), Ins1, glucagon, MAPK1, Snap-25 that may regulate insulin exocytosis, and Pdx1 (Barbosa et al. 2006).

Overexpression of INGAP in pancreatic acinar cells also caused a significant increase in both the β-cell mass and pancreatic insulin content, which was mainly contributed by increased number of small islets. These mice were resistant to β-cell destruction, hyperglycemia, or hypoinsulinemia following streptozotocin treatment and had a markedly preserved islet structure (Taylor-Fishwick et al. 2006a). Meanwhile, targeted expression of INGAP to pancreatic β-cells (IP-INGAP) in mice

enhanced glucose tolerance and significantly delayed the development of hyperglycemia caused by streptozotocin. Isolated islets from these INGAP-overexpressing mice displayed increased insulin release in response to glucose stimulation in the presence of streptozotocin. This is partially due to a decreased induction of apoptosis and oxidative stress in the islets of the transgenic mice, indicated by lower levels of caspase 3 and NADPH oxidase-1 (NOX1), respectively (Hashimoto et al. 2006).

The application of INGAP peptide in diabetic intervention has been explored in a report on the 65th Scientific Sessions of the American Diabetes Association (2005). Ratner RE et al. reported a double-blind, placebo-controlled trial on INGAP peptide therapy which induced islet neogenesis and improved insulin secretion both in T1D and T1D patients, which suggests that INGAP can be used as an effective therapy alone or in combination with other antidiabetic drugs. The detailed experiment was further published, indicating INGAP peptide (600 mg/d) increased C-peptide secretion in T1D and reduced HbA1c levels in T2D patients (Dungan et al. 2009). However, similar result has not been peer-reviewed and reported.

Antibacterial Reg3γ

Consistent to the role of most Reg proteins, Reg3γ expression was activated in the skeletal muscles and innervating nerves after different models of tissue injury in rat (Klasan et al. 2013). In the intestines, Reg3γ is produced together with lysozyme and cryptdin by Paneth cells, which constitute innate intestinal immunity. In the ileal tissue after 2-day food deprivation, Reg3γ mRNA level was decreased significantly, and the decrease in protein content and localization were confirmed by Western blot and immunohistochemistry. This decrease was associated with increased bacterial translocation into the mesenteric lymph nodes (Hodin et al. 2011). Similar to Reg3β, Reg3γ secreted by specialized epithelial cells is involved in limiting the epithelial contact with bacteria in the small intestine. In Reg3γ-deficient mice, more bacterial reach the small intestinal epithelium; Reg3γ specifically affects Gram-positive bacteria (Johansson and Hansson 2011; Vaishnava et al. 2011). But so far, there is no direct evidence indicating the involvement of Reg3γ in islet cells proliferation.

Reg4

Reg4 is a distinct isoform in the family, starting from its structure and chromosomal location to its high expression in colon cancer. Human Reg4, also named RELP (regenerating protein-like protein), was discovered from a high-throughput screening of inflammatory large bowel disease library. The Reg4 cDNA consists of 7 exons rather than the 6 found in other Reg family genes (Kamarainen et al. 2003). The protein differs in the five-amino-acid insertion (P-N/D-G-E/D-G) present in Reg3 proteins and the six residues (S/A-Q-T-E-L-P) near the N-terminus found in all other Reg family proteins. Its chromosome location also differs from the rest,

e.g., chromosome 1 vs. 2p12 in the human and chromosome 3 vs. 6C in the mouse. Reg4 is normally expressed in the prostate, testes, stomach, duodenum, jejunum, ileum, and colon (Hartupee et al. 2001; Oue et al. 2005). Cells positive for Reg4 are mostly enteroendocrine and mucin-producing goblet cells, e.g., immunofluorescent dual labeling demonstrated its colocalization with chromogranin A in the neuroendocrine cells of the duodenal epithelium (Violette et al. 2003).

Reg4 expression in normal islets has not been determined. In a single report, positive Reg4 staining was shown in mouse insulin-producing islet cells (Oue et al. 2005), but we could not confirm it in normal or malignant pancreas (data not shown), nor was the Human Protein Atlas (www.proteinatlas.org). In islet-derived neuroendocrine tumor, Reg4 is totally negative, while a distinct positive staining for Hath1 (helix-loop-helix transcription factor, regulating differentiation of neural and intestinal secretory cells) was found in peri-islet cells. The same pattern was followed in liver and lymph node metastases from islets (Heiskala et al. 2010). So far, there is no evidence showing that Reg4 is related to cell proliferation of the pancreatic islets.

Reg4 expression is increased in various types of human diseases, especially cancer. In the Atlas, Reg4 is clearly demonstrated in the glandular cells of the small intestines, colon, and rectum. The protein is strongly expressed in the cryptal epithelium of ulcerative colitis, and to a lesser extent in the parietal cells of the gastric corpus mucosa. It was also upregulated in the goblet cells of the glands representing intestinal metaplasia in the esophagus and the gastric antrum (Kamarainen et al. 2003). Reg4 overexpression in human gastric cancer cells caused an increased number and size of tumors and worsened survival of nude mice with peritoneal metastasis, whereas Reg4 knockdown improved survival (Heald et al. 2006). Consequently, Reg4 is highly expressed in drug-resistant colon cancers vs. drug-sensitive ones. Compared to low or no expression of other Reg genes (Reg1A, Reg1B, Reg3), Reg4 is expressed in 71 % colorectal tumors (Violette et al. 2003; Zhang et al. 2003). Reg4 positive colorectal cancer patients have a significantly worse prognosis than those negative for it (Maake and Reinecke 1993). Reg4 also plays important roles in tumor progression and deterioration of prostate cancer (Ohara et al. 2008). High serum Reg4 level in patients with pancreatic cancer was associated with poor response to chemoradiotherapy (Quaife et al. 1989). Applications of specific Reg4 antibodies or small interfering RNAs against Reg4 resulted in increased cell apoptosis and decreased proliferation, leading to decreased tumor growth and increased host animal survival (Bishnupuri et al. 2010). These data indicate that Reg4 may play a role in tumor formation, diagnosis, and/or treatment. More discussion on pancreatic tumors can be found in the chapter entitled "▶ Pancreatic Neuroendocrine Tumors."

Possible Role of Reg Proteins on β-Cell Neogenesis

The stem cells have the ability of differentiating from one precursor cell to multiple specialized terminal types, whereas progenitor cells can only be differentiated into their (one and only) direct targets. In adult pancreas, β-cells preserve a limited

ability to replicate and generate new cells under stress or injury. Therefore, identifying those progenitor cells and factors influencing their differentiating outcome represents a promising avenue toward rescuing diabetes. Recent studies revealed that progenitor cells located in the ductal epithelium can be induced to express Ngn3 and become new β-cells after partial duct ligation in mouse pancreas (Xu et al. 2008).

Reg1 expression was detected in mouse embryonic stem cells (ESCs) and can be activated by Wnt/β-catenin signaling pathway; the latter is important for the maintenance of ESC in an undifferentiated state (Parikh et al. 2012). It indicates that Reg1 might play an important role during embryonic development. Attempts have been made to assess the effect of Reg1 protein on ESC differentiation by adding recombinant protein and overexpressing Reg1 gene. Unfortunately, no significant effect was observed in cell growth compared with those untreated. Nevertheless, the potential effect of Reg1 on stem cell proliferation should be reexamined. If it has protective and/or proliferative effects on stem cells, it can be used along with other transcriptional factors to facilitate the differentiation into β-cells.

In β-cell differentiation, INGAP seems to play a more active role than other Reg family proteins. INGAP promoter activations driven by pancreatic protein 1 (Pan-1), phorbol myristate acetate (PMA), or leukemia inhibitory factor (LIF) can be inhibited by Pdx1 through direct promoter binding (Taylor-Fishwick et al. 2006b). Further studies revealed that the repressing effect of Pdx1 is dependent on its interaction with Pan-1/NeuroD (Taylor-Fishwick et al. 2010); Pdx1 may be part of a negative feedback mechanism to control islet expansion. Similar to Reg1, INGAP-positive cells were also present in mouse embryonic pancreatic buds (Hamblet et al. 2008). Based on that INGAP and some growth factors are essential for islet development, a strategy was proposed by using a combination of INGAP and growth factors including EGF and GLP-1 to cause the expansion of embryonic stem cells and pancreatic progenitor cells. Moreover, administration of INGAP peptide intraperitoneally to hamsters, which received multiple low doses of streptozotocin, stimulated the growth of new endocrine cells with mature islets appearance. The INGAP peptide can also normalize blood glucose and insulin levels; the mechanism of this protective effect seems to include increased expression of Pdx1 in ductal and islet cells (Rosenberg et al. 2004). As for other members of Reg family, Reg2 expression was highly induced in HFD-fed mice or after 70 % pancreatectomy (16, 139, 171); Reg1 and Reg3β exhibited similar changes. Whether they can be considered for islet neogenesis or differentiation needs to be assessed.

Transdifferentiation of other endogenous pancreatic cells into β-cells is now considered as another mechanism of islet regeneration. In response to appropriate stimuli, ductal epithelial and acinar cells can be induced to become regenerating islet cells (Pittenger et al. 2009a). Both direct acinar/ductal to islet transformation and indirect acinar to ductal and then to islet transformation have been proposed to increase β-cell numbers and mass in vitro (Bonner-Weir et al. 2008; Rooman et al. 2002; Schmied et al. 2001). In the early stages of streptozotocin-induced

diabetes, both Reg1 expression and BrdU incorporation can be induced in residual β-cells, indicating a role in β-cell proliferation (Anastasi et al. 1999). In the meantime, co-expression of Reg1 and cytokeratin 19 in acini-ductal cells suggests that Reg1 may participate in the transdifferentiation of acinar and/or ductal cells to islet cells (Tezel et al. 2004).

Compared to other isoforms, the role of INGAP in islet transdifferentiation is better established. In acinar-derived, duct-like cells cultured with a mixture of gastrin, HGF, and INGAP, the mass of islet-like clusters was significantly increased (Lipsett et al. 2007b). Several transcriptional factors, which were crucial for β-cell differentiation, were introduced in order to promote the transdifferentiation of ductal cells into islet-like clusters (ILCs) (Li et al. 2009). A 4-step strategy using nicotinamide, exendin-4, TGF-β, and INGAP peptide was tested. The mass of ILCs was much larger in the group treated with INGAP than that with a scrambled peptide, with increased expression of Pdx1, insulin, and glucagon. Furthermore, the ILCs with INGAP treatment secreted higher levels of insulin and C-peptide during the differentiation process, illustrating a gain in the secreting capacity. INGAP or INGAP peptide can stimulate the proliferation of ductal cells, thereby maintaining a pool of possible precursors of islet cells. In another study, short-term incubation of the primitive duct-like structures derived from quiescent adult human islets with INGAP peptide induced a re-differentiation back to islet-like structures. Those newly generated islets resembled freshly isolated islets with respect to the number and topological arrangement of cell types within an islet and the capacity of glucose-stimulated insulin secretion. Furthermore, these islet-like structures also express the islet-specific transcription factors Pdx1, ISL-1, and Nkx-2.2 and the islet-specific proteins GLUT2 and C-peptide to levels comparable to freshly isolated islets (Rafaeloff et al. 1997). Thus, limited in vitro evidence supports the role of INGAP and Reg1 not only in β-cell proliferation but, more interestingly, also in new β-cell formation, especially through a process of transdifferentiation.

General and Isoform-Specific Functions of Reg Proteins

Different isoforms also share some similar biological effects in the pancreatic development and pathophysiological conditions due to structural resemblances. Both Reg1 and INGAP contribute to pancreatic cell protection against apoptosis during oxidative stress. The level of Reg1 and INGAP transcripts was increased significantly in hamsters with chronic diabetes induced by streptozotocin and was slightly decreased upon administration of the antioxidant probucol. The latter caused an increase in number of insulin-positive cells in the pancreata of diabetic hamsters (Takatori et al. 2003). As well, both Reg1 and INGAP were suggested to play a role in the differentiation of stem cells into the islet cells and transdifferentiating endogenous pancreatic cells to islet cells.

However, members of the Reg family also possess isoform-specific properties. For example, several Reg proteins can be induced in the pancreas in response to

injury, such as pancreatectomy or caerulein-induced acute pancreatitis, in an isoform-specific fashion (Graf et al. 2002; De Leon et al. 2006). Upon caerulein or sodium taurocholate treatment, the level of Reg3 proteins, especially Reg3β, showed an acute increase in the first day after the induction of pancreatitis that rapidly returned to baseline, while Reg1 exhibited a persistent elevation and did not return to normal level after 35 days of caerulein injection. Post-injury level of Reg3β, but not Reg1, showed the highest induction and was significantly correlated with the severity of the pancreatic injury and the mortality rate (Graf et al. 2002; Zenilman et al. 2000). Administration of anti-Reg1 and/or anti-Reg3α antibody to rats with established pancreatitis increased pancreatic wet weight, indicating worsened tissue inflammation and cell necrosis (Viterbo et al. 2009). Thus, endogenous Reg1 and Reg3α, but not Reg3β, seem to be protective against the onset of acute pancreatitis.

Both the synthesis and secretion of Reg1 and Reg3 proteins (Reg3α, Reg3β, Reg3δ) were increased after 9 weeks of age in the chronic pancreatic WBN/Kob rat and peaked at 6 months. Elevations of these proteins correlated with the disease progression and coincided with increased cell apoptosis and tissue fibrosis (Bimmler et al. 2004). Using immunoreactivity, both Reg1 and Reg3 proteins were increased and colocalized to the same areas of pancreatic acinar cells displaying active inflammation and fibrosis. The immunogold technique revealed the intracellular localization of Reg1 and Reg3 proteins in the secretory apparatus. However, despite the anti-inflammatory and antiapoptotic effect of Reg3 proteins, the acinar damage in the WBN/Kob rat might be attributed more to Reg1, as supported by the elongated structures with fibrillar contents formed from fusion of Reg1-positive zymogen granules (Meili et al. 2003).

In a subset of gastric cancers, both Reg1A and Reg4 were overexpressed, suggesting their involvement in gastric carcinogenesis. Reg1A expression was closely related to the venous invasion and tumor stage, whereas Reg4 showed no such clear relationship (Yamagishi et al. 2009). Although several Reg proteins have been demonstrated to promote islet cell growth, survival, and/or function, there are signs of isoform specificities. Their pattern of activation in the rat intestine following antidiabetic duodenal-jejunal bypass was isoform and segment specific (Li et al. 2013b). For isoforms of Reg3, we and others have found that Reg3α promoted cell proliferation in vitro (Cui et al. 2009); overexpressed Reg3β and Reg3δ (INGAP) protected the β-cells in vivo against streptozotocin-induced diabetes (Taylor-Fishwick et al. 2006a; Xiong et al. 2011; Chang et al. 2011); although the expression of Reg3γ in regenerating pancreas was significantly induced, its direct effect on β-cells has not been studied (Rankin and Kushner 2010; De Leon et al. 2006).

Molecular Factors Regulating the Expression of Reg Family Genes

The molecular mechanisms regulating Reg protein expression are not fully understood yet. Regulating factors and their interactions may differ depending on the tissue and specific isoform under study. Reg proteins are induced by several inflammatory cytokines, including IL-1β, IL-6, and TNF-α (Dusetti et al. 1995). Reg1 mRNA expression is induced by the combination of IL-6 and dexamethasone,

but not by the treatment with an individual pro- or anti-inflammatory factor, such as IL-1β, TNF-α, IFNγ, dexamethasone, or IL-6. Induction of Reg1 mRNA levels by the combined IL-6/dexamethasone is further enhanced by the addition of nicotinamide or 3-aminobenzamide, the inhibitors for PARP which normally binds to a 12-bp cis element on Reg1 promoter (TGCCCCTCCCAT) and inhibits the formation of protein/DNA complex (Akiyama et al. 2001). Thus, PARP is a negative regulator of Reg1 transcription in the β-cells. The highly conserved element at -81-bp/-70-bp region of the Reg1 promoter has been proven essential for the activations by both IL-6/dexamethasone and IL-6/dexamethasone/nicotinamide treatments (Akiyama et al. 2001).

Both human Reg1A and Reg3A proteins are downstream targets of the Wnt/β-catenin pathway during liver tumorigenesis (Cavard et al. 2006). Upregulation of Reg1A and Reg3A gene expression was confirmed in the liver by Northern blot analysis and immunohistochemistry. In the adenoma and hepatocellular carcinoma, a strong immunoreactivity was detected for Reg3A and a less pronounced signal for Reg1A. Using the Huh7 hepatoma cell line, the Reg3A gene was upregulated upon the activation of Wnt/β-catenin signaling by stabilizing β-catenin in its unphosphorylated form with lithium chloride (LiCl). This induction was abolished by inhibition of β-catenin signaling with siRNA (Cavard et al. 2006). The overexpression of Reg1A and Reg3A and the activation of the Wnt/β-catenin pathway were also detected in colon adenoma from familial adenomatous polyposis, but not in the pediatric liver tumor hepatoblastoma (Cavard et al. 2006).

Interestingly, Reg3β gene expression can be induced during both TNF–αinduced cell apoptosis and Cdx1-induced cell proliferation. Long-term incubation of acinar AR42J cells with TNF-α induced cell apoptosis. In the meantime, Reg3β gene expression was induced by a MAPK/MEK1-mediated pathway which antagonized TNF-α-induced cell death (Harrison et al. 1998). Reg3β prevents the activation of macrophages by TNF-α, probably through inhibition of the nuclear translocation of NF-κB (Vasseur et al. 2004). In intestinal epithelial cells, Reg3β gene expression was increased at both mRNA and protein levels by Cdx1 overexpression. Cdx1 may directly activate the Reg3β promoter through a putative Cdx1-response element. Overexpression of the Reg3β gene or in vitro administration of Reg3β protein significantly increased the proliferation of intestinal cells (Zhao et al. 1997).

In addition, IL-22 also caused a robust induction of Reg3β mRNA in an acinar cell line, but not in a β-cell line, presumably through activation of STAT3 and resulting changes in gene transcription. Ex vivo incubation of isolated acinar cells with IL-22 also induced a substantial upregulation of Reg3β mRNA and a modest effect on the expression of osteopontin (OPN), a proliferative glycoprotein that can be induced by Reg3β itself (Xiong et al. 2011). Deficiency of the IL-22 receptor in mice resulted in loss of Reg3β mRNA upregulation upon IL-22 injection (Aggarwal et al. 2001). The activation of toll-like receptor 2 (TLR2) is required for *Yersinia*-induced expression of Reg3β and the subsequent clearance of the bacterial load in Peyer's patches or aggregated lymphoid nodules (Dessein et al. 2009). Reg3β can also increase its own transcription by increasing the binding of the nuclear factors C/EBPβ, P-CREB, P-ELK1, EGR1, STAT3, and ETS2 to its own promoters. This

self-activation seems to involve MAPK signaling and the activation of p44/p42, p38, and JNK (Ferrés-Masó et al. 2009).

The implantation of Reg3β-expressing hepatocytes into SCID mice enhanced liver regeneration following hepatectomy, probably by modulating the effects of TNF-α, IL-6, and STAT3, thus shortening the cell cycles of hepatocytes (Lieu et al. 2005). In Reg3β-deficient mice, there was a delayed ERK and AKT signaling but persistent activation of TNF-α/IL-6/STAT3 pathway during liver regeneration following hepatectomy, which may induce delayed liver regeneration and persistently inflammatory condition (Lieu et al. 2006).

Perspective

After two decades of research, more Reg family proteins have been characterized with relevance to the function of endocrine and exocrine pancreas. We remain hopeful that some isoforms of Reg proteins under certain circumstances will be proved to promote islet cell growth, regeneration, or survival against harsh conditions. There are plenty of indirect evidences showing associations of the changes in Reg proteins with islet regeneration or protection; more transgenic and knockout mice are being developed; however, in vitro direct incubations with the islet cells and recombinant proteins need to be carefully performed. We hope one day to demonstrate that Reg proteins are secreted extracellularly and act on cell membrane receptor(s); however, the only evidence of Reg1 receptor is still thin. The classification of Reg family proteins in Table 1 and our preliminary analysis on their regulation, effects, and isoform specificity should help to expand our research toward establishing the biological relevance of Reg proteins.

Acknowledgments Our research activity was supported by the Canadian Diabetes Association (OG-3-11-3469-JL) and the China Scholarship Council (201208370055).

Cross-References

▶ Mechanisms of Pancreatic β-Cell Apoptosis in Diabetes and Its Therapies
▶ Pancreatic Neuroendocrine Tumors
▶ Stem Cells in pancreatic Islets

References

Abe M et al (2000) Identification of a novel Reg family gene, Reg IIIdelta, and mapping of all three types of Reg family gene in a 75 kilobase mouse genomic region. Gene 246 (1–2):111–122

Acquatella-Tran Van Ba I et al (2012) Regenerating islet-derived 1α (Reg-1α) protein is new neuronal secreted factor that stimulates neurite outgrowth via exostosin Tumor-like 3 (EXTL3) receptor. J Biol Chem 287(7):4726–4739

Aggarwal S et al (2001) Acinar cells of the pancreas are a target of interleukin-22. J Interferon Cytokine Res 21(12):1047–1053
Akiyama T et al (2001) Activation of Reg gene, a gene for insulin-producing β-cell regeneration: poly(ADP-ribose) polymerase binds Reg promoter and regulates the transcription by autopoly (ADP-ribosyl)ation. Proc Natl Acad Sci USA 98(1):48–53
Algul H et al (2007) Pancreas-specific RelA/p65 truncation increases susceptibility of acini to inflammation-associated cell death following cerulein pancreatitis. J Clin Invest 117 (6):1490–1501
Anastasi E et al (1999) Expression of Reg and cytokeratin 20 during ductal cell differentiation and proliferation in a mouse model of autoimmune diabetes. Eur J Endocrinol 141(6):644–652
Ashcroft FJ et al (2004) Control of expression of the lectin-like protein Reg-1 by gastrin: role of the Rho family GTPase RhoA and a C-rich promoter element. Biochem J 381(Pt 2):397–403
Astorri E et al (2010) Circulating Reg1α proteins and autoantibodies to Reg1α proteins as biomarkers of β-cell regeneration and damage in type 1 diabetes. Horm Metab Res 42 (13):955–960
Baeza N et al (1997) Specific reg II gene overexpression in the non-obese diabetic mouse pancreas during active diabetogenesis. FEBS Lett 416(3):364–368
Baeza N et al (2001) Pancreatitis-associated protein (HIP/PAP) gene expression is upregulated in NOD mice pancreas and localized in exocrine tissue during diabetes. Digestion 64(4):233–239
Barbosa H et al (2006) Islet Neogenesis Associated Protein (INGAP) modulates gene expression in cultured neonatal rat islets. Regul Pept 136(1–3):78–84
Bartoli C et al (1993) A gene homologous to the reg gene is expressed in the human pancreas. FEBS Lett 327(3):289–293
Bartoli C et al (1998) Expression of peptide-23/pancreatitis-associated protein and Reg genes in human pituitary and adenomas: comparison with other fetal and adult human tissues. J Clin Endocrinol Metab 83(11):4041–4046
Billestrup N, Nielsen JH (1991) The stimulatory effect of growth hormone, prolactin, and placental lactogen on β-cell proliferation is not mediated by insulin-like growth factor-I. Endocrinology 129(2):883–888
Bimmler D et al (1999) Regulation of PSP/reg in rat pancreas: immediate and steady-state adaptation to different diets. Pancreas 19(3):255–267
Bimmler D et al (2004) Coordinate regulation of PSP/reg and PAP isoforms as a family of secretory stress proteins in an animal model of chronic pancreatitis. J Surg Res 118 (2):122–135
Bishnupuri KS et al (2010) Reg IV regulates normal intestinal and colorectal cancer cell susceptibility to radiation-induced apoptosis. Gastroenterology 138(2):616.e1–626.e2
Bonner-Weir S (2000a) Life and death of the pancreatic β cells. Trends Endocrinol Metab 11 (9):375–378
Bonner-Weir S (2000b) Perspective: postnatal pancreatic β cell growth. Endocrinology 141 (6):1926–1929
Bonner-Weir S et al (2004) The pancreatic ductal epithelium serves as a potential pool of progenitor cells. Pediatr Diabetes 5:16–22
Bonner-Weir S et al (2008) Transdifferentiation of pancreatic ductal cells to endocrine β-cells. Biochem Soc Trans 36(Pt 3):353–356
Borelli MI et al (2005) INGAP-related pentadecapeptide: its modulatory effect upon insulin secretion. Regul Pept 131(1–3):97–102
Bouwens L et al (1994) Cytokeratins as markers of ductal cell differentiation and islet neogenesis in the neonatal rat pancreas. Diabetes 43(11):1279–1283
Cavard C et al (2006) Overexpression of regenerating islet-derived 1 α and 3 α genes in human primary liver tumors with β-catenin mutations. Oncogene 25(4):599–608
Chakraborty C et al (1995) Age-related changes in peptide-23/pancreatitis-associated protein and pancreatic stone protein/reg gene expression in the rat and regulation by growth hormone-releasing hormone. Endocrinology 136(5):1843–1849

Chang TJ et al (2011) Targeted expression of INGAP to β cells enhances glucose tolerance and confers resistance to streptozotocin-induced hyperglycemia. Mol Cell Endocrinol 335 (2):104–109
Cheng CM et al (2000) Insulin-like growth factor 1 regulates developing brain glucose metabolism. Proc Natl Acad Sci USA 97(18):10236–10241
Choi JH et al (2010) Isolation of genes involved in pancreas regeneration by subtractive hybridization. Biol Chem 391(9):1019–1029
Choi SM et al (2013) Innate Stat3-mediated induction of the antimicrobial protein Reg3gamma is required for host defense against MRSA pneumonia. J Exp Med 210(3):551–561
Christa L et al (1996) HIP/PAP is an adhesive protein expressed in hepatocarcinoma, normal Paneth, and pancreatic cells. Am J Physiol 271(6 Pt 1):G993–G1002
Closa D, Motoo Y, Iovanna JL (2007) Pancreatitis-associated protein: from a lectin to an anti-inflammatory cytokine. World J Gastroenterol 13(2):170–174
Cozar-Castellano I et al (2004) Induction of β-cell proliferation and retinoblastoma protein phosphorylation in rat and human islets using adenovirus-mediated transfer of CDK4 and cyclin D1. Diabetes 53(1):149–159
Cui W et al (2009) Overexpression of Reg3α increases cell growth and the levels of cyclin D1 and CDK4 in insulinoma cells. Growth Factors 27(3):195–202
D'Amour KA et al (2006) Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotechnol 24(11):1392–1401
D'Ercole AJ (1999) Actions of IGF system proteins from studies of transgenic and gene knockout models. In: Rosenfeld RG, Roberts J (eds) The IGF system: molecular biology, physiology, and clinical applications. Humana Press, Totowa, pp 545–576
De Leon DD et al (2006) Identification of transcriptional targets during pancreatic growth after partial pancreatectomy and exendin-4 treatment. Physiol Genomics 24(2):133–143
Dessein R et al (2009) Toll-like receptor 2 is critical for induction of Reg3β expression and intestinal clearance of *Yersinia pseudotuberculosis*. Gut 58(6):771–776
Dheen ST, Rajkumar K, Murphy LJ (1997) Islet cell proliferation and apoptosis in insulin-like growth factor binding protein-1 in transgenic mice. J Endocrinol 155(3):551–558
Diehl JA et al (1998) Glycogen synthase kinase-3β regulates cyclin D1 proteolysis and subcellular localization. Genes Dev 12(22):3499–3511
Dor Y et al (2004) Adult pancreatic β-cells are formed by self-duplication rather than stem-cell differentiation. Nature 429(6987):41–46
Dungan KM, Buse JB, Ratner RE (2009) Effects of therapy in type 1 and type 2 diabetes mellitus with a peptide derived from islet neogenesis associated protein (INGAP). Diabetes Metab Res Rev 25(6):558–565
Dusetti NJ et al (1994) Molecular cloning, genomic organization, and chromosomal localization of the human pancreatitis-associated protein (PAP) gene. Genomics 19(1):108–114
Dusetti NJ et al (1995) Pancreatitis-associated protein I (PAP I), an acute phase protein induced by cytokines. Identification of two functional interleukin-6 response elements in the rat PAP I promoter region. J Biol Chem 270(38):22417–22421
Ferrés-Masó M et al (2009) PAP1 signaling involves MAPK signal transduction. Cell Mol Life Sci 66(13):2195–2204
Folch-Puy E et al (2006) Pancreatitis-associated protein I suppresses NF-kappa B activation through a JAK/STAT-mediated mechanism in epithelial cells. J Immunol 176(6):3774–3779
Frigerio JM et al (1993a) Identification of a second rat pancreatitis-associated protein. Messenger RNA cloning, gene structure, and expression during acute pancreatitis. Biochemistry 32 (35):9236–9241
Frigerio JM et al (1993b) The pancreatitis associated protein III (PAP III), a new member of the PAP gene family. Biochim Biophys Acta 1216(2):329–331
Fujishiro M et al (2012) Regenerating gene (REG) 1 α promotes pannus progression in patients with rheumatoid arthritis. Mod Rheumatol 22(2):228–237

Gedulin BR et al (2005) Exenatide (exendin-4) improves insulin sensitivity and β-cell mass in insulin-resistant obese fa/fa Zucker rats independent of glycemia and body weight. Endocrinology 146(4):2069–2076
George M et al (2002) β cell expression of IGF-I leads to recovery from type 1 diabetes. J Clin Invest 109(9):1153–1163
Georgia S, Bhushan A (2004) β cell replication is the primary mechanism for maintaining postnatal β cell mass. J Clin Invest 114(7):963–968
Gigoux V et al (2008) Reg genes are CCK2 receptor targets in ElasCCK2 mice pancreas. Regul Pept 146(1–3):88–98
Gironella M et al (2007) Experimental acute pancreatitis in PAP/HIP knock-out mice. Gut 56 (8):1091–1097
Gironella M et al (2013) Reg3β deficiency impairs pancreatic tumor growth by skewing macrophage polarization. Cancer Res 73(18):5682–5694
Graf R et al (2001) A family of 16-kDa pancreatic secretory stress proteins form highly organized fibrillar structures upon tryptic activation. J Biol Chem 276(24):21028–21038
Graf R et al (2002) Coordinate regulation of secretory stress proteins (PSP/reg, PAP I, PAP II, and PAP III) in the rat exocrine pancreas during experimental acute pancreatitis. J Surg Res 105 (2):136–144
Graf R et al (2006) Exocrine meets endocrine: pancreatic stone protein and regenerating protein–two sides of the same coin. J Surg Res 133(2):113–120
Gross DJ et al (1998) Amelioration of diabetes in nonobese diabetic mice with advanced disease by linomide-induced immunoregulation combined with Reg protein treatment. Endocrinology 139(5):2369–2374
Gu G, Brown JR, Melton DA (2003) Direct lineage tracing reveals the ontogeny of pancreatic cell fates during mouse embryogenesis. Mech Dev 120(1):35–43
Gurr W et al (2002) A Reg family protein is overexpressed in islets from a patient with new-onset type 1 diabetes and acts as T-cell autoantigen in NOD mice. Diabetes 51(2):339–346
Gurr W et al (2007) RegII is a β-cell protein and autoantigen in diabetes of NOD mice. Diabetes 56 (1):34–40
Hamblet NS et al (2008) The Reg family member INGAP is a marker of endocrine patterning in the embryonic pancreas. Pancreas 36(1):1–9
Hansson A, Thoren M (1995) Activation of MAP kinase in Swiss 3T3 fibroblasts by insulin-like growth factor-I. Growth Regul 5(2):92–100
Hansson A, Hehenberger K, Thoren M (1996) Long-term treatment of Swiss 3T3 fibroblasts with dexamethasone attenuates MAP kinase activation induced by insulin-like growth factor- I (IGF-I). Cell Biochem Funct 14(2):121–129
Harbeck MC et al (1996) Expression of insulin receptor mRNA and insulin receptor substrate 1 in pancreatic islet β-cells. Diabetes 45(6):711–717
Harrison M et al (1998) Growth factor protection against cytokine-induced apoptosis in neonatal rat islets of Langerhans: role of Fas. FEBS Lett 435(2–3):207–210
Hartupee JC et al (2001) Isolation and characterization of a cDNA encoding a novel member of the human regenerating protein family: Reg IV. Biochim Biophys Acta 1518(3):287–293
Hashimoto N et al (2006) Ablation of PDK1 in pancreatic β cells induces diabetes as a result of loss of β cell mass. Nat Genet 38(5):589–593
He S-Q et al (2010) Inflammation and nerve injury induce expression of pancreatitis-associated protein-II in primary sensory neurons. Mol Pain 6:23
Heald A, Stephens R, Gibson JM (2006) The insulin-like growth factor system and diabetes–an overview. Diabet Med 23(Suppl 1):19–24
Heiskala K et al (2010) Expression of Reg IV and Hath1 in neuroendocrine neoplasms. Histol Histopathol 25(1):63–72
Hervieu V et al (2006) HIP/PAP, a member of the reg family, is expressed in glucagon-producing enteropancreatic endocrine cells and tumors. Hum Pathol 37(8):1066–1075

Hill DJ et al (1999) Insulin-like growth factors prevent cytokine-mediated cell death in isolated islets of Langerhans from pre-diabetic non-obese diabetic mice. J Endocrinol 161(1):153–165
Hodin CM et al (2011) Starvation compromises Paneth cells. Am J Pathol 179(6):2885–2893
Honda H, Nakamura H, Otsuki M (2002) The elongated PAP II/Reg III mRNA is upregulated in rat pancreas during acute experimental pancreatitis. Pancreas 25(2):192–197
Hu G et al (2011) Reg4 protects against acinar cell necrosis in experimental pancreatitis. Gut 60 (6):820–828
Huszarik K et al (2010) Adjuvant immunotherapy increases β cell regenerative factor Reg2 in the pancreas of diabetic mice. J Immunol 185(9):5120–5129
Iovanna J et al (1991) Messenger RNA sequence and expression of rat pancreatitis-associated protein, a lectin-related protein overexpressed during acute experimental pancreatitis. J Biol Chem 266(36):24664–24669
Iovanna JL et al (1993) PAP, a pancreatic secretory protein induced during acute pancreatitis, is expressed in rat intestine. Am J Physiol 265(4 Pt 1):G611–G618
Itoh T, Teraoka H (1993) Cloning and tissue-specific expression of cDNAs for the human and mouse homologues of rat pancreatitis-associated protein (PAP). Biochim Biophys Acta 1172 (1–2):184–186
Jamal AM et al (2005) Morphogenetic plasticity of adult human pancreatic islets of Langerhans. Cell Death Differ 12:702–712
Johansson ME, Hansson GC (2011) Microbiology. Keeping bacteria at a distance. Science 334 (6053):182–183
Jung EJ, Kim CW (2002) Interaction between chicken protein tyrosine phosphatase 1 (CPTP1)-like rat protein phosphatase 1 (PTP1) and p60(v-src) in v-src-transformed Rat-1 fibroblasts. Exp Mol Med 34(6):476–480
Kamarainen M et al (2003) RELP, a novel human REG-like protein with up-regulated expression in inflammatory and metaplastic gastrointestinal mucosa. Am J Pathol 163(1):11–20
Kamimura T, West C, Beutler E (1992) Sequence of a cDNA clone encoding a rat Reg-2 protein. Gene 118(2):299–300
Kapur R et al (2012) Short-term effects of INGAP and Reg family peptides on the appearance of small β-cells clusters in non-diabetic mice. Islets 4(1):40–48
Keim V, Loffler HG (1986) Pancreatitis-associated protein in bile acid-induced pancreatitis of the rat. Clin Physiol Biochem 4(2):136–142
Keim V et al (1984) An additional secretory protein in the rat pancreas. Digestion 29(4):242–249
Kimura N et al (1992) Expression of human regenerating gene mRNA and its product in normal and neoplastic human pancreas. Cancer 70(7):1857–1863
Klasan GS et al (2013) Reg3G gene expression in regenerating skeletal muscle and corresponding nerve. Muscle Nerve 49(1):61–68
Kobayashi S et al (2000) Identification of a receptor for reg (regenerating gene) protein, a pancreatic β-cell regeneration factor. J Biol Chem 275(15):10723–10726
Konishi H et al (2013) N-terminal cleaved pancreatitis-associated protein-III (PAP-III) serves as a scaffold for neurites and promotes neurite outgrowth. J Biol Chem 288(15):10205–10213
Lai Y et al (2012) The antimicrobial protein REG3A regulates keratinocyte proliferation and differentiation after skin injury. Immunity 37(1):74–84
Lasserre C et al (1992) A novel gene (HIP) activated in human primary liver cancer. Cancer Res 52 (18):5089–5095
Lasserre C et al (1994) Structural organization and chromosomal localization of a human gene (HIP/PAP) encoding a C-type lectin overexpressed in primary liver cancer. Eur J Biochem 224 (1):29–38
Laurine E et al (2005) PAP IB, a new member of the Reg gene family: cloning, expression, structural properties, and evolution by gene duplication. Biochim Biophys Acta 1727 (3):177–187
Lee KS et al (2012) Helicobacter pylori CagA triggers expression of the bactericidal lectin REG3gamma via gastric STAT3 activation. PLoS One 7(2):e30786

Levetan CS et al (2008) Discovery of a human peptide sequence signaling islet neogenesis. Endocr Pract 14(9):1075–1083
Levetan CS et al (2010) Human Reg3a gene protein as a novel islet neogenesis therapy for reversal of type 1 and 2 diabetes. In: American Diabetes Association, 70th scientific sessions, Orlando
Li J et al (2009) Islet neogenesis-associated protein-related pentadecapeptide enhances the differentiation of islet-like clusters from human pancreatic duct cells. Peptides 30(12):2242–2249
Li L et al (2010) PSP/reg inhibits cultured pancreatic stellate cell and regulates MMP/TIMP ratio. Eur J Clin Invest 41(2):151–158
Li B et al (2013a) Intestinal adaptation and Reg gene expression induced by antidiabetic duodenal-jejunal bypass surgery in Zucker fatty rats. Am J Physiol Gastrointest Liver Physiol 304(7): G635–G645
Li B et al (2013b) Intestinal adaptation and Reg gene expression induced by anti-diabetic duodenal-jejunal bypass surgery in Zucker fatty rats. Am J Physiol Gastrointestin Liver Physiol 304(7):G635–G645
Lieu HT et al (2005) HIP/PAP accelerates liver regeneration and protects against acetaminophen injury in mice. Hepatology 42(3):618–626
Lieu HT et al (2006) Reg2 inactivation increases sensitivity to Fas hepatotoxicity and delays liver regeneration post-hepatectomy in mice. Hepatology 44(6):1452–1464
Lipsett M et al (2007a) The role of islet neogenesis-associated protein (INGAP) in islet neogenesis. Cell Biochem Biophys 48(2–3):127–137
Lipsett MA, Castellarin ML, Rosenberg L (2007b) Acinar plasticity: development of a novel in vitro model to study human acinar-to-duct-to-islet differentiation. Pancreas 34(4):452–457
Liu JL, Cui W (2007) Which gene, Reg2 or Reg3β, was targeted that affected liver regeneration? Hepatology 45(6):1584–1585
Liu JL et al (2008) Possible roles of reg family proteins in pancreatic islet cell growth. Endocr Metab Immune Disord Drug Targets 8(1):1–10
Liu Y et al (2009) β-Cells at the crossroads: choosing between insulin granule production and proliferation. Diab Obes Metab 11:54–64
Liu L, Liu JL, Srikant CB (2010) Reg2 protects mouse insulinoma cells from streptozotocin-induced mitochondrial disruption and apoptosis. Growth Factors 28(5):370–378
Luo C et al (2013) Transcriptional activation of Reg2 and Reg3β genes by glucocorticoids and interleukin-6 in pancreatic acinar and islet cells. Mol Cell Endocrinol 365(2):187–196
Maake C, Reinecke M (1993) Immunohistochemical localization of insulin-like growth factor 1 and 2 in the endocrine pancreas of rat, dog, and man, and their coexistence with classical islet hormones. Cell Tissue Res 273(2):249–259
Mally MI et al (1994) Developmental gene expression in the human fetal pancreas. Pediatr Res 36 (4):537–544
Marselli L et al (2010) Gene expression profiles of β-cell enriched tissue obtained by laser capture microdissection from subjects with Type 2 diabetes. PLoS One 5(7):e11499
Mauras N et al (2000) Recombinant human insulin-like growth factor I has significant anabolic effects in adults with growth hormone receptor deficiency: studies on protein, glucose, and lipid metabolism. J Clin Endocrinol Metab 85(9):3036–3042. [MEDLINE record in process]
Meier JJ et al (2008) β-cell replication is the primary mechanism subserving the postnatal expansion of β-cell mass in humans. Diabetes 57(6):1584–1594
Meili S et al (2003) Secretory apparatus assessed by analysis of pancreatic secretory stress protein expression in a rat model of chronic pancreatitis. Cell Tissue Res 312(3):291–299
Moriizumi S et al (1994) Isolation, structural determination and expression of a novel reg gene, human regI β. Biochim Biophys Acta 1217(2):199–202
Moriscot C et al (1996) Absence of correlation between reg and insulin gene expression in pancreas during fetal development. Pediatr Res 39(2):349–353
Moses A et al (1996) Recombinant human insulin-like growth factor I increases insulin sensitivity and improves glycemic control in type II diabetes. Diabetes 45(1):91–100

Mueller CM, Zhang H, Zenilman ME (2008) Pancreatic reg I binds MKP-1 and regulates cyclin D in pancreatic-derived cells. J Surg Res 150(1):137–143
Multigner L et al (1983) Pancreatic stone protein, a phosphoprotein which inhibits calcium carbonate precipitation from human pancreatic juice. Biochem Biophys Res Commun 110 (1):69–74
Namikawa K et al (2005) Expression of Reg/PAP family members during motor nerve regeneration in rat. Biochem Biophys Res Commun 332(1):126–134
Narushima Y et al (1997) Structure, chromosomal localization and expression of mouse genes encoding type III Reg, RegIII α, RegIII β, RegIII γ. Gene 185(2):159–168
Nata K et al (2004) Molecular cloning, expression and chromosomal localization of a novel human REG family gene, REG III. Gene 340(1):161–170
Nguyen KT et al (2006) Essential role of Pten in body size determination and pancreatic β-cell homeostasis in vivo. Mol Cell Biol 26(12):4511–4518
Nishimune H et al (2000) Reg-2 is a motoneuron neurotrophic factor and a signalling intermediate in the CNTF survival pathway. Nat Cell Biol 2(12):906–914
O'Hara A et al (2013) The role of proteasome β subunits in gastrin-mediated transcription of plasminogen activator inhibitor-2 and regenerating protein1. PLoS One 8(3):e59913
Ochiai K et al (2004) Activated pancreatic enzyme and pancreatic stone protein (PSP/reg) in bile of patients with pancreaticobiliary maljunction/choledochal cysts. Dig Dis Sci 49 (11–12):1953–1956
Ohara S et al (2008) Reg IV is an independent prognostic factor for relapse in patients with clinically localized prostate cancer. Cancer Sci 99(8):1570–1577
Okamoto H (1999) The Reg gene family and Reg proteins: with special attention to the regeneration of pancreatic β-cells. J Hepatobiliary Pancreat Surg 6(3):254–262
Orelle B et al (1992) Human pancreatitis-associated protein. Messenger RNA cloning and expression in pancreatic diseases. J Clin Invest 90(6):2284–2291
Ose T et al (2007) Reg I-knockout mice reveal its role in regulation of cell growth that is required in generation and maintenance of the villous structure of small intestine. Oncogene 26 (3):349–359
Oue N et al (2005) Expression and localization of Reg IV in human neoplastic and non-neoplastic tissues: Reg IV expression is associated with intestinal and neuroendocrine differentiation in gastric adenocarcinoma. J Pathol 207(2):185–198
Parikh A, Stephan AF, Tzanakakis ES (2012) Regenerating proteins and their expression, regulation and signaling. Biomol Concept 3(1):57–70
Pelengaris S et al (2004) Brief inactivation of c-Myc is not sufficient for sustained regression of c-Myc-induced tumours of pancreatic islets and skin epidermis. BMC Biol 2:26
Perfetti R et al (1996a) Regenerating (reg) and insulin genes are expressed in prepancreatic mouse embryos. J Mol Endocrinol 17(1):79–88
Perfetti R et al (1996b) Differential expression of reg-I and reg-II genes during aging in the normal mouse. J Gerontol A Biol Sci Med Sci 51(5):B308–B315
Petropavlovskaia M et al (2012) Mechanisms of action of islet neogenesis-associated protein: comparison of the full-length recombinant protein and a bioactive peptide. Am J Physiol Endocrinol Metab 303(7):E917–E927
Pittenger GL et al (2007) Intramuscular injection of islet neogenesis-associated protein peptide stimulates pancreatic islet neogenesis in healthy dogs. Pancreas 34(1):103–111
Pittenger GL, Taylor-Fishwick D, Vinik AI (2009a) The role of islet neogenesis-associated protein (INGAP) in pancreatic islet neogenesis. Curr Protein Pept Sci 10(1):37–45
Pittenger GL, Taylor-Fishwick D, Vinik AI (2009b) A role for islet neogenesis in curing diabetes. Diabetologia 52(5):735–738
Pospisilik JA et al (2003) Dipeptidyl peptidase IV inhibitor treatment stimulates β-cell survival and islet neogenesis in streptozotocin-induced diabetic rats. Diabetes 52(3):741–750
Quaife CJ et al (1989) Histopathology associated with elevated levels of growth hormone and insulin-like growth factor I in transgenic mice. Endocrinology 124(1):40–48

Rafaeloff R et al (1997) Cloning and sequencing of the pancreatic islet neogenesis associated protein (INGAP) gene and its expression in islet neogenesis in hamsters. J Clin Invest 99 (9):2100–2109

Rane SG, Reddy EP (2000) Cell cycle control of pancreatic β cell proliferation. Front Biosci 5:D1–D19

Rane SG et al (1999) Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in β-islet cell hyperplasia. Nat Genet 22(1):44–52

Rankin MM, Kushner JA (2010) Aging induces a distinct gene expression program in mouse islets. Islets 2(6):4–11

Reichert M, Rustgi AK (2011) Pancreatic ductal cells in development, regeneration, and neoplasia. J Clin Invest 121(12):4572–4578

Rooman I, Lardon J, Bouwens L (2002) Gastrin stimulates β-cell neogenesis and increases islet mass from transdifferentiated but not from normal exocrine pancreas tissue. Diabetes 51 (3):686–690

Rosenberg L et al (2004) A pentadecapeptide fragment of islet neogenesis-associated protein increases β-cell mass and reverses diabetes in C57BL/6J mice. Ann Surg 240(5):875–884

Rouimi P et al (1988) The disulfide bridges of the immunoreactive forms of human pancreatic stone protein isolated from pancreatic juice. FEBS Lett 229(1):171–174

Rouquier S et al (1991) Rat pancreatic stone protein messenger RNA. Abundant expression in mature exocrine cells, regulation by food content, and sequence identity with the endocrine reg transcript. J Biol Chem 266(2):786–791

Sanchez D et al (2000) Overexpression of the reg gene in non-obese diabetic mouse pancreas during active diabetogenesis is restricted to exocrine tissue. J Histochem Cytochem 48 (10):1401–1410

Sanchez D et al (2001) Preferential expression of reg I β gene in human adult pancreas. Biochem Biophys Res Commun 284(3):729–737

Sanchez D et al (2004) Implication of Reg I in human pancreatic duct-like cells in vivo in the pathological pancreas and in vitro during exocrine dedifferentiation. Pancreas 29 (1):14–21

Sandgren EP et al (1990) Overexpression of TGF α in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. Cell 61(6):1121–1135

Sasahara K et al (2000) Molecular cloning and tissue-specific expression of a new member of the regenerating protein family, islet neogenesis-associated protein-related protein. Biochim Biophys Acta 1500(1):142–146

Scharfmann R, Corvol M, Czernichow P (1989) Characterization of insulin-like growth factor I produced by fetal rat pancreatic islets. Diabetes 38(6):686–690

Schiesser M et al (2001) Conformational changes of pancreatitis-associated protein (PAP) activated by trypsin lead to insoluble protein aggregates. Pancreas 22(2):186–192

Schmied BM et al (2001) Transdifferentiation of human islet cells in a long-term culture. Pancreas 23(2):157–171

Seaberg RM et al (2004) Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. Nat Biotechnol 22(9):1115–1124

Sherr CJ (2001) The INK4a/ARF network in tumour suppression. Nat Rev Mol Cell Biol 2 (10):731–737

Shervani NJ et al (2004) Autoantibodies to REG, a β-cell regeneration factor, in diabetic patients. Eur J Clin Invest 34(11):752–758

Sieradzki J et al (1988) Stimulatory effect of insulin-like growth factor-I on [^{3}H]thymidine incorporation, DNA content and insulin biosynthesis and secretion of isolated pancreatic rat islets. J Endocrinol 117(1):59–62

Skarnes WC et al (2011) A conditional knockout resource for the genome-wide study of mouse gene function. Nature 474(7351):337–342

Smith FE et al (1991) Enhanced insulin-like growth factor I gene expression in regenerating rat pancreas. Proc Natl Acad Sci USA 88(14):6152–6256

Smith FE et al (1994) Pancreatic Reg/pancreatic stone protein (PSP) gene expression does not correlate with β-cell growth and regeneration in rats. Diabetologia 37(10):994–999
Song SY et al (1999) Expansion of Pdx1-expressing pancreatic epithelium and islet neogenesis in transgenic mice overexpressing transforming growth factor α. Gastroenterology 117 (6):1416–1426
Spak E et al (2010) Changes in the mucosa of the Roux-limb after gastric bypass surgery. Histopathology 57(5):680–688
Suarez-Pinzon WL et al (2008) Combination therapy with glucagon-like peptide-1 and gastrin restores normoglycemia in diabetic NOD mice. Diabetes 57(12):3281–3288
Suzuki Y et al (1994) Structure and expression of a novel rat RegIII gene. Gene 144(2):315–316
Takasawa S et al (2006) Cyclin D1 activation through ATF-2 in Reg-induced pancreatic β-cell regeneration. FEBS Lett 580(2):585–591
Takatori A et al (2003) Protective effects of probucol treatment on pancreatic β-cell function of SZ-induced diabetic APA hamsters. Exp Anim 52(4):317–327
Taylor-Fishwick DA et al (2006a) Islet neogenesis associated protein transgenic mice are resistant to hyperglycemia induced by streptozotocin. J Endocrinol 190(3):729–737
Taylor-Fishwick DA et al (2006b) PDX-1 can repress stimulus-induced activation of the INGAP promoter. J Endocrinol 188(3):611–621
Taylor-Fishwick DA et al (2008) Pancreatic islet immunoreactivity to the Reg protein INGAP. J Histochem Cytochem 56(2):183–191
Taylor-Fishwick DA et al (2010) Pdx-1 regulation of the INGAP promoter involves sequestration of NeuroD into a non-DNA-binding complex. Pancreas 39(1):64–70
Terazono K et al (1988) A novel gene activated in regenerating islets. J Biol Chem 263 (5):2111–2114
Terazono K et al (1990) Expression of reg protein in rat regenerating islets and its co-localization with insulin in the β cell secretory granules. Diabetologia 33(4):250–252
Teta M et al (2007) Growth and regeneration of adult β cells does not involve specialized progenitors. Dev Cell 12(5):817–826
Tezel E et al (2004) REG I as a marker for human pancreatic acinoductular cells. Hepatogastroenterology 51(55):91–96
Tourrel C et al (2001) Glucagon-like peptide-1 and exendin-4 stimulate β-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. Diabetes 50(7):1562–1570
Unno M et al (1993) Structure, chromosomal localization, and expression of mouse reg genes, reg I and reg II. A novel type of reg gene, reg II, exists in the mouse genome. J Biol Chem 268 (21):15974–15982
Unno M et al (2002) Production and characterization of Reg knockout mice: reduced proliferation of pancreatic β-cells in Reg knockout mice. Diabetes 51(Suppl 3):S478–S483
Vaishnava S et al (2011) The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. Science 334(6053):255–258
Vasseur S et al (2004) p8 improves pancreatic response to acute pancreatitis by enhancing the expression of the anti-inflammatory protein pancreatitis-associated protein I. J Biol Chem 279 (8):7199–7207
Violette S et al (2003) Reg IV, a new member of the regenerating gene family, is overexpressed in colorectal carcinomas. Int J Cancer 103(2):185–193
Viterbo D et al (2009) Administration of anti-Reg I and anti-PAPII antibodies worsens pancreatitis. JOP 10(1):15–23
Waelput W et al (2000) Identification and expression analysis of leptin-regulated immediate early response and late target genes. Biochem J 348(Pt 1):55–61
Wang TC et al (1993) Pancreatic gastrin stimulates islet differentiation of transforming growth factor α-induced ductular precursor cells. J Clin Invest 92(3):1349–1356
Wang RN et al (1997) Expression of gastrin and transforming growth factor-α during duct to islet cell differentiation in the pancreas of duct-ligated adult rats. Diabetologia 40(8):887–893

Wang F et al (2011) Identification of RegIV as a novel GLI1 target gene in human pancreatic cancer. PLoS One 6(4):e18434
Watanabe T et al (1990) Complete nucleotide sequence of human reg gene and its expression in normal and tumoral tissues. The reg protein, pancreatic stone protein, and pancreatic thread protein are one and the same product of the gene. J Biol Chem 265(13):7432–7439
Watanabe T et al (1994) Pancreatic β-cell replication and amelioration of surgical diabetes by Reg protein. Proc Natl Acad Sci USA 91(9):3589–3592
Wilding Crawford L et al (2008) Gene expression profiling of a mouse model of pancreatic islet dysmorphogenesis. PLoS One 3(2):e1611
Xiong X et al (2011) Pancreatic islet-specific overexpression of Reg3β protein induced the expression of pro-islet genes and protected mice against streptozotocin-induced diabetes. Am J Physiol Endocrinol Metab 300:E669–E680
Xu G et al (1999) Exendin-4 stimulates both β-cell replication and neogenesis, resulting in increased β-cell mass and improved glucose tolerance in diabetic rats. Diabetes 48 (12):2270–2276
Xu G et al (2006) GLP-1/exendin-4 facilitates [β]-cell neogenesis in rat and human pancreatic ducts. Diabetes Res Clin Pract 73(1):107–110
Xu X et al (2008) β cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 132(2):197–207
Yamagishi H et al (2009) Expression profile of REG family proteins REG I[α] and REG IV in advanced gastric cancer: comparison with mucin phenotype and prognostic markers. Mod Pathol 22(7):906–913
Yamaoka T et al (2000) Diabetes and tumor formation in transgenic mice expressing Reg I. Biochem Biophys Res Commun 278(2):368–376
Ying LS et al (2013) Enhanced RegIV expression predicts the intrinsic 5-fluorouracil (5-FU) resistance in advanced gastric cancer. Dig Dis Sci 58(2):414–422
Zenilman ME et al (1996a) Pancreatic regeneration (reg) gene expression in a rat model of islet hyperplasia. Surgery 119(5):576–584
Zenilman ME et al (1996b) Pancreatic thread protein is mitogenic to pancreatic-derived cells in culture. Gastroenterology 110(4):1208–1214
Zenilman ME et al (1997) Pancreatic reg gene expression is inhibited during cellular differentiation. Ann Surg 225(3):327–332
Zenilman ME, Chen J, Magnuson TH (1998) Effect of reg protein on rat pancreatic ductal cells. Pancreas 17(3):256–261
Zenilman ME et al (2000) Comparison of reg I and reg III levels during acute pancreatitis in the rat. Ann Surg 232(5):646–652
Zhang Y et al (2003) Reg IV, a differentially expressed gene in colorectal adenoma. Chin Med J (Engl) 116(6):918–922
Zhao AZ et al (1997) Attenuation of insulin secretion by insulin-like growth factor 1 is mediated through activation of phosphodiesterase 3B. Proc Natl Acad Sci USA 94(7):3223–3228
Zhou J, Bievre M, Bondy CA (2000) Reduced GLUT1 expression in $Igf1^{-/-}$ null oocytes and follicles. Growth Horm IGF Res 10(3):111–117

Inflammatory Pathways Linked to β Cell Demise in Diabetes

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_30,

Abstract

Inflammation is proposed to play a key role in the development of both type 1 diabetes (T1D) and type 2 diabetes (T2D). It is well established that autoimmunity against β cells is responsible for massive loss of β cells in T1D. Recently, it has been recognized that chronic low-grade inflammation is not limited to insulin target organs but is also seen in islets in T2D. In T1D, T lymphocytes are primed to destroy the β cells. However, the process that leads to the development of self-reactive T lymphocytes remains elusive and is an area of intense research. A complex interplay between genetic and environmental factors, β cells, and immune cells is likely involved in the process. Immunomodulatory therapies have been attempted with some promises in animal models of T1D but have not yielded satisfactory effects on humans. Recent initiatives to evaluate T1D pathology in human donor pancreata hold promise to increase our knowledge in the coming years. In T2D, overnutrition results in metabolic stress including glucolipotoxicity, endoplasmic reticulum stress, and oxidative stress that potentially trigger an inflammatory response in the islets. Substantial evidence exists for an increase in humoral inflammatory mediators and an accumulation of macrophages in the islets of T2D subjects. Anti-inflammatory therapies targeting IL-1β and NF*k*B have shown improvement in β cell functions in small short term studies of T2D humans, providing a proof of principle for targeting islet inflammation in T2D. However, the nature of islet inflammation in T2D needs better characterization to tailor anti-inflammatory therapies that are effective and durable for T2D.

Keywords

Type 1 diabetes • Type 2 diabetes • Cytokines • Immune cells • 12-Lipoxygenase

Abbreviation

12/15LO	12/15-Lipoxygenase
12-HETE	12-Hyrdroxyeicosatetraenoic acid
12LO	12-Lipoxygenase
A1c	Hemoglobin A1c
AAb	Autoantibody
ACE	Angiotensin-converting enzyme
AdipoR1	Adiponectin receptor 1
AMPK	AMP-activated kinase
ANGPTL8	Angiopoietin-like 8

AP-1	Activator protein-1
ARb	Angiotensin receptor blockers
AT	Adipose tissue
ATF6	Activating transcription factor-6
CANTOS	Canakinumab Anti-inflammatory Thrombosis Outcomes Study
CB1	Cannabinoid receptor type 1
CCL	Chemokine (C-C motif) ligand
CDKAL1	CDK5 regulatory subunit-associated protein-1-like
CHOP	C/EBP homology protein
CLECL1	C-type leptin-like 1
CVD	Cardiovascular disease
CXCL	Chemokine(C-X-C motif) ligand
DAISY	Diabetes Association in Support of Youth
DC	Dendritic cells
DEXI	Dexamethasone induced
DHA	Docosahexaenoic acid
DMT1	Divalent metal transporter 1
DPPIV	Dipeptidyl peptidase-IV
EIF2AK3	Eukaryotic translation initiation factor 2α kinase-3
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinases
FasL	Fas ligand
GAD	Glutamic acid decarboxylase
GIP	Gastric inhibitory polypeptide
GK rat	Goto-Kakizaki rat
GLIS3	Gli-similar 3
GLP	Glucagon-like peptide
GWAS	Genome-wide association study
HLA	Human leukocyte antigen
HOMA	Homeostasis Model Assessment
hsCRP	High sensitivity C-reactive protein
IA2	Islet antigen 2
IAA	Insulin autoantibody
IAPP	Islet amyloid polypeptide
ICA	Islet cell antibody
IFN	Interferon
IL	Interleukin
IL-18RAP	IL-18R accessory protein
IL-1Ra	IL-1 receptor antagonist
iNKT	Invariant natural killer T cells
IRE1	Inositol requiring enzyme-1
IRS	Insulin receptor substrate
JDRF	Juvenile Diabetes Research Foundation
JNK	c-Jun N-terminal kinase

KIR	Killer immunoglobulin-like receptor
LADA	Latent autoimmune diabetes of adults
Lp	*Lactobacillus plantarum*
LPS	Lipopolysaccharide
MΦ	Macrophages
MAPK	Mitogen-activated protein kinase
MHC	Major histocompatibility complex
MIP	Macrophage Inflammatory Proteins
NADPH	Nicotinamide adenine dinucleotide phosphate
NEFA	Non-esterified fatty acids
NFκB	Nuclear factor kappa light chain enhancer of activated B cells
NK	Natural killer
NLRP3	NLR family, pyrin domain containing 3
NOD	Nonobese diabetic
Non-DM	Nondiabetic
nPOD	Network for the Pancreatic Organ Donor with Diabetes
OM	Omental
PA	Palmitic acids
PERK	PKR-like eukaryotic initiation factor 2α kinase
PEVNET	Persistent Virus Infection in Diabetes Network
PI3K	Phosphoinositide 3-kinase
PKB	Protein kinase B
PPAR	Peroxisome proliferator-activated receptor
PTB1B	Protein tyrosine phosphatase 1B
PTEN	Phosphatase and tensin homolog
qRT-PCR	Real-time reverse transcription polymerase chain reaction
ROS	Reactive oxygen species
SC	Subcutaneous
SLE	Systemic lupus erythematosus
SOCS3	Suppressor of cytokine signaling 3
STAT	Signal transducer and activator of signal transduction
STZ	Streptozotocin
SUOX	Sulfite oxidase
SVF	Seminal vesicle fluid
T1D	Type 1 diabetes
T2D	Type 2 diabetes
Th	T helper
TINSAL-T2D	Targeting-Inflammation Using Salsalate in Type 2 Diabetes
TLR4	Toll receptor 4
TNF	Tumor necrosis factor
TXNIP	Thioredoxin-interacting protein
UKPDS	United Kingdom Prospective Diabetes Study
UPR	Unfolded protein response
WFS1	Wolfram syndrome 1

Introduction

The incidence of diabetes, both T1D and T2D, is on the rise globally, creating an urgent need to understand their pathogenesis and to identify effective therapeutic targets. There is a substantial volume of evidence supporting the idea that inflammatory responses play an active role in the development of T1D and T2D. Inflammation serves as a defense system to protect a host by removing pathogens, foreign bodies, toxins, and damaged cells/tissues and promoting healing of tissues. Damaged or infected host cells produce humoral factors that communicate with immune cells to orchestrate inflammatory responses to achieve the removal of "harm" and to restore the normal architecture and function of cells. Innate immune responses involve immune cells without specified antigens, while adaptive immune responses target specified antigens through recognition by lymphocytes. It is well established that β cell loss in T1D results from an adaptive immune response against β cells. However, cross talk between β cells and variety of leukocytes, from both innate and adaptive immune responses, is required to establish self-reactive lymphocytes.

T2D was originally regarded as nonimmune-mediated diabetes. However, inflammation involving both innate and adaptive immune cells has been shown in insulin target tissues and peripheral circulation in T2D. More recently, signs of inflammation have been shown in human islets affected by T2D. Here, we will discuss the nature of islet inflammation in T1D and T2D, the communication of islets with other metabolic organs in T1D and T2D, and the current status of anti-inflammatory therapy for T1D and T2D.

Autoimmunity-Based Inflammation in T1D

General Introduction

Although the exact cause of T1D remains unknown, a great number of contributing factors have been appreciated. Many of these factors play into the development of autoimmunity and contribute to the inflammatory responses that result in the loss of β cells in T1D. Especially of note are the roles played by certain genetic haplotypes, potential environmental triggers (including viral infections, interactions of the immune system with the gut microbiome, and diet), immune cells (Mϕ, dendritic cells, B cells, T cells, and NK cells), and defects in the pancreatic islets themselves. This section will provide an overview of the natural progression of the disease as it is seen in the clinic, as well as the involvement of key cell types as we currently understand.

T1D is an inflammatory autoimmune disease in which the body's T cells are primed to destroy the β cells of the pancreatic islets. This autoimmune destruction leads to the ultimate reliance on exogenous sources of insulin for survival.

There are many facets to this disease, including altered T cell development (Rosmalen et al. 2002; Tanaka et al. 2010), the development of AAb (Ziegler and Nepom 2010), increased pancreatic inflammation (Arif et al. 2004; Saha and Ghosh 2012; Willcox et al. 2009), and deficiencies in regulatory T cells (Lindley et al. 2005). However, T cells alone are not solely responsible for diabetes development. There is also ample evidence to support the idea that the β cells themselves have defects in coping with ER stress (Marhfour et al. 2012; Tersey et al. 2012b). Yet more evidence suggests that β cells normally undergo apoptosis without autoimmunity arising, but in the presence of inflammatory insults, this apoptosis initiates the autoimmune cascade (Carrington et al. 2011; Roggli et al. 2012). When combined, all of these processes can lead to autoimmunity against the islets and a lifetime reliance on exogenous sources of insulin.

Perhaps the greatest indicator of potential diabetes development is the expression of certain susceptibility genes. Genetic susceptibility to T1D is defined by a multitude of genes, although the most important are those in the HLA haplotype. These genes determine the interactions of the T cell with MHC, which include the antigenic peptides that bind within the grooves of the MHC molecules. It appears that in the case of T1D, certain haplotypes lead to less stringent negative selection of T cells in the thymus, thereby increasing the number of circulating mature T cells that recognize self-antigens (Thorsby and Ronningen 1993). The HLA alleles most strongly associated with disease development are DRB1, DQA1, and DQB1, which encode HLA class II proteins involved in $CD4^+$ T cell recognition of antigens (Todd 2010).

For many years, it has been proposed that T1D is initiated by viral infection in at least a portion of those who develop T1D. Indeed, it is quite clear that environmental factors play a role in T1D development, as the concordance rate between monozygotic twins is only 30–50 %, indicating that there is a significant environmental contribution above genetic susceptibility (Redondo et al. 1999). The group of viruses most commonly associated with T1D development is that of the enteroviruses, which are members of the *Picornaviridae* family. Enteroviruses are RNA viruses able to form four structural and seven functional proteins (Jaidane and Hober 2008). There are several lines of evidence correlating infection with Coxsackie B viruses and the development of T1D (Dotta et al. 2007; Roivainen et al. 1998; Ylipaasto et al. 2012). Based on these data, the nPOD-Virus Group, a consortium involving 35 investigators worldwide, has formed in order to address this question with a multitude of complementary approaches that will be applied to the joint study of human pancreas from T1D patients identified by the JDRF nPOD. It is anticipated that the study will provide conclusive data regarding the type of virus strongly associated with T1D development. Many of the same investigators are also involved with the PEVNET, or Persistent Virus Infection in Diabetes Network, which also seeks answers to this question.

Beyond the mounting evidence that viruses may play a role in the development of T1D, it has also become evident that alterations in the gut microbiome are associated with disease progression. This will be discussed in further detail in a subsequent section of this chapter. A future goal will be to identify biomarkers of risk for progression to T1D.

T1D is on the rise, especially in children younger than 5 years of age. A recent prospective study (named the DAISY study) focused on infant diet to determine the risk of developing T1D (Frederiksen et al. 2013). Genetically susceptible children are at greater risk for developing T1D when either the father or siblings are T1D. Genetically susceptible patients showed a significant correlation with disease progression based upon their exposure to certain foods in infancy. Infants with relatives affected by T1D were studied for a history of breastfeeding, formula feeding, and timing of exposure to solid foods recorded. Both early (before 4 months of age) and late exposures (after 6 months of age) to any cereal led to an increased risk of T1D. Late exposure to non-gluten cereals specifically increased the risk to T1D, but early exposure did not. Gluten-containing cereals did not increase the risk when separated out from non-gluten-containing cereals. Additionally, early exposure to fruits increased the risk of T1D. The age at first exposure to cow's milk did not correlate with diabetes risk. Overall, the study recommends that new food antigens be introduced concurrently with breastfeeding between 4 and 6 months of age to minimize the risk of developing T1D, as breastfeeding provides protection from disease development.

To date, the best indicator of disease progression to overt T1D is the expression of AAb. These are antibodies produced against antigens related directly to β cells and insulin production. In another prospective study, 13,377 children were assessed for seroconversion, or development of AAb against insulin, GAD65, and IA2, and the risk of progression to T1D (Ziegler et al. 2013). 7.9 % of the children seroconverted, while 92.1 % remained free of islet AAb. It is not uncommon for children at high risk to develop AAb prior to developing full-blown diabetes. This occurs faster in children that develop multiple AAb prior to 3 years of age, with the 10-year risk being 74.9 % versus 60.9 % in those children who develop AAb after the age of 3. The development of multiple AAb is, by itself, predictive of the development of T1D in children, although the time to disease onset varied greatly. It has been suggested that islet inflammation develops by the deposition of autoantigens binding with islet AAb to form pro-inflammatory immune complexes.

Beyond the production of AAb, there is no good way of detecting T1D clinically before most patients present with hyperglycemia. Unless a family history dictates closer evaluation of potential disease progression, most patients are not tested for their HLA genotypes, production of AAb, or loss of the first-phase insulin response in a glucose tolerance test. From laboratory research, scientists have begun to piece together the events leading up to this clinical presentation.

Development of Autoimmunity Against β Cells

While it is still unclear what initiates autoimmunity against β cells, there are certainly key events that must occur in order for diabetes to fully develop. For example, it is understood that T cells are needed in order for T1D to develop, but it is unclear what recruits the autoimmune T cells to the pancreas in order to react against the β cells. Recent evidence suggests that there are metabolic defects within the pancreatic islets that might lead to increased susceptibility to autoimmune-mediated destruction (Tersey et al. 2012b). Perhaps stress signals supplied to the resident Mϕ and dendritic cells by the inherently stressed islets are sufficient to cause upregulation of inflammatory cytokine and chemokine production, which then leads to recruitment of additional immune effectors.

Research in this area is complicated by the fact that there are limited means of studying human disease. While our understanding has been greatly increased by the formation of the nPOD and PEVNET consortia, we still cannot study the disease as it develops within a single patient, and we are limited to snapshots of the disease or extrapolation of in vitro data in hopes that the same scenarios play out in vivo. Research in this area would benefit greatly from the further development of imaging modalities that allow us to study what is going on in vivo in real time. Some strides have been made in this arena (Antkowiak et al. 2013; Brom et al. 2010); however, in the absence of sufficient means to fully study human disease, we must use alternative models to test certain hypotheses. The following section provides details of our understanding of both rodent and human disease, and how they compare to one another.

Rodent Models

The use of mouse and rat models affords some insight into human disease. However, these models are not by any means perfect substitutes for the development of T1D in humans. We know that Mϕs and dendritic cells are recruited to the islets early in the process (between 3 and 6 weeks of age) of diabetes progression in NOD mouse model, the most frequently used model of T1D (Charre et al. 2002). The 12/15LO, a mouse gene for leukocyte 12LO implicated in inflammation (discussed in detail below), has been linked to β cell damage and T1D. Mϕ levels are significantly decreased in the absence of the 12/15LO enzyme (a mouse isoform of 12 LO), which metabolizes arachidonic acid to pro-inflammatory mediators (McDuffie et al. 2008). The reduction of Mϕ was associated with decrease in diabetes incidence in NOD mice with 12/15LO deletion. Additionally, NOD mice have increased numbers of pro-inflammatory circulating Mϕ (Nikolic et al. 2005). Evidence of aberrant dendritic cell development and function in NOD mice suggests one mechanism for the downstream development of autoimmune T cells in the periphery of these mice (Boudaly et al. 2002). Both types of antigen-presenting cells are potent cytokine producers and therefore contribute substantially to the development of β cell autoimmunity. The role of cytokines will be reviewed in greater detail in subsequent sections.

Additional early responders include NK cells, which are capable of producing large amounts of IFN-γ (Brauner et al. 2010). However, a recent study suggests that while more numerous in diseased mice, NK cells are dispensable in disease progression of NOD mice, as depleting NOD NK cells do not alter the disease course (Beilke et al. 2012). In non-autoimmune strains, NK cells are present in the pancreas and thought to function as sentinel cells rather than effectors of autoimmunity.

T cells begin to appear in the NOD pancreas by 6–8 weeks of age, with a mixture of $CD4^+$, $CD8^+$, and $Foxp3^+$ T cells making up the bulk of the insulitis. While regulatory $Foxp3^+$ T cells make an appearance in the pancreas, they are unable to stop the ensuing autoimmunity (D'Alise et al. 2008). In NOD models, the effector T cells ($CD4^+$ and $CD8^+$ cells) cause invasive and rapid destruction of most islets in affected mice. One significant difference between rodent and human research is the level of insulitis present around the islets during the autoimmune response. Murine T cells completely overwhelm the islet, while examples of human insulitis are much more subdued (Atkinson et al. 2013).

In the NOD model, it has been suggested that $CD4^+$ T cells, but not $CD8^+$ T cells, contribute to the development of autoimmunity by producing IFN-γ. $CD8^+$ T cells, however, tend to use either the granzyme/perforin pathway or the Fas/FasL pathway (Scott et al. 2010; Varanasi et al. 2012). Increased levels of granzyme A and Fas are able to overcome a granzyme B deficiency in the NOD model, such that disease incidence is not reduced or slowed (Kobayashi et al. 2013). Progression of research in diabetes has enabled us to more accurately assess which cells play a pathogenic role in the development of T1D. Recently, CD27-γδ T cells have been implicated in the development of diabetes in NOD mice by contributing to IL-17 production and playing a role in the development of islet inflammation (Markle et al. 2013b).

Humans

The appearance of islet-specific AAb occurs early in the progression of T1D relative to clinical evidence of β cell failure. While antibodies serve as a marker of autoimmunity, their role in the disease progression is not fully understood. As the antigens they recognize are not cell surface antigens, it does not appear that islet AAb cause direct destruction of the pancreas. Perhaps the AAb serve merely as indicators of underlying autoimmunity (Skyler and Sosenko 2013); however, some believe that they could form immune complexes that promote islet inflammation (Achenbach et al. 2004; Bonifacio et al. 1990; Orban et al. 2009). There is evidence that some diabetic patients develop secondary vascular complications based on the formation of immune complexes (Nicoloff et al. 2004).

Concurrently with AAb production, some patients begin to display impaired glucose tolerance characterized by loss of first-phase insulin secretion, indicating metabolic deficiencies present in the islets (Scheen 2004). Unfortunately, due to a lack of imaging modalities, we are unable to determine if these deficiencies correlate with increased T cell mediated damage in the islets.

Examination of peripheral blood cells has shown that plasmacytoid dendritic cell balance is significantly skewed in recent onset T1D patients (Allen et al. 2009). These cells are able to enhance the development of the autoimmune T cell response to the islet autoantigens. Dendritic cells show some promise as therapeutic targets in tolerizing protocols in which treatment with mammalian glycolipids increases IL-10 production, reduces IL-12 production, and reduces autoreactive T cell development by dendritic cells (Buschard et al. 2012).

Many have studied monocytes and Mϕ in order to discern their role in T1D development in humans. $CD68^+$ Mϕ has been detected in the islets of diabetic patients at both early and late time points in disease progression (Willcox et al. 2009). In one study, Bradshaw et al. found that monocytes from T1D patients were more likely to produce cytokines that stimulated the development of Th17 cells (Bradshaw et al. 2009). Another study has shown that IL-1β expression due to signaling through toll-like receptors was increased in monocytes of AAb + patients as compared to AAb- controls (Alkanani et al. 2012), implicating alterations in innate immune pathways in the development of T1D (Meyers et al. 2010). Furthermore, there are several candidate genes expressed by monocytes and monocyte-derived cells that have been suggested as contributors to diabetes pathogenesis, including CD226, CLECL1, DEXI, and SUOX (Wallace et al. 2012).

There is evidence that the IL-18R accessory protein (IL-18RAP) is altered on the surface of NK cells of T1D patients, which can lead to increased IFN-γ production by NK cells in some patients, which may stimulate downstream T cell responses (Myhr et al. 2013). In children diagnosed before the age of 5, NK cell phenotyping based on KIR gene and HLA class I gene haplotypes has shown that there is an increased representation of genotypes predictive of NK cell activation, which may indicate a role for these cells in some cases of T1D (Mehers et al. 2011). Additional evidence supports the idea that NK cell frequency and activation states are altered in both classical T1D and LADA patients (Akesson et al. 2010; Rodacki et al. 2007). There may be functional differences in NK cells due to expression of particular alleles and how they interact with HLA-C1 molecules (Ramos-Lopez et al. 2009).

Downstream of AAb production, T cells are recruited to the islets, and it is thought that $CD8^+$ T cells use cytotoxic mechanisms to destroy the pancreatic β cells. This destruction appears to occur mainly through cytotoxic degranulation involving perforin and granzymes, although maximal destruction is dependent upon the strength of the signal through the T cell receptor (Knight et al. 2013).

It is clear that pro-inflammatory cytokines can directly affect islet health (Eizirik and Mandrup-Poulsen 2001). In healthy humans, there is a subset of IL-10 producing T cells that appear to regulate pro-inflammatory cytokine production, thereby limiting the damaging effects of these cytokines (Tree et al. 2010). Below, we review our knowledge about cytokines involved in β cell demise.

Pathway by Which Cytokines Mediate β Cell Death

Elevated inflammatory cytokines have been reported for both T1D and T2D (Al-Maskari et al. 2010; Catalan et al. 2007; Eizirik and Mandrup-Poulsen 2001; Igoillo-Esteve et al. 2010; Jorns et al. 2005; Kang et al. 2010; Steinberg 2007; Su et al. 2010; Tilg and Moschen 2008). Acute exposure of ex vivo islets and/or β cell lines to a variety of inflammatory cytokines that are elevated in diabetes induces β cell dysfunction. Key inflammatory cytokine interactions include those mediated by IL-1β, IFN-γ, or TNF-α. Paired or triple combinations of these cytokines that include IL-1β are widely reported to induce β cell failure and promote apoptosis. Single cytokine treatment of the islets does not induce β cell dysfunction. A synergy in intracellular signaling pathways mediated by cytokines is required (Eizirik and Mandrup-Poulsen 2001; Rabinovitch et al. 1990).

Ex vivo studies on human or mouse primary islets show that a brief (6 h) exposure to three inflammatory cytokines (TNF-α, IL-1β, IFN-γ) is sufficient to result in loss of glucose-stimulated insulin secretion, increased inflammatory gene expression, and induction of apoptosis (Taylor-Fishwick et al. 2013; Weaver et al. 2012). Dissecting the intracellular events that mediate the transition of a β cell from functional to dysfunctional in an inflammatory environment may offer new approaches to preserve β cells exposed to inflammation. This strategic approach would be relevant to slowing diabetes progression as a monotherapy or reversing diabetes as a combinatorial approach with an islet regeneration strategy, either endogenous stimulation or exogenous repopulation (cell transplantation, encapsulation, xenotransplantation, etc.) (Taylor-Fishwick and Pittenger 2010; Taylor-Fishwick et al. 2008).

Literature reports describe many gene changes in β cells or islets that result from exposure to pro-inflammatory cytokines (Eizirik et al. 2012). Our own studies have identified changes in gene expression and enzyme activation that co-associate with pro-inflammatory cytokine-induced β cell dysfunction. The development of targeted inhibitors will allow description of a framework to integrate these pathways.

12LO enzymes are associated with the development of T1D. Genetic deletion of 12/15LO (a mouse 12LO) in the diabetes-prone NOD mouse confers protection to T1D onset relative to control (wild type) NOD mice (McDuffie et al. 2008). Islets from 12/15LO-deficient mice are resistant to β cell dysfunction induced by pro-inflammatory cytokines (Bleich et al. 1999). 12LO oxidizes cellular polyunsaturated acids to form lipid mediators termed eicosanoids. By definition, 12LO oxidizes carbon-12 of arachidonic acid. There are several isozymes that catalyze the reactions including leukocyte 12LO and platelet 12LO (Imai et al. 2013b). A major stable bioactive metabolite of 12LO activity is 12-HETE. Stimulation of human donor islets with pro-inflammatory cytokines increases the gene expression for 12LO. The active lipid product of 12LO activity, 12-HETE, reproduces, in part, β cell dysfunction mediated by pro-inflammatory cytokine stimulation (Ma et al. 2010). Direct addition of 12-HETE results in a loss of glucose-stimulated

insulin secretion and promotes β cell apoptosis. Newly described small-molecular-weight compounds selectively inhibit the activity of 12LO (Kenyon et al. 2011).

These selective inhibitors of 12LO preserve islets/β cell function in an inflammatory cytokine environment and inhibit apoptosis (Taylor-Fishwick and Nadler unpublished). These data connect pro-inflammatory cytokine stimulation with 12LO activation placing 12LO as one mediator of inflammatory cytokine-induced β cell dysfunction. Pro-inflammatory cytokines are potent inducers of cellular ROS, as are other serum conditions associated with the diabetic state, high free fatty acids, and elevated glucose (Cunningham et al. 2005; Inoguchi and Nawata 2005; Janciauskiene and Ahren 2000; Michalska et al. 2010; Morgan et al. 2007; Nakayama et al. 2005; Oliveira et al. 2003; Uchizono et al. 2006). Emerging contributors to sustained elevation in β cell ROS are NADPH oxidase enzymes. NADPH oxidase activity is regulated by 12LO activation (Weaver et al. 2012). A detailed description of NADPH oxidase enzymes in relation to β cell function is provided in the chapter by Taylor-Fishwick, "Role of NADPH Oxidase in β Cell Dysfunction."

Our studies provide an additional component to a molecular framework of intracellular β cell changes following inflammatory exposure. These discoveries support an emerging concept of an interactive interface between the islets and the immune system. IL-12 production and function has been described in β cells (Taylor-Fishwick et al. 2013). Expression of IL-12 gene and protein are observed following inflammatory cytokine stimulation (TNF-α, IL-1β, IFN-γ) of primary human and mouse islets, or β cell lines. Homogeneous β cell lines are devoid of any potential immune cell "contamination." While this is a concept that challenges established immune-based sources of IL-12, local production of IL-12 in the β cell could play a signsificant role in targeting the immune mediator recruitment. Separately, support for a paracrine function of IL-12 in β cells is also provided (Taylor-Fishwick et al. 2013). β cells, including human β cells, express the receptor for the IL-12 ligand and are responsive to IL-12-ligand/IL-12-receptor ligation. Administration of exogenous IL-12 directly mediated β cell dysfunction.

Observed were induction of apoptosis and disruption of glucose-stimulated insulin secretion (Taylor-Fishwick et al. 2013). Exogenous IL-12 induced a dose-dependent expression of IFN-γ in β cell lines suggesting a functional IL-12/STAT4/IFN axis. Previous studies have provided evidence of an active STAT4 signaling pathway in islet β cells (Yang et al. 2003, 2004). Importantly, neutralization of IL-12 with an IL-12 antibody blocked the β cell dysfunction induced by inflammatory cytokine stimulation (Taylor-Fishwick et al. 2013). A small molecule inhibitor of IL-12 prevented STAT4 activation, prevented T1D in NOD, and, in combination with exendin-4 or a β cell growth factor, reversed established diabetes (Yang et al. 2002, 2006). The functional defects mediated by IL-12 corresponded to those seen with inflammatory cytokine stimulation. Both receptor and ligand for IL-12 are upregulated in β cells exposed to inflammatory cytokine stimulation (TNF-α, IL-1β, IFN-γ). These studies suggest that pro-inflammatory cytokines induce local IL-12 expression that may be a mediator of inflammatory cytokine-induced β cell dysfunction. Lastly, selective inhibitors of 12LO

(Kenyon et al. 2011) suppress the induction of IL-12 ligand in islets and β cells exposed to inflammatory cytokine stimulation (Taylor-Fishwick and Nadler, unpublished). Inflammatory cytokine stimulation of 12LO activity is implied in the regulation of β cell IL-12 expression.

Beyond the key pro-inflammatory cytokines, there are others that likely affect disease progression of diabetes. The particular role of IL-17 in the development of T1D is rather controversial, as some have shown that it does not play a role (Joseph et al. 2012), while others maintain that it plays an important role in disease progression. Still others see a limited role for IL-17 in diabetes progression (Saxena et al. 2012). It has been suggested that IL-17 enhances the apoptotic response of β cells to TNF-α, IL-1β, and IFN-γ (Arif et al. 2011). The presence of Th22 (IL-22-producing helper T cells) and Th17 cells correlated strongly in T1D patients versus controls; however, it is unclear what the role of the Th22 cells is at this point in time (Xu et al. 2014). There is evidence in mice that IL-22 is increased under protective circumstances due to modulations in gut bacteria (Kriegel et al. 2011).

A conclusion from these observations is that β cells are not merely passive in terms of immune interactions. There appears a growing body of evidence that β cells actively interact with the immune system. Transcriptome analysis of islets exposed to pro-inflammatory cytokines further serves to reinforce the concept of an active dialog between the pancreatic islets and the immune system in T1D (Eizirik et al. 2012).

Metabolic Stress and Islet Inflammation: Implication in Islet Dysfunction Associated with T2D

T2D: The Most Common Form of Diabetes

T2D affects 347 million individuals in a recent worldwide study and has seen astonishing doubling of prevalence in less than 3 decades (Danaei et al. 2011). T2D is the most common form of diabetes that is characterized by insufficient insulin secretion to overcome insulin resistance. Extreme hyperglycemia itself could cause acute illness such as nonketotic hyperosmolar status. In addition, persistent hyperglycemia, even at modest degree, increases risks of macrovascular complications (represented by cardiovascular disease and stroke) and microvascular complications (retinopathy, nephropathy, and neuropathy) (Stratton et al. 2000). T2D is a leading cause of end-stage renal disease and blindness in developed countries and increases the risk of heart attack to two to four times. Thus, financial and emotional burdens of the disease are significant for affected individuals, as well as for society at large.

The recent rise in T2D incidence is largely attributed to environmental factors, including a sedentary lifestyle and excessive energy intake, which result in insulin resistance commonly seen in obesity (Danaei et al. 2011). In order to maintain normoglycemia, the body will compensate for insulin resistance by increasing the islet mass. However, a fraction of insulin-resistant subjects fails to produce enough

insulin and develops T2D, highlighting the critical role of the islets in the pathogenesis of T2D (Kahn et al. 2006). Indeed, many of the T2D susceptibility genes identified from GWAS are related to the regulation of insulin secretion, rather than insulin resistance (Petrie et al. 2011). Also, the natural history of T2D is characterized by gradual worsening of the disease due to progressive islet dysfunction, rather than a continuous increase in insulin resistance (Kahn et al. 2006; Prentki and Nolan 2006). Both a reduction in islet function and an eventual reduction in mass are noted in T2D. One early sign of islet dysfunction is the impairment of first-phase insulin secretion, which is an acute rise in insulin in response to glucose (Kahn et al. 2009). Forty to fifty percent of islet mass is already lost by the time T2D subjects present with hyperglycemia. An increase in β cell death and possibly dedifferentiation of β cells is considered to be responsible for the loss of islet mass (reviewed in Weir and Bonner-Weir (2013)). Here, we will discuss the potential mechanisms that lead to reduction of functional β cell mass and the evidence supporting involvement of inflammatory pathways in the islets during the development of T2D.

Does Metabolic Stress Cause Islet Inflammation in T2D?

Insulin resistance and the associated metabolic stress have profound effects on the health of the islets, which is discussed in a chapter, "Pancreatic β Cells in Metabolic Syndrome," and in a recent review (Imai et al. 2013a). Here, we will discuss the potential mechanisms by which metabolic stress provokes inflammatory responses in the islets.

Hyperglycemia and Dyslipidemia

Obesity, the most common cause of insulin resistance, is characterized by excessive energy intake from carbohydrate and lipids. Thus, hyperglycemia and dyslipidemia are potentially responsible for the loss of islet mass and function in T2D. In experimental conditions, it is well documented that prolonged exposure to elevated levels of glucose impairs β cell function and survival through oxidative stress, ER stress, enhancement of the hexosamine pathway, and others (Bensellam et al. 2012; Poitout and Robertson 2008). The activation of ER stress and oxidative stress could provoke inflammatory responses in the islets (discussed below); however, there are conflicting reports as to whether high levels of glucose directly induce the islet production of IL-1β, a cytokine that is proposed to play a key role in chronic inflammation associated with T2D (Bensellam et al. 2012). It has been widely accepted that exposure of β cell lines or islets to saturated fatty acids, especially PA, in culture conditions negatively affects insulin secretion and viability of β cells (Poitout and Robertson 2008). Increased production of ROS and ceramides, alterations in the integrity of ER, and aberrant protein palmitoylation are proposed mechanisms by which fatty acids elicit deleterious effects on the islets (Baldwin et al. 2012; Boslem et al. 2012; Cnop et al. 2010; Poitout and Robertson 2008). Fatty acids have been shown to provoke

inflammatory responses directly in the islets in experimental settings. Treatment of human islets with PA increased the expression of multiple cytokines including IL-1β, TNF-α, IL-6, IL-8, CXCL1, and CCL2 (Igoillo-Esteve et al. 2010). In this study, the blockade of IL-1β in human islets treated with PA prevented the rise of inflammatory cytokines but did not prevent apoptosis, indicating that inflammatory responses may not be solely responsible for the cytotoxic effects of PA in human islets ex vivo (Igoillo-Esteve et al. 2010). Interestingly, an in vivo study that raised serum PA levels by infusion in C57Bl/6 mice caused islet dysfunction, which seemed to result from cross talk between β cells and pro-inflammatory Mϕs that were recruited to the islets (Eguchi et al. 2012). A similar result was obtained in prediabetic, diabetes-prone BioBreeding rats, a model for T1D. A 48-h infusion of Intralipid (lipid emulsion), used to increase serum fatty acids, induced CCL2, IL-1β, TNF-α, IFN-γ, and IL-10 expression in the islets, caused mononuclear cell accumulation in islets, and impaired insulin secretion in prediabetic, diabetes-prone BioBreeding rats, but not in nondiabetes-prone rats (Tang et al. 2013). Therefore, recruitment and activation of Mϕ into the islets may play an important role in islet dysfunction in the setting of hyperlipidemia in rodent models. However, it still remains controversial whether fatty acids directly impair islet function and survival in humans in vivo, as the increase in serum fatty acids causes insulin resistance, which complicates the interpretation of islet function (Giacca et al. 2011). Finally, we need to bear in mind that cytotoxic effects of saturated fatty acids are not specific to the islets but seen in a wide range of cells. These effects are substantially lessened when cells are treated with a mixture of saturated and unsaturated fatty acids that simulates the typical lipid profile in vivo (van Raalte and Diamant 2011).

Oxidative Stress

The association of oxidative stress with islet failure in T2D was implicated in a study that demonstrated differential expression of genes related to oxidative stress in the islets obtained by laser microdissection of human pancreata from T2D donors (Marselli et al. 2010). Although ROS serves as an intracellular signal at a physiological level, excessive ROS is detrimental to the β cells (Pi and Collins 2010), the cells that are vulnerable to oxidative stress due to low antioxidant capacity (Lenzen 2008). It has been shown that high glucose, fatty acids, and IAPP (discussed below) result in an increased production of ROS by mitochondria and NADPH oxidase in the islets (Koulajian et al. 2013; Lightfoot et al. 2012; Rolo and Palmeira 2006; Zraika et al. 2009). Mitochondria are critically important for glucose-stimulated insulin secretion and a highly active organelle in β cells. Defects in their function and morphology are commonly seen in T2D and result in oxidative stress (Supale et al. 2012). A role of NADPH oxidase in oxidative stress is further discussed in chapter "NADPH Oxidase in β Cell Dysfunction." As ROS is a strong signal to provoke pro-inflammatory response in immune cells and nonimmune cells, ROS is a potential pathway connecting metabolic stress and inflammation in T2D islets through activation of JNK, p38-MAPK, NF*k*B, and AP-1 and subsequent

production of cytokines including IL-1β, TNF-α, and CCL2 (Lamb and Goldstein 2008; Padgett et al. 2013).

ER Stress

The ER is an intracellular organelle with key roles in proper peptide synthesis and maturation (folding), in Ca^{2+} homeostasis, and in the regulation of carbohydrate and lipid metabolism (Hotamisligil 2010). The UPR is a programmed response to protect the integrity of the ER from accumulation of misfolded proteins and metabolic stress by orchestrating an adaptive response or to produce cell death signals due to overwhelming pressures on the ER (Oslowski and Urano 2010). Three ER membrane-bound proteins, PERK, IRE1*a*, and ATF6, function as sensors of ER stress and coordinator of the UPR response (Oslowski and Urano 2010; Scheuner and Kaufman 2008). The importance of UPR response for the maintenance of β cell health is well known through studies of humans and mice with rare mutations. Misfolding of insulin due to gene mutations results in diabetes due to ER stress (Scheuner and Kaufman 2008). Similarly, Wolfram syndrome and Wolcott-Rallison syndrome both result from mutations affecting ER homeostasis and lead to the development of diabetes: the former due to defects in the WFS1 protein that regulates Ca^{2+} store in the ER and the latter due to defects in PERK (EIF2AK3) (Scheuner and Kaufman 2008). Interestingly, a polymorphism of CDKAL1, which is associated with T2D in GWAS, may introduce an insulin mutation at translation level, providing another example in humans in which misfolding of insulin may contribute to T2D development (Wei et al. 2011).

Aside from mutations, the insulin-resistant status associated with T2D could provoke ER stress in the β cells through several pathways. The increased demand for insulin secretion under insulin-resistant status necessitates upregulation of insulin production, which could account for 30–50 % of protein synthesis in the β cells (Scheuner and Kaufman 2008). In addition, high glucose levels, fatty acids, oxidative stress, mitochondrial dysfunction, and IAPP (see below) all induce ER stress (Hotamisligil 2010; Scheuner and Kaufman 2008). Indeed, a contribution of ER stress to islet failure has been implicated in both human and mouse studies. CHOP, a proapoptotic transcription factor upregulated in ER stress, was increased in the β cells of pancreata from human T2D donors (Huang et al. 2007). *Ob*/*ob* mice and db/db mice are both insulin resistant and obese due to lack of leptin signaling but show different capacities for β cell compensation. Interestingly, the expression of genes related to adaptive UPR was increased in *ob*/*ob* mice that compensate for insulin resistance well, but those genes failed to increase in db/db mice that progressively lose the β cells due to failure to alleviate ER stress (Chan et al. 2013).

Once the UPR, a programmed response to ER stress, is activated to a certain level, a member of UPR proteins initiates inflammatory responses in many cells including the β cells (Eizirik et al. 2013; Garg et al. 2012; Zhang and Kaufman 2008). In brief, PERK reduces translation of I*k*B, while IRE1*a* promotes degradation of I*k*B, both resulting in activation of NF*k*B through removal of inhibition by I*k*B (Zhang and Kaufman 2008). PERK and IRE1*a* also induces TXNIP (also

discussed in below) expression that could increase IL-1β secretion through an interaction of TXNIP with NLRP3 (Lerner et al. 2012; Oslowski et al. 2012).

A Potential Role of TXNIP in Islet Dysfunction and Inflammation in T2D

TXNIP regulates cellular redox status by inhibiting thioredoxin in a redox state-dependent manner (Spindel et al. 2012). A series of studies has revealed that TXNIP may be one of the mediators of β cell demise in diabetes. The expression of TXNIP is upregulated by glucose and increased in diabetic islets. TXNIP provokes apoptosis and impairs islet function through several pathways, including induction of mitochondrial apoptosis pathway and regulation of insulin transcription via microRNA (Saxena et al. 2010; Xu et al. 2013). Interestingly, as discussed in above, TXNIP is also induced by ER stress and contributes to ER stress-induced inflammation by activating an inflammasome (Lerner et al. 2012; Oslowski et al. 2012). Therefore, TXNIP may initiate β cell demise in response to elevated glucose, ROS, and ER stress through a multitude of pathways including inflammatory pathway.

A Potential Role of IAPP in Islet Dysfunction and Inflammation in T2D

One of the distinct characteristics of human islets affected by T2D is the accumulation of amyloid that was first noted in 1900 (Weir and Bonner-Weir 2013). Aggregates derived from IAPP, which is co-secreted with insulin from the β cells, are the major component of amyloid found in T2D islets. IAPP has physiological functions in the regulation of gastric emptying and satiety (Cao et al. 2013). Although human IAPP is soluble in monomeric form, it typically forms amyloid fibrils through a multistep self-driven reaction, a process not seen in mouse IAPP that does not possess an amyloidogenic sequence (Westermark et al. 2011). Several transgenic mice that express human IAPP have been created to determine whether human IAPP actively participates in β cell demise in T2D or is a by-product of β cell death. In a recent review, cytotoxic effects of human IAPP in transgenic mice were noted to be most apparent when overexpression of human IAPP is combined with some stressors such as high-fat diet (Montane et al. 2012). In humans, the deposition of islet amyloid correlates with β cell apoptosis indicating a close association between islet amyloid and β cell death (Jurgens et al. 2011).

Rapid accumulation of amyloid is also seen in human islet transplants and considered to contribute a graft failure (Westermark et al. 2011). Although the mechanisms by which IAPP elicits toxicity are not fully understood and could be multifactorial, the involvement of inflammatory pathways has been implicated. The activation of JNK pathway was shown to play a key role in inducing apoptosis in the islets overexpressing human IAPP cultured in high glucose (Subramanian et al. 2012). Furthermore, it has been shown that the islets exposed to human IAPP fibril increase the expression of CCL2 and CXCL1, which can potentially initiate recruitment of leukocytes (Westwell-Roper et al. 2011). Interestingly, human IAPP fibrils also induce the activation of inflammasomes and the production

of IL-1β and other cytokines from bone marrow derived-monocytes (Masters et al. 2010; Westwell-Roper et al. 2011). Indeed, mouse islets overexpressing human IAPP transplanted into STZ-induced diabetic NOD.scid mice showed increased recruitment of F4/80-positive Mϕ, supporting the idea that human IAPP fibril provokes an inflammatory response in the islets (Westwell-Roper et al. 2011).

Other Pathways Implicated in T2D Pathogenesis That Potentially Induce Islet Inflammation

There are several other factors that may contribute to islet dysfunction in T2D and may provoke inflammatory responses in the islets. Autophagy, or degradation and recycling of cytoplasmic components, is upregulated in the β cells under insulin resistance and appears to be important for the adaptive increase seen in β cell mass in mouse studies (Fujitani et al. 2010). Recently, a role for autophagy in chronic inflammation has gained attention, as defects in proteins regulating autophagy are associated with Crohn's disease, SLE, and others (Levine et al. 2011). Future studies are needed to determine whether or not human T2D is associated with defects in autophagy in the islets. If present, it needs to be addressed whether defects in autophagy modulate inflammatory responses in the islets. The alteration in gut microbiota is another pathway implicated in T2D pathogenesis, and bacteria may produce inflammatory signals for the islets, such as LPS. This is discussed further in below.

Role of Inflammation as an Amplifier of Metabolic Stress

As we discussed, there are multiple pathways that could generate inflammatory signals under metabolic stress in the islets (Fig. 1). Importantly, once induced, islet inflammation, in turn, can exacerbate oxidative stress, ER stress, and mitochondrial dysfunction and further increase islet inflammation in T2D, as well as in T1D. Pro-inflammatory cytokines including IL-1β, TNF-α, and IFN-γ impair ER homeostasis through nitric oxide-dependent and nitric oxide-independent mechanisms (Eizirik et al. 2013). 12LO is another pro-inflammatory mediator that potentially amplifies islet inflammation (Imai et al. 2013b). 12LO, a lipoxygenase upregulated in the islets of an animal model of T2D (Imai et al. 2013b) and in some humans with T2D (Galkina and Imai, unpublished), leads to the production of pro-inflammatory lipid metabolites, such as 12-HETE. 12-HETE, in turn, will induce additional pro-inflammatory cytokines such as IL-12, ER stress, and oxidative stress (Cole et al. 2012a; Weaver et al. 2012). Recently DMT1, an iron transporter, was shown to mediate oxidative stress in response to IL-1β in the islets (Hansen et al. 2012b). DMT1 is upregulated by IL-1β in the islets, and increases iron-catalyzed formation of ROS providing another pathway to amplify an inflammatory response (Hansen et al. 2012b). Importantly, deletion of both DMT1 and 12LO in mice was protective against development of diabetes in both T1D and T2D models, supporting the significant contribution of these key molecules that may serve as amplifiers of inflammatory responses in the islets (Hansen et al. 2012b; Imai et al. 2013b).

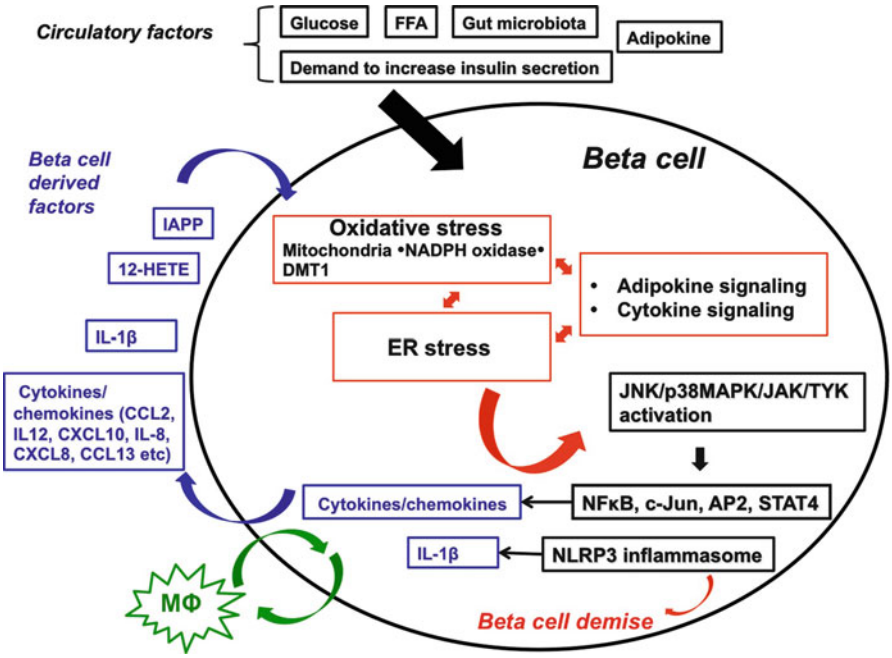

Fig. 1 Major inflammatory mediators and signaling pathways implicated in β cell demise in type 2 diabetes. Metabolic stress is considered to trigger inflammation in islets in type 2 diabetes. Increased glucose, excess saturated fatty acids (*FFA*), and higher demand for insulin secretion result in ER stress and oxidative stress. The alteration in adipokines and gut microbiota may also provoke islet inflammation. Cytokines produced locally or from circulation activate inflammatory pathways such as JAK/TYK, p38MAPK, and JNK via specific cytokine receptors in β cells. Oxidative stress and ER stress also result in the activation of JAK/TYK, p38MAPK, and JNK. In β cells under metabolic stress, there is extensive cross talk between signaling molecules involved in oxidative stress, ER stress, and inflammatory responses. Ultimately the activation of downstream transcription factors NFκB, c-Jun, and AP2 (downstream of JNK) will increase the production of pro-inflammatory mediators such as CCL2, IL-12, IL-8, CXCL10, CXCL8, and CCL13. The production of 12-HETE (via 12LO activation) and IL-1β (via Nlrp3 inflammasome) will be also increased in β cells under stress. These pro-inflammatory mediators produced by β cells will further increase oxidative stress, ER stress, and cytokine signaling creating a feedforward loop to exacerbate the inflammatory response. IL-1β increases the expression of NADPH oxidase and DMT1, which increases iron transport; both lead to an increase in reactive oxygen species and, together with IAPP, contribute to increased oxidative stress. 12-HETE will induce the production of IL-12, oxidative stress, and ER stress. IL-12 activates STAT4 signaling. Chemokine produced by β cells will also recruit macrophages (Mϕ) into the islets that further augment inflammatory response in islets

Evidence of Islet Inflammation in T2D

Humoral Mediators of Inflammation in the Islets of T2D

An increase in several humoral mediators of inflammation has been reported in human T2D islets. Gene arrays comparing the islets obtained by laser capture microdissection of frozen pancreata sections in 9 non-DM and 10 T2D donors

found upregulation of IL-1β and IL-8 in the T2D group. While the expression of IL-1β determined by qRT-PCR was barely detectable in the islets from non-DM donors, six out of ten T2D donors showed high IL-1β expression in the islets (56-fold increase over the controls) (Boni-Schnetzler et al. 2008). There was a trend of increased IL-8 qRT-PCR expression in the same study (Boni-Schnetzler et al. 2008). Reanalysis of the gene array above performed using the same cohorts plus one additional non-DM showed upregulation of CCL2 and CCL13 (Igoillo-Esteve et al. 2010). No difference was noted in IFN-γ. In both studies, reflecting the heterogeneity of the T2D population, the expression levels of cytokines varied widely in the T2D cohort compared with non-DM control.

CXCL10 is a chemokine that has been shown to play an important role in early insulitis associated with T1D. Both mRNA levels, determined by qRT-PCR, and secretion of CXCL10 were increased in human islets from three T2D donors (Schulthess et al. 2009). The protein levels of heterodimeric IL-12 and the phosphorylation of downstream signaling molecule, STAT4, were increased in human islets from three T2D donors implicating the activation of IL-12 pathways (Taylor-Fishwick et al. 2013). More recently, microarray gene expression data from 48 human donors including 10 T2D donors were analyzed using a principle of weighted gene co-expression network to identify a group of genes that are associated with T2D traits. The study found that a group containing IL-1 related genes correlates with A1c (Mahdi et al. 2012). Collectively, human islets affected by T2D tend to show an increase in inflammatory humoral factors, which potentially contributes to a reduced islet function and viability. It has been shown that IL-12 and CXCL10 impair insulin secretion in human islets ex vivo (Schulthess et al. 2009; Taylor-Fishwick et al. 2013). The effects of IL-1β on the islets have been studied extensively, as it has been considered one of the major mediators of insulitis in T1D (Donath and Shoelson 2011).

The activation of IL-1β results in the production of additional pro-inflammatory cytokines in the islets and impairs islet functions (Donath and Shoelson 2011). In addition to direct toxicity, CCL2 and CXCL10 are known chemoattractants that may amplify inflammation through recruitment of Mϕ, lymphocytes, and other leukocytes. In a transgenic mouse model, CCL2 overexpression (20- to 700-fold greater than wild-type control) resulted in the accumulation of monocytes and DC in the islets and caused hyperglycemia (Martin et al. 2008). On the other hand, the overexpression of CCL2 in NOD mice was protective against the development of diabetes due to increased tolerogenic CD11b + CD11c + DC cells, indicating the complexity of immune regulation by CCL2 in the islets (Kriegel et al. 2012). Overall, the actual contributions of these factors in apoptosis and the impairment of islet function in T2D need to be further clarified.

Cellular Mediators of Inflammation in the Islets of T2D

Several rodent models of T2D show an increased accumulation of innate immune cells during the development of hyperglycemia. The GK rat, which is also discussed in chapter "Islet Structure and Function in GK Rat," shows an increase in MΦs and granulocytes in and around the islets (Homo-Delarche et al. 2006). An increase in

$CD68^+$ MΦs in the islets was also noted in a histological study of C57BL/6 J mice on a high-fat diet and in db/db mice (Ehses et al. 2007).

Elevation of serum PA over the course of a 14-h infusion led to the recruitment of CD11b + Ly-6C + M1-type pro-inflammatory Mϕs into the islets of C57Bl/6 mice, indicating that metabolic alterations may be sufficient to initiate Mϕ accumulation (Eguchi et al. 2012). In humans, two studies have reported an increase in $CD68^+$ Mϕs in the islets of pancreata from T2D donors (Ehses et al. 2007; Richardson et al. 2009). With the exception of one study that tested the effects of acute infusion of fatty acids (Eguchi et al. 2012), the majority of the aforementioned studies are histological analyses, leaving detailed characterization of Mϕ subtypes for future studies. Recently, an intriguing study demonstrated that Mϕ infiltration indeed may contribute to the impairment of β cell function in Zucker diabetic fatty rat, a rat model of T2D (Fig. 1). In this model, endocannabinoid that increases insulin resistance through CB1 receptor was also shown to impair the islet function by activating NLRP3 inflammasome in Mϕ that is recruited to the islets (Jourdan et al. 2013).

T2D might not show the classical criteria for autoimmune disease including loss of tolerance to self-tissue antigen, such as the β cells in case of T1D, and disease phenotypes transferable through pathological antibodies or immune cells (Velloso et al. 2013); however, immune cells classically associated with adaptive immune responses are altered in insulin target tissues and peripheral circulation of T2D patients and implicated in the development of insulin resistance (DeFuria et al. 2013; Nikolajczyk et al. 2012). Importantly, at least in rodent models, modulations that target these cells ameliorate insulin resistance (Nikolajczyk et al. 2012). Since islets are under metabolic stress that is similar to insulin target tissues, the involvement of lymphocytes and DC in islet dysfunction of T2D cannot be ruled out. The most intriguing data supporting the role of adaptive immune cells in islet dysfunction of T2D is a series of works that demonstrate T cells reactive to islet extract in the peripheral circulation of clinically defined T2D subjects (Brooks-Worrell et al. 2011, 2012; Brooks-Worrell and Palmer 2013). Currently, antigens recognized by these peripheral T cells are not defined, and the number of subjects studied is limited. If this phenomenon is to be detected in a larger population of T2D, it will support the involvement of lymphocytes in islet dysfunction of T2D.

Beyond peripheral lymphocytes, only limited information is currently available regarding the involvement of adaptive immune cells locally in the islets of T2D. The heterogeneity of islet DC has recently evaluated in detail in mice (Yin et al. 2012), but little is known about the profiles of intra-islet DC in human islets of healthy controls or T2D patients. Interestingly, significant lymphocyte infiltration consisting of $CD3^+$ T cells was noted in C57BL/6 mice placed on a high-fat diet at an advanced age (Omar et al. 2013). Although C57Bl/6 mice on a high-fat diet is a commonly used model of T2D, this inbred strain of mice is known to develop autoimmune lesions in multiple organs with advanced age, making any extrapolations of this mouse model to pathology of human T2D difficult (Hayashi et al. 1989). From histological studies, the infiltration of lymphocytes is considered to be relatively limited in human T2D in general (Ehses et al. 2007). As in the case

for Mϕs, detailed analysis of subset and functional status of lymphocytes and DC may demonstrate their contribution in T2D, especially the slow progressive decline in islet mass and function seen in T2D that may result from accumulation of subtle damage.

The Effects of Metabolic Alteration in Other Organs on Islet Function and Islet Inflammation

Glucose and energy homeostasis are regulated by cross talk between several key organs including the pancreatic islets, the AT, the liver, the brain, the skeletal muscle, and the gut. Here, we focus on how the AT, the liver, and the gut affect the function and health of the pancreatic islets.

The Regulation of Islet Function by AT in Health and Disease

AT is an active endocrine organ with a wide, biologically active secretome capable of controlling energy homeostasis through peripheral and central regulation. AT is also endowed with remarkable plasticity being able to respond in face of nutrient overload by more than doubling its mass and storage capacity. However, this metabolic adaptation to chronically positive energy balance leads to many pathogenic changes. In particular, glucotoxicity, lipotoxicity, and innate and adaptive immune responses in AT all lead to production of pro-inflammatory cytokines and a perturbed secretome reflected in quantitative changes of the various adipokines (Deng and Scherer 2010; Piya et al. 2013). These changes lead to the subclinical inflammation in AT that is a key pathogenic contributor to obesity-mediated insulin resistance and diabetes (Hotamisligil 2006; Ouchi et al. 2011). In this section, we will discuss the interaction between AT dysregulation and pancreatic islet inflammation leading to β cell functional demise. Since the interaction between the two organs relies on secretory factors produced by AT that both directly or indirectly affect the pancreatic islets, the focus will be on a causal relationship between metabolic dysregulation and changes in the adipocytokine production and how the latter could in turn influence β cell functional failure.

Metabolic Responses in AT Responsible for a Dysregulated Secretome

The AT secretome is composed of molecules commonly referred to as adipokines or adipocytokines. The list of these biologically active molecules is growing, as both novel and existing molecules secreted by adipocytes or AT are being discovered. It is becoming increasingly clear that adipokines form an important part of an adipo-insular axis, dysregulation of which may have key roles in β cell failure and the development of T2D (Dunmore and Brown 2013; Imai et al. 2013a).

The majority of the adipokines are peptides/proteins with hormone-like properties, including cytokines that are either specific to adipocytes or are also produced by other cells or endocrine organs. In this context, NEFA can also be considered adipokines. Phenotypically, an enlarged AT is associated with dysregulated adipokine secretion (Wellen and Hotamisligil 2003, 2005). However, the

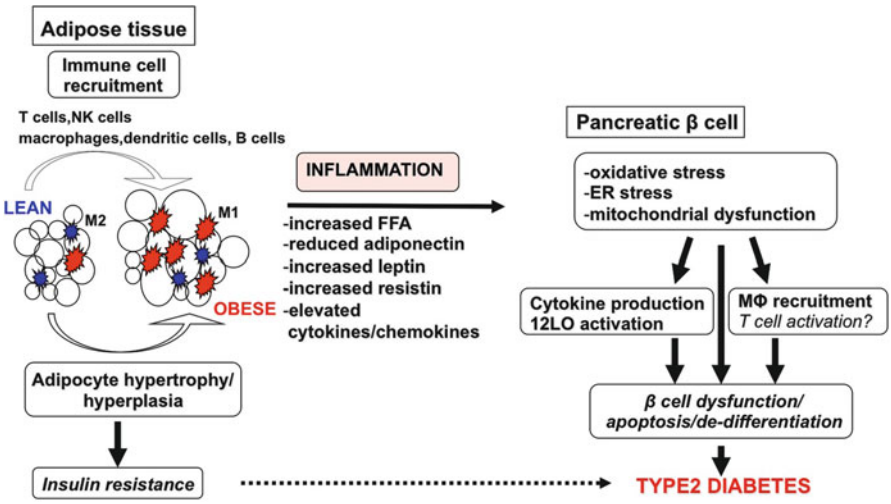

Fig. 2 Dysfunctional adipose tissue secretome in obesity drives β cell inflammation. Excess caloric excess in obesity leads to adipocyte hypertrophy and some degree of hyperplasia. Also, immune cell recruitment takes place in the adipose tissue and a macrophage (Mϕ) phenotypic shift occurs from the M2 anti-inflammatory (in *blue*) to M1 pro-inflammatory (in *red*) Mϕ. As a result adipocytes will generate a pro-inflammatory secretome: increased adipocyte leptin and resistin, reduced adiponectin, and increased chemokines and cytokine. In adipose tissue, M1 Mϕs, Th1CD4$^+$, CD8$^+$, and NK cells together will increase pro-inflammatory cytokines and chemokines. These circulatory factors will result in cellular stresses leading to increased production of pro-inflammatory cytokine and 12LO activation in the β cells. Also Mϕ seems to be recruited into the islets in response to cellular stresses. T cell activation may also take place in the islets under stress. The progressive inflammatory insult combined with cellular stresses will cause loss of β cell function and mass and result in type 2 diabetes when combined with insulin resistance

mechanisms leading to this dysregulation are complex and only partly understood. Both the hyperplastic adipocytes and other cells residing in the AT are significant contributors.

In Fig. 2 we illustrate some of the established key players and the effects on islet dysfunction. Adipocytes themselves can produce cytokines and chemokines in response to changes in their cell size (Skurk et al. 2007). Also, in response to increased saturated fatty acids, adipocytes activate TLR4 and NF*k*B, leading to downstream adipokine production (Youssef-Elabd et al. 2012). Hypoxia, associated with a reduced angiogenic response in the growing AT, induces pro-inflammatory responses in adipocytes including formation of adipokines such as CCL5 (Skurk et al. 2009). Other cytokines were reportedly produced by adipocytes in obesity including IL-18, CXCL10, and MIP-1 (Herder et al. 2007; Skurk et al. 2005a, b). These locally produced chemokines can contribute to the elevation of the circulating chemokine pool and hence may affect distant sites, including the pancreatic islets, the liver, and the muscle. Interestingly, epidemiologic studies showed significant correlations between systemic chemokine concentration and glucose

intolerance and T2D (Herder et al. 2005). Also, adipocyte chemokine secretion may contribute to the recruitment of immune cells into the inflamed AT.

Cell components of both innate and adaptive immunity populate AT in obesity and may themselves represent an important source of inflammatory cytokines (Lumeng and Saltiel 2011). Mϕs are abundantly present in AT in obesity in both rodents and humans (Chawla et al. 2011; Osborn and Olefsky 2012). In particular, the pro-inflammatory "M1-like" Mϕ subset accumulates in visceral fat in obesity due to progressive lipid accumulation (Nguyen et al. 2007; Prieur et al. 2011) (Fig. 2). These Mϕs, whose differentiation is promoted by lipopolysaccharide or IFN-γ, produce pro-inflammatory mediators such as TNF-α, IL-6, IL-1β, IL-12, etc. Alternatively, the activated "M2-like" Mϕ, the main subset present in the lean AT, secretes anti-inflammatory molecules such as IL-10 and IL-1 receptor antagonist (Osborn and Olefsky 2012). There are many cellular mechanisms and pathways responsible for the increased inflammation in both Mϕ and adipocytes in obesity. Some of these include the activation of TLR- or NLRP3-dependent pathways via NEFA or ceramides produced mainly in adipocytes (Nguyen et al. 2007; Vandanmagsar et al. 2011), as well as ER stress and autophagy (Martinez et al. 2013). Also, recent reports emphasized the pro-inflammatory roles of the incretin hormone GIP in adipocyte via increased production of cytokines and free fatty acids via lipolysis (Nie et al. 2012; Timper et al. 2013). Besides Mϕ, several other immune cells populate AT in obesity such as neutrophils, mast cells, $CD8^+$ and $CD4^+$ T cells, NK cells, and B cells (reviewed in (Mathis 2013)). All of these cells may contribute to production of pro-inflammatory cytokines and chemokines that could also affect islet function via their release in circulation. The mechanisms by which various cytokines and chemokines induce islet inflammation and β cell dysfunction were described earlier in this chapter and in a few recent reviews (Donath et al. 2013; Imai et al. 2013a).

Effects of AT Adipokines on Pancreatic β Cell Dysfunction

Besides cytokines, which can be produced by adipocytes and other cells, of particular interest are the adipokines that are uniquely produced by the adipocytes such as leptin, adiponectin, omentin, resistin, and visfatin, which can also act in an endocrine manner and affect the function or dysfunction of the islet cells (Fig. 2). Ample evidence shows that the adipokine balance is significantly altered in obesity, both in rodent models and in humans, showing an increase of leptin, resistin, and TNF-α and a reduction of adiponectin and visfatin.

Leptin is known to be key in the regulation of glucose homeostasis via central and peripheral actions. Human and rodent β cells express the long form of the leptin receptor that is required for intracellular signaling, as well as the truncated forms. In obesity, excess leptin production occurs due to an increase in adipose mass; however, the peripheral and central signaling of leptin is attenuated due to the phenomenon of leptin resistance (Patel et al. 2008). It has been proposed that the leptin resistance in the β cell may lead to dysfunction and could contribute to T2D in human obesity (Morioka et al. 2007). Although the leptin signaling in the β cells is similar to other leptin-responding tissues (Marroqui et al. 2012),

the mechanisms responsible for leptin resistance have not been yet studied. It is possible that increases in SOCS3 and PTP1B, reportedly associated with obesity and responsible for inhibition of leptin signaling in AT and hypothalamus, could be key (Myers et al. 2010). Additional effects of leptin on β cell mass generated contradictory results. As opposed to some rodent models, leptin in humans appears to have an antiproliferative effect on β cells that involves the PI3K pathway and inhibition of the protein and lipid phosphatase PTEN, which ultimately leads to increased activation of the K_{ATP} channels and inhibition of glucose-stimulated insulin secretion (Wang et al. 2010). Also, in human islets, leptin exerted a U-shape response on insulin secretion, with lower concentrations inhibiting insulin release and higher concentrations stimulating it (Dunmore and Brown 2013). Therefore, it is possible that in the early stages of insulin resistance, an increase in circulating leptin prior to the development of defects in signaling mechanism may be responsible for the compensatory hyperinsulinemia. In severe obesity, which is characterized by impaired leptin signaling, the latter may reduce insulin secretion and contribute to hyperglycemia and progression to T2D.

Another adipokine secreted exclusively by the adipocytes is adiponectin. Adiponectin improves insulin sensitivity and vascular function. Obesity is characterized by reduced adiponectin secretion by the hypertrophic adipocytes. Both of the adiponectin receptors are expressed in primary and clonal β cells with AdipoR1 expressed at significantly higher levels. Signaling involves AMPK, PPARγ, PPARα, and p38MAPK (Wijesekara et al. 2010). A wealth of evidence suggests a beneficial role of adiponectin in β cell function and survival. Increased adiponectin in both a GK model following gastric bypass and the clonal β cell lines BRIN BD11 and MIN1 following in vitro palmitate treatment (Brown et al. 2010a) was associated with a reduction of β cell apoptosis (Chai et al. 2011). In vivo mouse studies indicated that many of the adiponectin actions are mediated via activation of ceramidase activity, which leads to generation of sphingosine-1-phosphate (Holland et al. 2011). Furthermore, a recent report showed that adiponectin caused a significant increase in insulin content and secretion via a PPARγ-mediated effect and also had a proliferative effect independent of PPARγ in MIN6 cells (Rao et al. 2012). This latter effect apparently requires generation of reactive oxygen species that may have a beneficial role. Therefore, the combined antiapoptotic and proliferative effects of adiponectin suggest an important contribution of adiponectin to preserve β cell mass and function. The reduced levels of adiponectin found in obesity and T2D may negate these beneficial effects.

Another important adipokine that is increased in obesity and T2D is TNF-α. TNF-α can induce β cell apoptosis via the NFκB pathway and may have direct effects on insulin secretion (Ortis et al. 2012). Recently, TNF-α reportedly increased IAPP expression in β cells with no concurrent expression of proinsulin, potentially leading to amyloid production, subsequent β cell death, and systemic increase in insulin resistance (Cai et al. 2011).

Recent findings showed that DPPIV is produced and secreted by human adipocytes (Lamers et al. 2011) and therefore may reduce the half-life of GLP-1 with

important implications on the insulinotropic effects of this gastric hormone on the β cell. Although it is unclear if obesity is associated with increased levels of DPPIV, inhibition of the latter by sitagliptin in a rodent model of obesity and insulin resistance reduced inflammatory cytokine and chemokine production both in the pancreatic islets and in AT and improved the glucose-stimulated insulin secretion in the pancreatic islets in vitro (Dobrian et al. 2010c).

Resistin is an adipocyte-specific-secreted molecule shown to induce insulin resistance in rodents but not in humans (Steppan et al. 2001). However, human islets express resistin and its expression is upregulated in T2D (Al-Salam et al. 2011). In clonal β cells, resistin downregulated insulin receptor expression, decreased cell viability, induced insulin resistance in the pancreatic islets, and caused a subsequent reduction in glucose-stimulated insulin secretion (Nakata et al. 2007).

One of the more recently described adipokines, visfatin, is a phosphoribosyltransferase that is secreted from AT via a nonclassical pathway. A recent meta-analysis study indicated that visfatin can be used as a predictor for the development of insulin resistance and diabetes and is positively associated with obesity and T2D (Chang et al. 2011). Interestingly, visfatin can increase insulin secretion and directly induce activation of the β cell insulin receptors by increasing their phosphorylation (Brown et al. 2010b; Revollo et al. 2007). In a recent study, visfatin was shown to stimulate β cell proliferation and to reduce palmitate-induced β cell apoptosis via ERK1/2 and PI3K/PKB-mediated pathways (Cheng et al. 2011). Although most of the in vitro studies suggest a positive effect of visfatin on β cell function, a recent study suggests that the effect is concentration dependent with potentially deleterious effects at pathologically high concentrations (Brown et al. 2010b). Future research is needed to refine our understanding on the effects of visfatin on islet functional health.

In conclusion, several pro-inflammatory mechanisms in AT may influence pancreatic β cell inflammation, survival, and functions (Fig. 2). Additional data will be valuable in understanding whether various adipokines play a causative role in the development of diabetes and whether they contribute to the progression of T2D as well. Also, investigating the cross talk between various adipokines will refine our understanding of the delicate balance between the overall beneficial vs. deleterious effects of the AT secretome on pancreatic β cell apoptosis, proliferation, and insulin secretion. The information will provide new approaches to target the "adipo-insular" axis for more efficient future therapies.

Influence of the Gut Microbiome

There is unquestionably a role for the gut microbiome in the development of both T1D and T2D. We are increasingly appreciative of the effects of our symbiotic counterparts in the gut and how they can alter the levels of inflammation in our system by changing gut permeability to antigens and pathogens as well as altering how nutritional intake is processed. All of these factors combined can affect downstream immune responses, which may protect or exacerbate the inflammatory processes related to diabetes development and β cell demise.

T1D

Early postnatal time period plays a large role in the development of the autoimmune response leading to T1D. The antibiotic vancomycin may deplete deleterious bacteria and support *Akkermansia muciniphila*, which appears to be protective in NOD mice (Hansen et al. 2012a). It is thought that *Akkermansia* may stimulate gram-negative bacterial interactions with gut wall, leading to TLR4 stimulation. Segmented filamentous bacteria have also been shown to protect against diabetes development in NOD mice by directing immune responses in the small intestine lamina propria cells (Kriegel et al. 2011). Furthermore, it has been determined that sex differences play a role in the composition of the gut microbiome and may contribute to female predominance of diabetes development in NOD mice (Markle et al. 2013a). Gut microbiota from male NOD mice can confer protection to female mice and do so by increasing the testosterone levels in female mice.

In a rat model of virus-induced T1D, protection against diabetes development was afforded to virus-infected rats treated with trimethoprim and sulfamethoxazole (Sulfatrim), as the viral infection led to increases in *Bifidobacterium* and *Clostridium* (Hara et al. 2012). While the infection increased the level of inflammation due to innate immune cells in the Peyer's patches and pancreatic draining lymph nodes, Sulfatrim treatment decreased the level of inflammation. These data point to a role of the gut microbiota in the development of virus-induced T1D.

An additional rat model showed that supplementing diabetes-prone BioBreeding rats with *Lactobacillus johnsonii* afforded protection from diabetes development, in part by altering the expression of pro-inflammatory cytokines (TNF-α and IFN-γ) that are known to lead to β cell toxicity (Valladares et al. 2010).

Enteric bacterial pathogens may disrupt the gut barrier, causing acceleration of insulitis (Lee et al. 2010), as this is one mechanism that increases insulitis in the NOD mouse strain. $CD8^+$ T cells are activated by the loss of intestinal barrier and pathogenic bacteria. Additionally, there are several lines of evidence that suggest increased intestinal permeability in human T1D patients (Bosi et al. 2006; Secondulfo et al. 2004).

Studies of AAb + vs. AAb- children matched for HLA genotype, age, sex, and feeding history suggest that *Bifidobacterium* (Actinobacteria, decreased), *Bacteroides* (increased), and butyrate-producing (decreased) species are altered in those with β cell autoimmunity (de Goffau et al. 2013). These differential profiles of microbiota might affect the intestinal epithelial barrier function and thereby modify the levels of inflammation. The authors of this work discussed the advantage of performing fecal microbe profiling before diagnosis to eliminate the possible influence of diabetic status on the gut microbiota of individuals and thereby justifying the analysis of those that were AAb + vs. AAb-.

Several studies have shown that T1D patients exhibit skewed gut microbiota as compared to controls (Brown et al. 2011; Giongo et al. 2011). Once diagnosed with T1D, patients appear to have decreased *Bifidobacterium* and increased *Bacteroides*, indicating that this trend continues beyond disease onset (Murri et al. 2013).

In this study, patients were also matched by age, sex, dietary habits, race, mode of delivery at birth, and duration of breastfeeding. Furthermore, the *Bacteroides* genus appears to be associated with T1D patients, while the enterotype 2 groups (*Prevotella* genus) are more strongly affiliated with healthy patients.

It is certainly possible that the gut microbiome of T1D patients is directly affected by viral infections (i.e., enterovirus), leading to altered immunity and the development of T1D (Hara et al. 2012).

T2D

Obesity and T2D are physiological states shown to have altered gut microbiota (Larsen et al. 2010). Both states show significant decreases in *Akkermansia muciniphila*, which degrades the mucus layer of the gut. Supplementing obese and T2D mice with this prebiotic correlates with improved metabolic profile, including decreased AT inflammation, decreased insulin resistance, and increased levels of the gut barrier, gut peptide secretion, and endocannabinoids, which control inflammation (Everard et al. 2013). Perhaps treatment to increase this colonizer will aid in treating obesity and metabolic disorders. Additionally, selectively increasing *Bifidobacterium* via prebiotics in *ob*/*ob* mice appears to increase the GLP-2 production, which decreases intestinal permeability and inflammation that is associated with obesity (Cani et al. 2009). Furthermore, rodents on a high-fat diet are more likely to exhibit decreases in *Lactobacillus* strains and *Bifidobacterium*. Decreases in these two strains correlate inversely with plasma glucose levels, insulin sensitivity, and inflammation (Chen et al. 2011; Sakai et al. 2013). These two groups, along with others, have shown that dietary supplementation with certain *Lactobacillus* strains and *Bifidobacterium* strains can alleviate high blood glucose levels (Honda et al. 2012).

Another attempt to alter the composition of the gut microbiome toward a less inflammatory phenotype includes treating mice to decrease phenolic acids by prebiotic supplementation with green tea powder and Lp (Axling et al. 2012). This study also corroborates the idea that increased levels of *Akkermansia* are complicit in blunting inflammatory responses. The authors conclude that green tea powder and Lp exert their effects on different portions of the gut, with the green tea powder standardizing the flora in the small intestine and the Lp standardizing flora in the cecum. Among the parameters tested, green tea powder lowered the HOMA index and increased insulin sensitivity in treated mice, but did not improve oral glucose tolerance.

In human patients, compositional alterations have been noted in the gut microbiome of those who have normal, impaired, and diabetic glucose control (Karlsson et al. 2013). This study suggests that the risks for diabetes development can be assessed more accurately by studying the gut microbiome and metagenomic clusters of patients.

The overarching theme in this arena is that inflammation resulting from obesity can be downplayed by altering the endogenous gut microbiome. It remains unclear which change occurs first: are gut microbiota affecting metabolic markers, or are the changes in gut microbiota result from metabolic alterations? Furthermore, it appears that the changes in the gut microbiome of T1D and T2D patients do not

necessarily undergo the same kinds of changes. Little is known whether the gut microbiome directly contributes to islet dysfunction in T2D. This is an area for active future investigation.

The Communication Between the Liver and Islets

The liver plays the critical role in glucose and energy homeostasis and has close bidirectional relationship with pancreatic islets. Insulin being secreted from the islets is first delivered to the liver via the portal vein, and then the liver will take up 80 % of insulin to regulate the metabolic function of the liver (Meier et al. 2005). Indeed, pulsatility of insulin delivery in the liver was shown to be an important determinant of hepatic insulin sensitivity in an experimental setting (Matveyenko et al. 2012). Thus, islet dysfunction may be a proximal cause for insulin resistance in the liver. However, it remains to be determined whether the impairment of insulin secretion contributes to the development of hepatosteatosis or steatohepatitis in humans.

On the other hand, recent studies demonstrate that the liver regulates insulin secretion and β cell mass through neuronal and humoral mechanisms. The activation of ERK in response to insulin resistance (*ob*/*ob* leptin-deficient mouse) or insulin deficiency (STZ or Akita mouse) was shown to trigger an increase in β cell mass through a neuronal relay, connecting the liver and pancreas by involving afferent splanchnic and efferent pancreatic vagal nerves (Imai et al. 2008). The unique contribution of the liver in the determination of islet mass was also implicated by mice with tissue-targeted deletion of insulin receptors. The genetic deletion of insulin receptors from the liver, but not from the muscle or AT, results in an increase in islet mass (El Ouaamari et al. 2013). Interestingly, the blockade of insulin signaling, either pharmacologically or genetically, results in the increase of β cell proliferation at least partly through a humoral pathway (El Ouaamari et al. 2013; Yi et al. 2013). Recently, an effort to identify a factor responsible for humoral mitotic signal leads to betatrophin (also known as ANGPTL8, lipasin), a 22 kDa peptide secreted from the liver (Yi et al. 2013). Currently, little is known regarding the pathway by which betatrophin promotes β cell proliferation. Furthermore, translational value of betatrophin requires additional studies, as it is associated with an increase in serum triglycerides and hepatic cancer (Quagliarini et al. 2012). Also, it remains an important area of research to determine whether or not humoral and neuronal regulations of islet mass by the liver are impaired when integrity of the liver is affected, such as in nonalcoholic fatty liver disease.

T1D and T2D: Are They a Continuum or Distinct Entities?

T2D subjects include a heterogeneous population with variable degrees of insulin resistance and β cell dysfunction. On the other hand, T1D is regarded as an independent entity of disease characterized by autoimmune destruction of the β cells (Atkinson et al. 2013). However, the question has arisen whether these 2 forms of diabetes are completely different, as the chronic inflammation is not only limited

to insulin target tissues but also seen in the islets in T2D. Indeed, interventions targeting inflammatory pathways, such as IL-1β, DMT1, 12LO, and others, have shown efficacy in animal models of both T1D and T2D (Dobrian et al. 2011; Ehses et al. 2009; Hansen et al. 2012b; Tersey 2012a; Thomas et al. 2004), highlighting the possibility that particular anti-inflammatory therapy may become a unifying therapeutic tactic for both forms of diabetes (Imai et al. 2013a). To consider a continuum for the two forms of diabetes, there are two major questions to be addressed: whether insulin resistance modifies development of autoimmunity in T1D (Wilkin 2009) and whether the alteration in β cells during T2D development shares any commonalities with autoimmune insulitis associated with T1D.

Does Insulin Resistance Modify the Development of T1D?

One of the strongest arguments supporting the role of insulin resistance in T1D development comes from epidemiology. The recent global rise in obesity has been followed by increase in T1D incidence in many areas of the world (Atkinson et al. 2013). Meta-analysis based on five clinical studies supported association between obesity and subsequent development of T1D (pooled odds ratio of 1.25 with 95 % CI 1.04–1.51) (Verbeeten et al. 2011). Ex vivo and animal studies indicate that AT inflammation, lipotoxicity, ER stress, mitochondrial dysfunction, oxidative stress, and other pathways activated by obesity and insulin resistance could trigger inflammatory processes in the islets. It is intriguing to postulate that these insults may trigger or amplify autoimmune process in T1D. A recent report revealed signs of ER stress and NFκB activation along with early insulitis at a prediabetic stage of NOD, indicating that these cell distress pathways may interact and amplify autoimmune insulitis (Tersey et al. 2012b). Although not directly relevant to the pathogenesis of β cell demise in T1D, it is noteworthy that environmental pressure and advancement in insulin therapy have seen the substantial proportion of T1D subjects becoming insulin resistant as adults. The recent review of "double diabetes" (T1D exhibiting insulin resistance) provides excellent insight into the contribution of insulin resistance to cardiovascular risks in T1D population (Cleland et al. 2013).

Does Autoimmunity Contribute to Islet Dysfunction in T2D?

Increases in Mϕs and inflammatory cytokines localized in affected islets provide strong support for the involvement of innate immune responses in T2D development (Imai et al. 2013a). In contrast, the adaptive immune response against the islets has been considered to play a little role in T2D (Velloso et al. 2013). UKPDS and other large population-based studies have shown low levels of positivity for islet autoantibodies, including GADA, ICA, or IA-2A, in clinically defined T2D, UKPDS study being 11.6 % positive (Davis et al. 2005; Lohmann et al. 2001).

Those positive likely represent subjects with autoimmune diabetes or LADA, which shares pathogenesis with T1D but initially presents as non-insulin-requiring

diabetes in adulthood (Rolandsson and Palmer 2010). However, a series of studies by Brooks-Worrell et al. indicate that a significant proportion of clinically defined T2D patients may harbor autoimmunity against the islets (Brooks-Worrell and Palmer 2012; Brooks-Worrell et al. 2011). They detected islet antibodies, including ICA, GADA, IAA, and IA-2A, along with T cell responses against human islet extracts, in 36 phenotypical T2D of less than 5-year duration, and A1C below 8 % on one non-insulin, diabetic medication. They found 11/36 cases were positive for at least one AAb and 22/36 cases harbored T cells in peripheral circulation that showed proliferative responses against human islet extracts. The glucagon-stimulated C-peptide response was significantly lower in those with T cell responses against the islets, implicating an association of T cell responses and β cell demise. Of 11 antibody positive subjects, only 4 were positive for GADA, an antibody commonly used to diagnose LADA (Brooks-Worrell et al. 2011). Together with islet-reactive T cells, the study proposes the presence of adaptive immune responses in a significant population of clinically defined T2D who do not fit into the classic classification of autoimmune diabetes or LADA. Further studies will be required to test whether similar frequencies are seen in large populations of clinically defined adult T2D, especially those with circulatory islet-reactive T cells, as these have not been analyzed widely (Velloso et al. 2013).

In closing, it needs to be stressed that there are many distinctions between typical T1D and T2D that justified the classification of diabetes. Age of onset, rate of progression for β cell destruction and insulin deficiency, profiles of AAb, genetic susceptibility, and histology of the islets are clearly different between typical T1D and T2D (Brooks-Worrell et al. 2012; Cnop et al. 2005; Igoillo-Esteve et al. 2010). Additionally, genetic susceptibility foci identified by GWAS are mostly distinct between T1D and T2D. GLIS3, a Kruppel-like zinc finger protein important for the development of the pancreas and normal insulin secretion from adult β cells, is exceptional, being associated with both T1D and T2D (Nogueira et al. 2013). Also, neither T2D risk loci identified by GWAS nor body weight led to T1D progression, at least in the first-degree relatives of T1D, cautioning against attributing the increase in T1D incidence seen in recent years to the increase in the incidence of obesity (Winkler et al. 2012). Therefore, the field needs to continue to evaluate commonality and distinction between two forms of diabetes, so as to obtain more insights into the pathways involved in β cell demise in both forms of diabetes.

Inflammatory Pathways as a Therapeutic Target for Diabetes

Effort to Halt β Cell Loss Through Anti-inflammatory Therapy in T1D

The remission of newly diagnosed T1D by cyclosporine originally provided support for immunomodulatory therapy for T1D in 1980s (Staeva et al. 2013). As cyclosporine is not suitable for long-term therapy due to toxicity and side effects, many scientists have made an effort to treat and/or prevent T1D in humans by targeting the immune pathways. Thus far, little has come of these efforts despite the many

treatments that cure disease in NOD mice (reviewed in (Shoda et al. 2005)). More recent work has studied novel targets shown to be involved in the pathogenesis of T1D. The dipeptidyl peptidase-IV inhibitor, MK-626, has beneficial effects on β cell area, insulitis, and regulatory T cell populations when paired with the histone deacetylase inhibitor, vorinostat, in NOD mice (Cabrera et al. 2013). In an effort to stimulate regulatory T cells in vivo, but preventing the exacerbation of autoimmunity, the combination of rapamycin and IL-2 was tested in mice (Baeyens et al. 2013). While both show positive results alone, these results showed unexpected deleterious effects by stimulating NK cells and directly affecting β cell health. In humans, the β cell function was only transiently affected, while both regulatory T cells and NK cells were augmented in a persistent manner (Long et al. 2012).

One goal of new therapeutics to treat T1D is aimed at reducing the amount of inflammation locally in the pancreas of T1D patients, thus limiting toxicity to other organs. One means of accomplishing this goal is to use improved delivery systems that specifically target the pancreas. In one instance, nanoparticles have aided drug delivery in a targeted fashion (Ghosh et al. 2012). Others are working on the generation of tolerized autologous cells to squelch the adaptive immune response. Regulatory T cells have been the cells of choice for many years (Putnam et al. 2009). More recent studies are honing in on dendritic cells. In vitro development of vitamin D3-dexamethasone-modulated dendritic cells has significant hurdles to overcome before it could be considered as a potential therapy for T1D (Kleijwegt et al. 2013). The current studies have tolerized $CD8^+$ T cells to themselves, which works well with naïve $CD8^+$ T cells, but not with memory T cells.

A comprehensive list of human trials and outcomes was recently reviewed by von Herrath et al. (von Herrath et al. 2013). For the most part, few of these trials have shown any positive outcomes. The results are not fully available for all of the studies covered to date. Several studies concentrated on anti-inflammatory treatments, which might help protect β cells prior to disease onset (i.e., DHA treatment in infants at high risk (Miller et al. 2010, 2011)), or controlling diet in infants (removing gluten has minimal effect (Frederiksen et al. 2013; Hummel et al. 2011), but removal of bovine insulin shows decreased levels of islet AAb (Hummel et al. 2011; Vaarala et al. 2012)). Many studies looked at the effect of reducing the IL-1β levels; however, these treatments do not appear to halt disease progression in T1D (Moran et al. 2013).

Results from human islet transplantation studies have demonstrated the potential to reverse T1D by replacement of the functional β cell pool (Ryan et al. 2002; Shapiro et al. 2000). As a curative strategy, islet transplantation is currently limited by a number of factors including donor islet availability, donor-recipient ratio, and lack of long-term islet survival (Taylor-Fishwick et al. 2008). Dysfunction of the islet graft results, in part, from inadequate vascularization and inflammation-induced damage. The role of inflammation in β cell damage is recognized, but the cellular events that mediate the effects have been less well defined. Consequently, research with foci on pathogenic mechanisms resulting in β cell dysfunction and reduced β cell survival will improve the outcome of islet transplant.

A central contribution of inflammation and inflammatory cytokines to β cell dysfunction is anticipated to apply to T1D development and must be addressed in strategies to slow, halt, or reverse diabetes progression. Description of an integrated pathway associated with T1D development with discrete points for therapeutic intervention is encouraging when considering the future for T1D therapeutics. However, the restoration of β cell mass remains as the major challenge, since substantial β cell mass is already lost when T1D subjects present with hyperglycemia. The greatest promise for an effective therapy against T1D would be one that confers protection to β cells from the damaging effects of inflammation that is combined with a strategy to enhance β cell mass.

Therapeutics Targeting IL-1 Pathway

Several clinical trials have tested the efficacy of IL-1 pathway for treatment of both T1D and T2D. It is not surprising since the IL-1 pathway has been strongly implicated in both forms of diabetes. As several IL-1-targeted compounds are already in clinical use for systemic inflammatory diseases such as rheumatoid arthritis (anakinra), neonatal onset multisystem inflammatory disease (anakinra), cryopyrin-associated periodic syndromes (canakinumab), and systemic juvenile idiopathic arthritis (canakinumab), the availability of agents facilitated the initiation of clinical trials for their application to diabetes. As current status of IL-1-targeted therapy for T1D is discussed above, here, we focus on its application to T2D.

In 2007, Larsen et al. reported the first proof-of-concept study that showed the efficacy of targeting the IL-1 pathway to improve glycemic control in T2D (Larsen et al. 2007). Anakinra is a synthetic analog of the naturally occurring antagonist for IL-1Ra that blocks the action of IL-1α and IL-1β. It reduced A1c by 0.46 % in T2D who had pretreatment A1c of 8.7 % in a 13-week placebo-controlled, double-blinded, randomized trial. The reduction in hsCRP and IL-6 supported the idea that anakinra elicited anti-inflammatory effect in the study cohort. The improvement in insulin secretion was considered to contribute to reduction of A1c based on the increase in C-peptide and the reduction of the proinsulin/insulin ratio in the absence of significant improvement in insulin sensitivity (Larsen et al. 2007). Thirty-nine-week follow-up of the same cohort after cessation of anakinra showed that the improvement in proinsulin/insulin ratio was maintained, along with the reduction in hsCRP and IL-6. The increase in C-peptide was not maintained when all T2D were combined. However, a subgroup who initially responded to anakinra by reduction in A1c after 13 weeks of treatment continued to maintain higher C-peptide compared with nonresponders after termination of anakinra (Larsen et al. 2009).

IL-1β antibodies (gevokizumab (XOMA-052), canakinumab, LY2189102) are another class of therapeutics targeting the IL-1 pathway. IL-1β antibodies have benefit of a longer half-life compared with recombinant IL-1Ra, thereby allowing less frequent injections, and they block IL-1β without blocking IL-1α or other

cytokines. Gevokizumab administered to T2D with average A1c of ~9 % reduced A1c by 0.85 % and increased both C-peptide and insulin sensitivity after 3 months (Cavelti-Weder et al. 2012). Twelve weeks of phase II trial of LY2189102 in T2D showed ~0.3 % reduction in A1c along with the reduction in glycemia and inflammatory markers (Sloan-Lancaster et al. 2013). Diacerein is an anti-inflammatory agent marketed for osteoarthritis in some countries that also reduces IL-1β. In a 2-month randomized double-blinded, placebo-controlled trial, diacerein increased insulin secretion without changing insulin sensitivity in drug naïve T2D (Ramos-Zavala et al. 2011). Overall, IL-1-targeted therapies were well tolerated in diabetic subjects. However, it should be noted that studies so far include relatively small numbers of subjects treated for a limited period of time. Also, several of the IL-1-targeted trials did not show an improvement in glycemic control in T2D. There was no improvement in insulin sensitivity or β cell function following 4 weeks of treatment with anakinra in 19 prediabetic subjects (13 completed the study), despite the reduction in CRP and blood leukocyte counts (van Asseldonk et al. 2011). In another study, 4 weeks of treatment with canakinumab resulted in statistically significant improvement in insulin secretion upon meal challenge in subjects with impaired glucose tolerance; however, there were no statistically significant changes in subjects with well-controlled diabetes despite the reduction in hsCRP (Rissanen et al. 2012). It is apparent that a larger study with longer duration is needed to clarify the clinical benefits of IL-1-targeted therapy in T2D. CANTOS trial is a multinational, event-driven, intent-to-treat protocol enrolling 17,200 stable, post-MI subjects with persistent elevation of C-reactive protein (Ridker et al. 2011).

Although early reports following 4 months of canakinumab administration to relatively well-controlled T2D (A1c 7.4 %) from the CANTOS trial showed no improvement in A1c despite the reduction in CRP (Ridker et al. 2012), the trial will likely provide much needed data about the long-term efficacy of IL-1-targeted therapy on glycemic control. In addition, the trial monitors the prevalence of new-onset diabetes and provides the information regarding its efficacy in prevention of T2D. As the focus of trial is on cardiovascular outcomes, it will provide critical information about overall mortality and morbidity benefits for T2D. Drawbacks for IL-1-based therapies include the cost of currently available formulations and the fact that most, except for diacerein, need to be administered by injections.

From the basic research point of view, there also is some reservation for long-term benefit of IL-1 targeted therapy. PA, one of the major saturated fatty acids found in circulation in humans, provokes apoptosis of human islets in culture, a process considered to be involved in β cell demise in T2D. This process is accompanied by the production of multiple cytokines including IL-1β, TNF-α, CCL2, IL-6, CXCL1, and IL-8. Although IL-1β antagonism was able to abolish the upregulation of many cytokines upon PA treatment, it failed to prevent apoptosis (Igoillo-Esteve et al. 2010). Also IL-6, one of the cytokines induced by IL-1β in the islets, may be beneficial for the islets by increasing insulin secretion and islet function through GLP-1 secretion at least when tested in a noninflammatory condition (Ellingsgaard et al. 2011).

Nonacetylated Salicylate for T2D

Salicylic acid and its derivatives, salicylates, have been used to alleviate inflammatory conditions in humans for centuries. They are originally derived from plants where they act as hormones regulating growth, development, and defense against pathogens. The hypoglycemic effect of salicylates was documented in 1876 (reviewed in Goldfine et al. (2011)) but received little attention until its role as an inhibitor of the NFκB pathway attracted attention. Nonacetylated salicylates, such as salsalate, have very weak activity as cyclooxygenase inhibitors but suppress the NFκB pathway, which is known to be activated in animal models of obesity and T2D (Donath and Shoelson 2011). However, salicylates may elicit hypoglycemic effects through multiple mechanisms in addition to inhibition of NFκB pathway. Proposed targets of salicylates encompass a wide range of enzymes and transcription factors, including mitochondrial dehydrogenases involved in glucose metabolism (Hines and Smith 1964) and transcription factors implicated in immune cell activation (Aceves et al. 2004). Recently, the direct activation of AMPK was proposed as another mechanism responsible for at least part of the metabolic effects of salicylates (Hawley et al. 2012). Salicylates have also been shown to suppress the activation of 11-β-hydroxysteroid dehydrogenase in AT and may prevent glucocorticoid production and insulin resistance (Nixon et al. 2012). There were several clinical trials that tested efficacy of salsalate for glycemic control in T2D. TINSAL-T2D is a multicenter randomized clinical trial that tested the efficacy of salsalate for T2D. Stage I, involving 14 weeks of 3, 3.5, and 4 gm/day salsalate for T2D, showed a significant reduction of A1c ranging 0.3–0.5 % in all doses of salsalate tested (Goldfine et al. 2010).

For the stage II trial, 286 T2D patients were enrolled in double-blinded, randomized, prospective study of 3.5 gm salsalate daily for 48 weeks as an add-on to the diet or other therapies excluding injectables and thiazolidinedione. Although A1c reduction has been modest (0.37 %, 0.53–0.21 95 % CI, $p < 0.001$), there was a reduction in the use of diabetic medications. The treatment group showed increases in fasting insulin levels and a reduction in C-peptide. Reductions in uric acid, glucose, leukocytes, and triglycerides and an increase in adiponectin were noted, indicating an overall improvement in metabolic syndrome. Increases in LDL and body weight, however, were concerning. A reversible increase in urine microalbumin was also noted in the study (Goldfine et al. 2013b). Sixty drug naïve, newly diagnosed T2D treated for 12 weeks with 3 gm/kg of salsalate showed reductions in fasting glucose (6.3 ± 0.2 mmol/l to 5.4 ± 0.2 mmol/l), triglycerides, and WBC, and a 0.5 % decline in A1c. This was accompanied by increased fasting insulin (Faghihimani et al. 2013). A 12-week, randomized placebo-controlled study of salsalate in 71 subjects with impaired fasting glucose and/or impaired glucose tolerance showed 6 % reduction in fasting glucose along with 25 % reduction in triglyceride with 53 % increase in adiponectin (Goldfine et al. 2013a). Overall, the reductions in glucose and A1c were modest but were generally reproducible in multiple studies. Several mechanisms responsible for improvement in glycemia are proposed from clinical data. Fasting C-peptide was reduced, arguing against improvement in β cell function (Goldfine et al. 2013a).

In addition, euglycemic hyperinsulinemic clamp testing did not support the improvement in insulin sensitivity (Goldfine et al. 2013a). Several studies implicate salsalate in reducing the clearance of insulin, resulting in reduced glycemia in humans (Hundal et al. 2002; Koska et al. 2009). The short half-life of salsalate requires multiple doses per day, and mechanisms of action are not fully elucidated, especially at the high dose used in clinical trials. However, this low-cost and orally active compound holds potential to serve as T2D agent, especially if shown to have cardiovascular benefit as well. Currently, cardioprotective effects of salsalate are being evaluated in the ongoing TINSAL-CVD trial.

12LO as a Target for T1D and T2D

The role of 12LO in the development of T1D is gaining appreciation. Several models have shown that T1D pathogenesis is disrupted in the absence of 12/15LO (a mouse gene for 12LO) in mice (Bleich et al. 1999; McDuffie et al. 2008). Further research has shown that it is likely that both Mϕ and islet expression of 12LO contribute to disease development (Green-Mitchell et al. 2013). Ongoing studies will investigate the contribution of individual cells to diabetes development in the NOD mouse model. In humans, 12LO is expressed in the islets, although specific cellular expression has not yet been determined (Ma et al. 2010). 12LO has also been implicated in the pathogenesis of enteroaggregative E. coli infection (Boll et al. 2012), which leads to upregulation of inflammation and possible alterations of the flora in the gut. Therefore, it is possible that the 12LO pathway could be activated by changes in the gut microbiome. The collective works thus far have indicated that inhibitors of 12LO might suitably and selectively target a portion of the inflammatory pathway with relatively minor side effects, as knockout mice show no major ill effects. In vitro investigations have shown promise in protecting the islets from cytokine injury using newly developed inhibitors of the 12LO pathway (Weaver et al. 2012).

The role of 12LO is not limited to islet inflammation in T1D. 12LO and their products play important roles in many tissues and organs, including the vasculature, kidney, AT, brain, and pancreatic islet during the development of T2D and their complications (Dobrian et al. 2010a). There is emerging evidence that the various 12LO isoforms (platelet 12LO, 12/15LO) in both white and brown fats are expressed in multiple cell-type constituent of AT: adipocytes, vascular cells, macrophages, and pre-adipocytes. The presence and roles of 12LO in adipocytes have been extensively investigated by our group and by others in mouse models of obesity, insulin resistance, and T2D. C57BL/6 J mice that have been on a high-fat diet for 8–16 weeks exhibit increased expression of 12/15LO in isolated white adipocytes (Chakrabarti et al. 2009), and Zucker obese rats, a genetically induced rodent model of obesity and insulin resistance, also exhibit increased expression of 12/15LO in isolated white adipocytes compared to lean controls (Chakrabarti et al. 2011). Also, more recent data from our lab showed that in db/db mice, leukocyte and platelet 12LO in AT and pancreatic islets undergo expressional increases that coincide with the metabolic decline.

Addition of one of the primary 12LO metabolites, 12-HETE, to fully differentiated 3T3-L1 adipocyte cultures significantly induced pro-inflammatory gene expression and secretion of many pro-inflammatory cytokines, including TNF-α, CCL2, IL-6, and IL-12p40. Also, the anti-inflammatory adiponectin was significantly decreased under these conditions. Importantly, addition of the same metabolites led to an increase in activation of c-Jun, while insulin-mediated activation of key insulin signaling proteins such as Akt and IRS1 was decreased. Addition of PA to 3T3-L1 adipocytes increased 12/15LO expression with concomitant increased cytokine expression. Also, recent evidence from our lab demonstrates that 12LO is a novel inflammatory pathway that mediates ER stress in the adipocyte (Cole et al. 2012a). Interestingly, in a 12/15LO conditional knockout in AT, the inflammation in response to high dietary fat was reduced not only in adipocytes but also in the pancreatic islets, and the glucose intolerance was restored, indicating the important role of 12/15LO expressed in AT for the islet functional demise (Cole et al. 2012b).

Relevant for the translational value of the therapeutic potential of various 12LO inhibitors, important differences were emphasized in the 12LO pathway between rodents and humans (Dobrian et al. 2010a). In a recent publication, we reported human 12LO mRNA and protein expression in human AT with exclusive localization in the SVF both in the SC and in the OM fat (Dobrian et al. 2010b). Increased expression of all of the 12LO enzyme isoforms in OM vs. SC AT suggests that the pathway may contribute to the pro-inflammatory milieu prominently associated to visceral fat in obesity (Dobrian et al. 2010b).

Recent evidence suggests a pro-inflammatory role of 12LO pathway in humans. Gene array analysis of AT showed that arachidonic acid metabolism is the second most significantly upregulated pathway in human omental compared to subcutaneous AT in human obese subjects. The very limited information on 12LO functional roles and changes with different pathological conditions in human AT grants future studies to identify the roles of different isoforms and the lipid mediators that are key for regulation of inflammation in human obesity and T2D.

While understanding the mechanisms that control the regulation of the 12LO pathway require substantial future efforts, the contribution of this pathway to inflammation in AT in obesity is well established and may constitute a valuable future therapeutic target. Therefore, 12LO inhibitors hold promise in T2D therapy by reducing insulin resistance and restoring insulin secretion.

Anti-inflammatory Effects of Therapies Currently Available for Diabetic Patients

Some of the drugs that are currently used to treat hyperglycemia in T2D (incretins, PPARγ agonists) or various diabetic complications, such as hypertension (ACE inhibitors, angiotensin receptor blockers), have anti-inflammatory effects that may contribute to their overall positive outcomes. Also, bariatric surgery performed in morbidly obese subjects in particular the Roux-en-Y procedure has anti-inflammatory effects in subjects with T2D that may be in part independent of weight loss.

GLP-1 is a gut incretin hormone that reduces hyperglycemia primarily via insulinotropic effects and by reducing postprandial excursions of plasma glucose (Drucker 2006). Modulation of the GLP-1 system is a current therapeutic option for the treatment of T2D and involves the use of either long-acting GLP-1 analogs, mimetic peptides acting as GLP-1 receptor agonists (Estall and Drucker 2006), or inhibitors of the DPPIV (such as sitagliptin and analogs) that extend the endogenous GLP-1 half-life (Drucker 2007). Both approaches proved successful for the control of glycemia in T2D. However, recent evidence unveiled off-target anti-inflammatory effects of the GLP-1 analogs and DPPIV inhibitors that may account for their overall positive therapeutic outcome independent of glucoregulation. A recent report showed that in a cohort of obese T2D subjects, 8 weeks after starting GLP-1 analog therapy, soluble CD163, a molecule released from inflammatory macrophage activation, was significantly reduced in the cohort that received therapy independent of reduction in A1c. Also, GLP-1 therapy in the cohort of T2D patients reduced the basal levels of pro-inflammatory cytokines TNF-α and IL-6 while increasing adiponectin levels (Hogan et al. 2013).

Also, GLP-1 overexpression via adenoviral GLP-1 delivery to *ob*/*ob* mice was shown to improve insulin sensitivity and glucose tolerance (Lee et al. 2012). The effect may be attributed to the anti-inflammatory effects in adipocytes and AT reflected by reduced JNK-related signaling and a bias toward the M2 anti-inflammatory macrophage phenotype in AT. GLP-1 agonism may also influence local inflammation by modulating innate immunity. iNKT cells have a rather controversial role in the pathogenesis of obesity-related insulin resistance with reports showing either a pro- or an anti-inflammatory role. A recent report showed a significant improvement in psoriasis plaques in subjects with obesity and T2D treated with the GLP-1 agonist exenatide (Hogan et al. 2011). Interestingly, the effect was mediated via activation of iNKT cells that have been shown to have insulin-sensitizing effects in AT. This opens the possibility to modulate the NK-cell phenotype by using GLP-1 agonists which may enhance the insulin-sensitizing effects of the treatment. DPPIV inhibitors represent another class of drugs that emerged as potent anti-inflammatory agents in various tissues. DPPIV is expressed in the immune cells, vasculature, pancreatic islets, and AT. Aside from its enzymatic activity, DPPIV has multiple nonenzymatic effects including stimulating T cell proliferation and monocyte migration through the endothelium, cytokine production, and processing of various chemokines.

The multiplicity of functions and targets suggests that DPPIV may play a distinct role aside from its effects on the incretin axis (Zhong et al. 2013). Several recent studies emphasized anti-inflammatory actions of DPPIV inhibitors in various tissues. Our lab was among the first to report a reduction in pro-inflammatory cytokine production in the AT, adipocytes, and pancreatic islets of C67Bl6 mice on high-fat diet treated for 12 weeks with the DDPIV inhibitor sitagliptin (Dobrian et al. 2010c). Other studies also showed anti-inflammatory effects of vildagliptin in the islets and enhanced β cell function in an advanced-aged diet-induced obesity mouse model (Omar et al. 2013) as well as reversal of new-onset diabetes in NOD mice through modulation of inflammation in the islets and stimulation of β cell

replication (Jelsing et al. 2012). Clinical trials are currently ongoing aiming to establish the beneficial anti-inflammatory effects of different DPPIV inhibitors on β cell function and on the cardiac and vascular function.

Hypertension is a common complication of obesity and T2D. Inhibition of the renin-angiotensin axis via ACE inhibitors or ARb represents the preferred approach in patients with obesity and T2D. Modulation of the renin-angiotensin system may also have anti-inflammatory effects aside from the blood pressure-lowering effect. A number of studies with ARbs found a reduction in systemic inflammation with reduced levels of TNF-α, IL-6, and C-reactive protein in hypertensive patients with T2D (Fliser et al. 2004; Pavlatou et al. 2011). The angiotensin receptor type 1 is expressed in several immune cells including T cells, monocytes, and macrophages. Not surprisingly, the ARb telmisartan was found to modulate AT macrophage polarization toward an M2-like anti-inflammatory phenotype in diet-induced obese mice (Fujisaka et al. 2011).

Importantly, several clinical trials have indicated that ACEs and ARbs reduce the incidence of new-onset T2D in high-risk populations. This effect was in part independent from the blood pressure-lowering activity and attributed to improved insulin signaling in the muscle and adipocyte (van der Zijl et al. 2012). Whether these effects are attributable to reduced inflammation remains to be determined. Additional anti-inflammatory drugs currently used for other conditions may have off-target anti-inflammatory effects and could be selected in the future keeping in mind the convincing evidence for the safety profiles in humans.

Concluding Remarks

Substantial progress has been made in recent years for the characterization of inflammatory response in the islets of both T1D and T2D. It is clear that the loss of tolerance to β cells in T1D is established through complex interaction between genetic and environmental factors, β cells, humoral mediators, and wide ranges of immune cells. Importantly, metabolic stress classically associated with T2D may modify immune response and may be an important contributing factor for the development of T1D. For T2D, metabolic stress such as glucolipotoxicity, ER stress, and oxidative stress is likely responsible for inflammatory response in the islets that in turn amplifies ER stress and oxidative stress, accelerating loss of β cell mass and function. Mϕs and humoral mediators such as IL-1β, CCL2, 12LO, IL-12, and CXCL10 are implicated in islet inflammation in T2D, but the role of adaptive immunity in the islets of T2D has not been firmly established. Islet functions and inflammatory response in the islets are likely under effects of metabolic status of other tissues such as the AT, the liver, and the gut. Further research is warranted in this area. Anti-inflammatory/immune therapy for T1D has been challenging despite the numerous efforts. The area may benefit from analyses of human donor pancreata to better understand pathogenesis of T1D in humans. Two major anti-inflammatory therapies tested for T2D to date are IL-1β-targeted therapy and salsalate. Both show promise and support the contribution of inflammatory

response in β cell dysfunction of T2D. However, the most effective target for anti-inflammatory therapy to halt heterogeneous and the slow progressive decline in the islets in T2D is yet to be determined. Thus, islet inflammation will likely remain an important area of research for the coming years.

Acknowledgement Human studies in unpublished data were approved by the Institutional Review Board at EVMS. Human islets in unpublished data were provided by Integrated Islet Distribution Program (IIDP). Funding support for the authors includes Juvenile Research Foundation grant (Nadler, Taylor-Fishwick), American Diabetes Association (Morris), National Institutes of Health (R01-DK090490 to Imai, R15-HL114062 to Dobrian, R01-HL112605 to Nadler), Astra Zeneca (Dobrian, Nadler), and Congressionally Directed Medical Research Program, Department of Defense (PR093521 to Taylor-Fishwick).

Cross-References

- ▶ Clinical Approaches to Preserve β-Cell Function in Diabetes
- ▶ Current Approaches and Future Prospects for the Prevention of β-Cell Destruction in Autoimmune Diabetes
- ▶ High Fat Programming of β-Cell Dysfunction
- ▶ Immunology of β-Cell Destruction
- ▶ Islet Structure and Function in the GK Rat
- ▶ Mechanisms of Pancreatic β-Cell Apoptosis in Diabetes and Its Therapies
- ▶ Pancreatic β Cells in Metabolic Syndrome
- ▶ Role of Mitochondria in β-cell Function and Dysfunction
- ▶ Role of NADPH Oxidase in β Cell Dysfunction
- ▶ The β-Cell in Human Type 2 Diabetes

References

Aceves M, Duenas A, Gomez C, San Vicente E, Crespo MS, Garcia-Rodriguez C (2004) A new pharmacological effect of salicylates: inhibition of NFAT-dependent transcription. J Immunol 173:5721–5729

Achenbach P, Warncke K, Reiter J, Naserke HE, Williams AJ, Bingley PJ, Bonifacio E, Ziegler AG (2004) Stratification of type 1 diabetes risk on the basis of islet autoantibody characteristics. Diabetes 53:384–392

Akesson C, Uvebrant K, Oderup C, Lynch K, Harris RA, Lernmark A, Agardh CD, Cilio CM (2010) Altered natural killer (NK) cell frequency and phenotype in latent autoimmune diabetes in adults (LADA) prior to insulin deficiency. Clin Exp Immunol 161:48–56

Alkanani AK, Rewers M, Dong F, Waugh K, Gottlieb PA, Zipris D (2012) Dysregulated Toll-like receptor-induced interleukin-1β and interleukin-6 responses in subjects at risk for the development of type 1 diabetes. Diabetes 61:2525–2533

Allen JS, Pang K, Skowera A, Ellis R, Rackham C, Lozanoska-Ochser B, Tree T, Leslie RD, Tremble JM, Dayan CM, Peakman M (2009) Plasmacytoid dendritic cells are proportionally expanded at diagnosis of type 1 diabetes and enhance islet autoantigen presentation to T-cells through immune complex capture. Diabetes 58:138–145

Al-Maskari M, Al-Shukaili A, Al-Mammari A (2010) Pro-inflammatory cytokines in Omani type 2 diabetic patients presenting anxiety and depression. Iran J Immunol 7:124–129

Al-Salam S, Rashed H, Adeghate E (2011) Diabetes mellitus is associated with an increased expression of resistin in human pancreatic islet cells. Islets 3:246–249

Antkowiak PF, Stevens BK, Nunemaker CS, McDuffie M, Epstein FH (2013) Manganese-enhanced magnetic resonance imaging detects declining pancreatic β-cell mass in a cyclophosphamide-accelerated mouse model of type 1 diabetes. Diabetes 62:44–48

Arif S, Tree TI, Astill TP, Tremble JM, Bishop AJ, Dayan CM, Roep BO, Peakman M (2004) Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. J Clin Invest 113:451–463

Arif S, Moore F, Marks K, Bouckenooghe T, Dayan CM, Planas R, Vives-Pi M, Powrie J, Tree T, Marchetti P, Huang GC, Gurzov EN, Pujol-Borrell R, Eizirik DL, Peakman M (2011) Peripheral and islet interleukin-17 pathway activation characterizes human autoimmune diabetes and promotes cytokine-mediated β-cell death. Diabetes 60:2112–2119

Atkinson MA, Eisenbarth GS, Michels AW (2013) Type 1 diabetes. Lancet 383(9911):69–82

Axling U, Olsson C, Xu J, Fernandez C, Larsson S, Strom K, Ahrne S, Holm C, Molin G, Berger K (2012) Green tea powder and Lactobacillus plantarum affect gut microbiota, lipid metabolism and inflammation in high-fat fed C57BL/6J mice. Nutr Metab 9:105

Baeyens A, Perol L, Fourcade G, Cagnard N, Carpentier W, Woytschak J, Boyman O, Hartemann A, Piaggio E (2013) Limitations of IL-2 and rapamycin in immunotherapy of type 1 diabetes. Diabetes 62(9):3120–3131

Baldwin AC, Green CD, Olson LK, Moxley MA, Corbett JA (2012) A role for aberrant protein palmitoylation in FFA-induced ER stress and β-cell death. Am J Physiol Endocrinol Metab 302:E1390–E1398

Beilke JN, Meagher CT, Hosiawa K, Champsaur M, Bluestone JA, Lanier LL (2012) NK cells are not required for spontaneous autoimmune diabetes in NOD mice. PloS One 7:e36011

Bensellam M, Laybutt DR, Jonas JC (2012) The molecular mechanisms of pancreatic β-cell glucotoxicity: recent findings and future research directions. Mol Cell Endocrinol 364:1–27

Bleich D, Chen S, Zipser B, Sun D, Funk CD, Nadler JL (1999) Resistance to type 1 diabetes induction in 12-lipoxygenase knockout mice. J Clin Invest 103:1431–1436

Boll EJ, Struve C, Sander A, Demma Z, Krogfelt KA, McCormick BA (2012) Enteroaggregative *Escherichia coli* promotes transepithelial migration of neutrophils through a conserved 12-lipoxygenase pathway. Cell Microbiol 14:120–132

Bonifacio E, Bingley PJ, Shattock M, Dean BM, Dunger D, Gale EA, Bottazzo GF (1990) Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. Lancet 335:147–149

Boni-Schnetzler M, Thorne J, Parnaud G, Marselli L, Ehses JA, Kerr-Conte J, Pattou F, Halban PA, Weir GC, Donath MY (2008) Increased interleukin IL-1β messenger ribonucleic acid expression in β-cells of individuals with type 2 diabetes and regulation of IL-1β in human islets by glucose and autostimulation. J Clin Endocrinol Metab 93:4065–4074

Bosi E, Molteni L, Radaelli MG, Folini L, Fermo I, Bazzigaluppi E, Piemonti L, Pastore MR, Paroni R (2006) Increased intestinal permeability precedes clinical onset of type 1 diabetes. Diabetologia 49:2824–2827

Boslem E, Meikle PJ, Biden TJ (2012) Roles of ceramide and sphingolipids in pancreatic β-cell function and dysfunction. Islets 4:177–187

Boudaly S, Morin J, Berthier R, Marche P, Boitard C (2002) Altered dendritic cells (DC) might be responsible for regulatory T cell imbalance and autoimmunity in nonobese diabetic (NOD) mice. Eur Cytokine Netw 13:29–37

Bradshaw EM, Raddassi K, Elyaman W, Orban T, Gottlieb PA, Kent SC, Hafler DA (2009) Monocytes from patients with type 1 diabetes spontaneously secrete proinflammatory cytokines inducing Th17 cells. J Immunol 183:4432–4439

Brauner H, Elemans M, Lemos S, Broberger C, Holmberg D, Flodstrom-Tullberg M, Karre K, Hoglund P (2010) Distinct phenotype and function of NK cells in the pancreas of nonobese diabetic mice. J Immunol 184:2272–2280

Brom M, Andralojc K, Oyen WJ, Boerman OC, Gotthardt M (2010) Development of radiotracers for the determination of the β-cell mass in vivo. Curr Pharm Des 16:1561–1567

Brooks-Worrell B, Palmer JP (2012) Immunology in the Clinic Review Series; focus on metabolic diseases: development of islet autoimmune disease in type 2 diabetes patients: potential sequelae of chronic inflammation. Clin Exp Immunol 167:40–46

Brooks-Worrell BM, Palmer JP (2013) Attenuation of islet-specific T cell responses is associated with C-peptide improvement in autoimmune type 2 diabetes patients. Clin Exp Immunol 171:164–170

Brooks-Worrell BM, Reichow JL, Goel A, Ismail H, Palmer JP (2011) Identification of autoantibody-negative autoimmune type 2 diabetic patients. Diabetes Care 34:168–173

Brooks-Worrell B, Narla R, Palmer JP (2012) Biomarkers and immune-modulating therapies for type 2 diabetes. Trends Immunol 33:546–553

Brown JE, Conner AC, Digby JE, Ward KL, Ramanjaneya M, Randeva HS, Dunmore SJ (2010a) Regulation of β-cell viability and gene expression by distinct agonist fragments of adiponectin. Peptides 31:944–949

Brown JE, Onyango DJ, Ramanjaneya M, Conner AC, Patel ST, Dunmore SJ, Randeva HS (2010b) Visfatin regulates insulin secretion, insulin receptor signalling and mRNA expression of diabetes-related genes in mouse pancreatic β-cells. J Mol Endocrinol 44:171–178

Brown CT, Davis-Richardson AG, Giongo A, Gano KA, Crabb DB, Mukherjee N, Casella G, Drew JC, Ilonen J, Knip M, Hyoty H, Veijola R, Simell T, Simell O, Neu J, Wasserfall CH, Schatz D, Atkinson MA, Triplett EW (2011) Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. PLoS One 6: e25792

Buschard K, Mansson JE, Roep BO, Nikolic T (2012) Self-glycolipids modulate dendritic cells changing the cytokine profiles of committed autoreactive T cells. PLoS One 7:e52639

Cabrera SM, Colvin SC, Tersey SA, Maier B, Nadler JL, Mirmira RG (2013) Effects of combination therapy with dipeptidyl peptidase-IV and histone deacetylase inhibitors in the non-obese diabetic mouse model of type 1 diabetes. Clin Exp Immunol 172:375–382

Cai K, Qi D, Wang O, Chen J, Liu X, Deng B, Qian L, Liu X, Le Y (2011) TNF-α acutely upregulates amylin expression in murine pancreatic β cells. Diabetologia 54:617–626

Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM (2009) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 58:1091–1103

Cao P, Marek P, Noor H, Patsalo V, Tu LH, Wang H, Abedini A, Raleigh DP (2013) Islet amyloid: from fundamental biophysics to mechanisms of cytotoxicity. FEBS letters 587:1106–1118

Carrington EM, Kos C, Zhan Y, Krishnamurthy B, Allison J (2011) Reducing or increasing β-cell apoptosis without inflammation does not affect diabetes initiation in neonatal NOD mice. Eur J Immunol 41:2238–2247

Catalan V, Gomez-Ambrosi J, Ramirez B, Rotellar F, Pastor C, Silva C, Rodriguez A, Gil MJ, Cienfuegos JA, Fruhbeck G (2007) Proinflammatory cytokines in obesity: impact of type 2 diabetes mellitus and gastric bypass. Obes Surg 17:1464–1474

Cavelti-Weder C, Babians-Brunner A, Keller C, Stahel MA, Kurz-Levin M, Zayed H, Solinger AM, Mandrup-Poulsen T, Dinarello CA, Donath MY (2012) Effects of gevokizumab on glycemia and inflammatory markers in type 2 diabetes. Diabetes Care 35:1654–1662

Chai F, Wang Y, Zhou Y, Liu Y, Geng D, Liu J (2011) Adiponectin downregulates hyperglycemia and reduces pancreatic islet apoptosis after roux-en-y gastric bypass surgery. Obes Surg 21:768–773

Chakrabarti SK, Cole BK, Wen Y, Keller SR, Nadler JL (2009) 12/15-lipoxygenase products induce inflammation and impair insulin signaling in 3T3-L1 adipocytes. Obesity (Silver Spring) 17(9):1657–1663

Chakrabarti SK, Wen Y, Dobrian AD, Cole BK, Ma Q, Pei H, Williams MD, Bevard MH, Vandenhoff GE, Keller SR, Gu J, Nadler JL (2011) Evidence for activation of inflammatory

lipoxygenase pathways in visceral adipose tissue of obese zucker rats. Am J Physiol Endocrinol Metab 300(1):E175–E187

Chan JY, Luzuriaga J, Bensellam M, Biden TJ, Laybutt DR (2013) Failure of the adaptive unfolded protein response in islets of obese mice is linked with abnormalities in β-cell gene expression and progression to diabetes. Diabetes 62:1557–1568

Chang YH, Chang DM, Lin KC, Shin SJ, Lee YJ (2011) Visfatin in overweight/obesity, type 2 diabetes mellitus, insulin resistance, metabolic syndrome and cardiovascular diseases: a meta-analysis and systemic review. Diabetes Metab Res Rev 27:515–527

Charre S, Rosmalen JG, Pelegri C, Alves V, Leenen PJ, Drexhage HA, Homo-Delarche F (2002) Abnormalities in dendritic cell and macrophage accumulation in the pancreas of nonobese diabetic (NOD) mice during the early neonatal period. Histol Histopathol 17:393–401

Chawla A, Nguyen KD, Goh YP (2011) Macrophage-mediated inflammation in metabolic disease. Nat Rev Immunol 11:738–749

Chen JJ, Wang R, Li XF, Wang RL (2011) Bifidobacterium longum supplementation improved high-fat-fed-induced metabolic syndrome and promoted intestinal Reg I gene expression. Exp Biol Med (Maywood) 236:823–831

Cheng Q, Dong W, Qian L, Wu J, Peng Y (2011) Visfatin inhibits apoptosis of pancreatic β-cell line, MIN6, via the mitogen-activated protein kinase/phosphoinositide 3-kinase pathway. J Mol Endocrinol 47:13–21

Cleland SJ, Fisher BM, Colhoun HM, Sattar N, Petrie JR (2013) Insulin resistance in type 1 diabetes: what is 'double diabetes' and what are the risks? Diabetologia 56:1462–1470

Cnop M, Welsh N, Jonas JC, Jorns A, Lenzen S, Eizirik DL (2005) Mechanisms of pancreatic β-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes 54(Suppl 2):S97–S107

Cnop M, Ladriere L, Igoillo-Esteve M, Moura RF, Cunha DA (2010) Causes and cures for endoplasmic reticulum stress in lipotoxic β-cell dysfunction. Diabetes Obes Metab 12(Suppl 2):76–82

Cole BK, Kuhn NS, Green-Mitchell SM, Leone KA, Raab RM, Nadler JL, Chakrabarti SK (2012a) 12/15-lipoxygenase signaling in the endoplasmic reticulum stress response. Am J Physiol Endocrinol Metab 302:E654–E665

Cole BK, Morris MA, Grzesik WJ, Leone KA, Nadler JL (2012b) Adipose tissue-specific deletion of 12/15-lipoxygenase protects mice from the consequences of a high-fat diet. Mediators Inflamm 2012:851798

Cunningham GA, McClenaghan NH, Flatt PR, Newsholme P (2005) L-alanine induces changes in metabolic and signal transduction gene expression in a clonal rat pancreatic β-cell line and protects from pro-inflammatory cytokine-induced apoptosis. Clin Sci (Lond) 109:447–455

D'Alise AM, Auyeung V, Feuerer M, Nishio J, Fontenot J, Benoist C, Mathis D (2008) The defect in T-cell regulation in NOD mice is an effect on the T-cell effectors. Proc Natl Acad Sci USA 105:19857–19862

Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, Lin JK, Farzadfar F, Khang YH, Stevens GA, Rao M, Ali MK, Riley LM, Robinson CA, Ezzati M (2011) National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 - country-years and 2.7 million participants. Lancet 378:31–40

Davis TM, Wright AD, Mehta ZM, Cull CA, Stratton IM, Bottazzo GF, Bosi E, Mackay IR, Holman RR (2005) Islet autoantibodies in clinically diagnosed type 2 diabetes: prevalence and relationship with metabolic control (UKPDS 70). Diabetologia 48:695–702

de Goffau MC, Luopajarvi K, Knip M, Ilonen J, Ruohtula T, Harkonen T, Orivuori L, Hakala S, Welling GW, Harmsen HJ, Vaarala O (2013) Fecal microbiota composition differs between children with β-cell autoimmunity and those without. Diabetes 62:1238–1244

DeFuria J, Belkina AC, Jagannathan-Bogdan M, Snyder-Cappione J, Carr JD, Nersesova YR, Markham D, Strissel KJ, Watkins AA, Zhu M, Allen J, Bouchard J, Toraldo G, Jasuja R, Obin MS, McDonnell ME, Apovian C, Denis GV, Nikolajczyk BS (2013) B cells promote

inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile. Proc Natl Acad Sci USA 110:5133–5138
Deng Y, Scherer PE (2010) Adipokines as novel biomarkers and regulators of the metabolic syndrome. Ann N Y Acad Sci 1212:E1–E19
Dobrian AD, Lieb DC, Cole BK, Taylor-Fishwick DA, Chakrabarti SK, Nadler JL (2010a) Functional and pathological roles of the 12- and 15-lipoxygenases. Prog Lipid Res 50:115–131
Dobrian AD, Lieb DC, Ma Q, Lindsay JW, Cole BK, Ma K, Chakrabarti SK, Kuhn NS, Wohlgemuth SD, Fontana M, Nadler JL (2010b) Differential expression and localization of 12/15 lipoxygenases in adipose tissue in human obese subjects. Biochem Biophys Res Commun 403:485–490
Dobrian AD, Ma Q, Lindsay JW, Leone KA, Ma K, Coben J, Galkina EV, Nadler JL (2010c) Dipeptidyl peptidase IV inhibitor sitagliptin reduces local inflammation in adipose tissue and in pancreatic islets of obese mice. Am J Physiol Endocrinol Metab 300:E410–E421
Dobrian AD, Lieb DC, Cole BK, Taylor-Fishwick DA, Chakrabarti SK, Nadler JL (2011) Functional and pathological roles of the 12- and 15-lipoxygenases. Prog Lipid Res 50:115–131
Donath MY, Shoelson SE (2011) Type 2 diabetes as an inflammatory disease. Nat Rev Immunol 11:98–107
Donath MY, Dalmas E, Sauter NS, Boni-Schnetzler M (2013) Inflammation in obesity and diabetes: islet dysfunction and therapeutic opportunity. Cell Metab 17:860–872
Dotta F, Censini S, van Halteren AG, Marselli L, Masini M, Dionisi S, Mosca F, Boggi U, Muda AO, Del Prato S, Elliott JF, Covacci A, Rappuoli R, Roep BO, Marchetti P (2007) Coxsackie B4 virus infection of β cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. Proc Natl Acad Sci USA 104:5115–5120
Drucker DJ (2006) The biology of incretin hormones. Cell Metab 3:153–165
Drucker DJ (2007) Dipeptidyl peptidase-4 inhibition and the treatment of type 2 diabetes: preclinical biology and mechanisms of action. Diabetes Care 30:1335–1343
Dunmore SJ, Brown JE (2013) The role of adipokines in β-cell failure of type 2 diabetes. J Endocrinol 216:T37–T45
Eguchi K, Manabe I, Oishi-Tanaka Y, Ohsugi M, Kono N, Ogata F, Yagi N, Ohto U, Kimoto M, Miyake K, Tobe K, Arai H, Kadowaki T, Nagai R (2012) Saturated fatty acid and TLR signaling link β cell dysfunction and islet inflammation. Cell Metab 15:518–533
Ehses JA, Perren A, Eppler E, Ribaux P, Pospisilik JA, Maor-Cahn R, Gueripel X, Ellingsgaard H, Schneider MK, Biollaz G, Fontana A, Reinecke M, Homo-Delarche F, Donath MY (2007) Increased number of islet-associated macrophages in type 2 diabetes. Diabetes 56:2356–2370
Ehses JA, Lacraz G, Giroix MH, Schmidlin F, Coulaud J, Kassis N, Irminger JC, Kergoat M, Portha B, Homo-Delarche F, Donath MY (2009) IL-1 antagonism reduces hyperglycemia and tissue inflammation in the type 2 diabetic GK rat. Proc Natl Acad Sci USA 106:13998–14003
Eizirik DL, Mandrup-Poulsen T (2001) A choice of death – the signal-transduction of immune-mediated β-cell apoptosis. Diabetologia 44:2115–2133
Eizirik DL, Sammeth M, Bouckenooghe T, Bottu G, Sisino G, Igoillo-Esteve M, Ortis F, Santin I, Colli ML, Barthson J, Bouwens L, Hughes L, Gregory L, Lunter G, Marselli L, Marchetti P, McCarthy MI, Cnop M (2012) The human pancreatic islet transcriptome: expression of candidate genes for type 1 diabetes and the impact of pro-inflammatory cytokines. PLoS Genet 8:e1002552
Eizirik DL, Miani M, Cardozo AK (2013) Signalling danger: endoplasmic reticulum stress and the unfolded protein response in pancreatic islet inflammation. Diabetologia 56:234–241
El Ouaamari A, Kawamori D, Dirice E, Liew CW, Shadrach JL, Hu J, Katsuta H, Hollister-Lock J, Qian WJ, Wagers AJ, Kulkarni RN (2013) Liver-derived systemic factors drive β cell hyperplasia in insulin-resistant states. Cell Rep 3:401–410
Ellingsgaard H, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT, Eppler E, Bouzakri K, Wueest S, Muller YD, Hansen AM, Reinecke M, Konrad D, Gassmann M, Reimann F, Halban PA, Gromada J, Drucker DJ, Gribble FM, Ehses JA, Donath MY (2011)

Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and α cells. Nat Med 17:1481–1489

Estall JL, Drucker DJ (2006) Glucagon and glucagon-like peptide receptors as drug targets. Curr Pharm Des 12:1731–1750

Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, de Vos WM, Cani PD (2013) Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci USA 110:9066–9071

Faghihimani E, Aminorroaya A, Rezvanian H, Adibi P, Ismail-Beigi F, Amini M (2013) Salsalate improves glycemic control in patients with newly diagnosed type 2 diabetes. Acta Diabetologica 50:537–543

Fliser D, Buchholz K, Haller H (2004) Antiinflammatory effects of angiotensin II subtype 1 receptor blockade in hypertensive patients with microinflammation. Circulation 110:1103–1107

Frederiksen B, Kroehl M, Lamb MM, Seifert J, Barriga K, Eisenbarth GS, Rewers M, Norris JM (2013) Infant exposures and development of type 1 diabetes mellitus: the Diabetes Autoimmunity Study in the Young (DAISY). JAMA Pediatr 167:808–815

Fujisaka S, Usui I, Kanatani Y, Ikutani M, Takasaki I, Tsuneyama K, Tabuchi Y, Bukhari A, Yamazaki Y, Suzuki H, Senda S, Aminuddin A, Nagai Y, Takatsu K, Kobayashi M, Tobe K (2011) Telmisartan improves insulin resistance and modulates adipose tissue macrophage polarization in high-fat-fed mice. Endocrinology 152:1789–1799

Fujitani Y, Ueno T, Watada H (2010) Autophagy in health and disease. 4. The role of pancreatic β-cell autophagy in health and diabetes. Am J physiol Cell Physiol 299:C1–C6

Garg AD, Kaczmarek A, Krysko O, Vandenabeele P, Krysko DV, Agostinis P (2012) ER stress-induced inflammation: does it aid or impede disease progression? Trends Mol Med 18:589–598

Ghosh K, Kanapathipillai M, Korin N, McCarthy JR, Ingber DE (2012) Polymeric nanomaterials for islet targeting and immunotherapeutic delivery. Nano Lett 12:203–208

Giacca A, Xiao C, Oprescu AI, Carpentier AC, Lewis GF (2011) Lipid-induced pancreatic β-cell dysfunction: focus on in vivo studies. Am J Physiol Endocrinol Metab 300:E255–E262

Giongo A, Gano KA, Crabb DB, Mukherjee N, Novelo LL, Casella G, Drew JC, Ilonen J, Knip M, Hyoty H, Veijola R, Simell T, Simell O, Neu J, Wasserfall CH, Schatz D, Atkinson MA, Triplett EW (2011) Toward defining the autoimmune microbiome for type 1 diabetes. ISME J 5:82–91

Goldfine AB, Fonseca V, Jablonski KA, Pyle L, Staten MA, Shoelson SE (2010) The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. Ann Intern Med 152:346–357

Goldfine AB, Fonseca V, Shoelson SE (2011) Therapeutic approaches to target inflammation in type 2 diabetes. Clin Chem 57:162–167

Goldfine AB, Conlin PR, Halperin F, Koska J, Permana P, Schwenke D, Shoelson SE, Reaven PD (2013a) A randomised trial of salsalate for insulin resistance and cardiovascular risk factors in persons with abnormal glucose tolerance. Diabetologia 56:714–723

Goldfine AB, Fonseca V, Jablonski KA, Chen YD, Tipton L, Staten MA, Shoelson SE (2013b) Salicylate (salsalate) in patients with type 2 diabetes: a randomized trial. Ann Intern Med 159:1–12

Green-Mitchell SM, Tersey SA, Cole BK, Ma K, Kuhn NS, Cunningham TD, Maybee NA, Chakrabarti SK, McDuffie M, Taylor-Fishwick DA, Mirmira RG, Nadler JL, Morris MA (2013) Deletion of 12/15-lipoxygenase alters macrophage and islet function in NOD-Alox15 (null) mice, leading to protection against type 1 diabetes development. PLoS One 8:e56763

Hansen CH, Krych L, Nielsen DS, Vogensen FK, Hansen LH, Sorensen SJ, Buschard K, Hansen AK (2012a) Early life treatment with vancomycin propagates Akkermansia muciniphila and reduces diabetes incidence in the NOD mouse. Diabetologia 55:2285–2294

Hansen JB, Tonnesen MF, Madsen AN, Hagedorn PH, Friberg J, Grunnet LG, Heller RS, Nielsen AO, Storling J, Baeyens L, Anker-Kitai L, Qvortrup K, Bouwens L, Efrat S, Aalund M, Andrews NC, Billestrup N, Karlsen AE, Holst B, Pociot F, Mandrup-Poulsen T (2012b)

Divalent metal transporter 1 regulates iron-mediated ROS and pancreatic β cell fate in response to cytokines. Cell Metab 16:449–461

Hara N, Alkanani AK, Ir D, Robertson CE, Wagner BD, Frank DN, Zipris D (2012) Prevention of virus-induced type 1 diabetes with antibiotic therapy. J Immunol 189:3805–3814

Hawley SA, Fullerton MD, Ross FA, Schertzer JD, Chevtzoff C, Walker KJ, Peggie MW, Zibrova D, Green KA, Mustard KJ, Kemp BE, Sakamoto K, Steinberg GR, Hardie DG (2012) The ancient drug salicylate directly activates AMP-activated protein kinase. Science 336:918–922

Hayashi Y, Utsuyama M, Kurashima C, Hirokawa K (1989) Spontaneous development of organ-specific autoimmune lesions in aged C57BL/6 mice. Clin Exp Immunol 78:120–126

Herder C, Haastert B, Muller-Scholze S, Koenig W, Thorand B, Holle R, Wichmann HE, Scherbaum WA, Martin S, Kolb H (2005) Association of systemic chemokine concentrations with impaired glucose tolerance and type 2 diabetes: results from the Cooperative Health Research in the Region of Augsburg Survey S4 (KORA S4). Diabetes 54(Suppl 2):S11–S17

Herder C, Hauner H, Kempf K, Kolb H, Skurk T (2007) Constitutive and regulated expression and secretion of interferon-gamma-inducible protein 10 (IP-10/CXCL10) in human adipocytes. Int J Obes (Lond) 31:403–410

Hines WJ, Smith MJ (1964) Inhibition of dehydrogenases by salicylate. Nature 201:192

Hogan AE, Tobin AM, Ahern T, Corrigan MA, Gaoatswe G, Jackson R, O'Reilly V, Lynch L, Doherty DG, Moynagh PN, Kirby B, O'Connell J, O'Shea D (2011) Glucagon-like peptide-1 (GLP-1) and the regulation of human invariant natural killer T cells: lessons from obesity, diabetes and psoriasis. Diabetologia 54:2745–2754

Hogan AE, Lynch L, Gaoatswe G, Woods C, Jackson R, O'Connell J, Moynagh PN, O'Shea D (2013) Glucagon-like peptide-1 (GLP-1) analogue therapy impacts inflammatory macrophages and cytokine increasing evidence for its anti-inflammatory actions. Endocr Rev 34:FP13–FP15

Holland WL, Miller RA, Wang ZV, Sun K, Barth BM, Bui HH, Davis KE, Bikman BT, Halberg N, Rutkowski JM, Wade MR, Tenorio VM, Kuo MS, Brozinick JT, Zhang BB, Birnbaum MJ, Summers SA, Scherer PE (2011) Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. Nat Med 17:55–63

Homo-Delarche F, Calderari S, Irminger JC, Gangnerau MN, Coulaud J, Rickenbach K, Dolz M, Halban P, Portha B, Serradas P (2006) Islet inflammation and fibrosis in a spontaneous model of type 2 diabetes, the GK rat. Diabetes 55:1625–1633

Honda K, Moto M, Uchida N, He F, Hashizume N (2012) Anti-diabetic effects of lactic acid bacteria in normal and type 2 diabetic mice. J Clin Biochem Nutr 51:96–101

Hotamisligil GS (2006) Inflammation and metabolic disorders. Nature 444:860–867

Hotamisligil GS (2010) Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. Cell 140:900–917

Huang CJ, Lin CY, Haataja L, Gurlo T, Butler AE, Rizza RA, Butler PC (2007) High expression rates of human islet amyloid polypeptide induce endoplasmic reticulum stress mediated β-cell apoptosis, a characteristic of humans with type 2 but not type 1 diabetes. Diabetes 56:2016–2027

Hummel S, Pfluger M, Hummel M, Bonifacio E, Ziegler AG (2011) Primary dietary intervention study to reduce the risk of islet autoimmunity in children at increased risk for type 1 diabetes: the BABYDIET study. Diabetes Care 34:1301–1305

Hundal RS, Petersen KF, Mayerson AB, Randhawa PS, Inzucchi S, Shoelson SE, Shulman GI (2002) Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. J Clin Invest 109:1321–1326

Igoillo-Esteve M, Marselli L, Cunha DA, Ladriere L, Ortis F, Grieco FA, Dotta F, Weir GC, Marchetti P, Eizirik DL, Cnop M (2010) Palmitate induces a pro-inflammatory response in human pancreatic islets that mimics CCL2 expression by β cells in type 2 diabetes. Diabetologia 53:1395–1405

Imai J, Katagiri H, Yamada T, Ishigaki Y, Suzuki T, Kudo H, Uno K, Hasegawa Y, Gao J, Kaneko K, Ishihara H, Niijima A, Nakazato M, Asano T, Minokoshi Y, Oka Y (2008) Regulation of pancreatic β cell mass by neuronal signals from the liver. Science 322:1250–1254

Imai Y, Dobrian AD, Morris MA, Nadler JL (2013a) Islet inflammation: a unifying target for diabetes treatment? Trends Endocrinol Metab: TEM 24:351–360

Imai Y, Dobrian AD, Weaver JR, Butcher MJ, Cole BK, Galkina EV, Morris MA, Taylor-Fishwick DA, Nadler JL (2013b) Interaction between cytokines and inflammatory cells in islet dysfunction, insulin resistance, and vascular disease. Diabetes Obes Metab 15(Suppl 3):117–129

Inoguchi T, Nawata H (2005) NAD(P)H oxidase activation: a potential target mechanism for diabetic vascular complications, progressive β-cell dysfunction and metabolic syndrome. Curr Drug Targets 6:495–501

Jaidane H, Hober D (2008) Role of coxsackievirus B4 in the pathogenesis of type 1 diabetes. Diabetes Metab 34:537–548

Janciauskiene S, Ahren B (2000) Fibrillar islet amyloid polypeptide differentially affects oxidative mechanisms and lipoprotein uptake in correlation with cytotoxicity in two insulin-producing cell lines. Biochem Biophys Res Commun 267:619–625

Jelsing J, Vrang N, van Witteloostuijn SB, Mark M, Klein T (2012) The DPP4 inhibitor linagliptin delays the onset of diabetes and preserves β-cell mass in non-obese diabetic mice. J Endocrinol 214:381–387

Jorns A, Gunther A, Hedrich HJ, Wedekind D, Tiedge M, Lenzen S (2005) Immune cell infiltration, cytokine expression, and β-cell apoptosis during the development of type 1 diabetes in the spontaneously diabetic LEW.1AR1/Ztm-iddm rat. Diabetes 54:2041–2052

Joseph J, Bittner S, Kaiser FM, Wiendl H, Kissler S (2012) IL-17 silencing does not protect nonobese diabetic mice from autoimmune diabetes. J Immunol 188:216–221

Jourdan T, Godlewski G, Cinar R, Bertola A, Szanda G, Liu J, Tam J, Han T, Mukhopadhyay B, Skarulis MC, Ju C, Aouadi M, Czech MP, Kunos G (2013) Activation of the Nlrp3 inflammasome in infiltrating macrophages by endocannabinoids mediates β cell loss in type 2 diabetes. Nat Med 19:1132–1140

Jurgens CA, Toukatly MN, Fligner CL, Udayasankar J, Subramanian SL, Zraika S, Aston-Mourney K, Carr DB, Westermark P, Westermark GT, Kahn SE, Hull RL (2011) β-cell loss and β-cell apoptosis in human type 2 diabetes are related to islet amyloid deposition. Am J Pathol 178:2632–2640

Kahn SE, Hull RL, Utzschneider KM (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444:840–846

Kahn SE, Zraika S, Utzschneider KM, Hull RL (2009) The β cell lesion in type 2 diabetes: there has to be a primary functional abnormality. Diabetologia 52:1003–1012

Kang YS, Song HK, Lee MH, Ko GJ, Cha DR (2010) Plasma concentration of visfatin is a new surrogate marker of systemic inflammation in type 2 diabetic patients. Diabetes Res Clin Pract 89:141–149

Karlsson FH, Tremaroli V, Nookaew I, Bergstrom G, Behre CJ, Fagerberg B, Nielsen J, Backhed F (2013) Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature 498:99–103

Kenyon V, Rai G, Jadhav A, Schultz L, Armstrong M, Jameson JB 2nd, Perry S, Joshi N, Bougie JM, Leister W, Taylor-Fishwick DA, Nadler JL, Holinstat M, Simeonov A, Maloney DJ, Holman TR (2011) Discovery of potent and selective inhibitors of human platelet-type 12-lipoxygenase. J Med Chem 54:5485–5497

Kleijwegt FS, Jansen DT, Teeler J, Joosten AM, Laban S, Nikolic T, Roep BO (2013) Tolerogenic dendritic cells impede priming of naive $CD8^+$ T cells and deplete memory $CD8^+$ T cells. Eur J Immunol 43:85–92

Knight RR, Kronenberg D, Zhao M, Huang GC, Eichmann M, Bulek A, Wooldridge L, Cole DK, Sewell AK, Peakman M, Skowera A (2013) Human β-cell killing by autoreactive preproinsulin-specific CD8 T cells is predominantly granule-mediated with the potency dependent upon T-cell receptor avidity. Diabetes 62:205–213

Kobayashi M, Kaneko-Koike C, Abiru N, Uchida T, Akazawa S, Nakamura K, Kuriya G, Satoh T, Ida H, Kawasaki E, Yamasaki H, Nagayama Y, Sasaki H, Kawakami A (2013) Genetic deletion of granzyme B does not confer resistance to the development of spontaneous diabetes in non-obese diabetic mice. Clin Exp Immunol 173:411–418

Koska J, Ortega E, Bunt JC, Gasser A, Impson J, Hanson RL, Forbes J, de Courten B, Krakoff J (2009) The effect of salsalate on insulin action and glucose tolerance in obese non-diabetic patients: results of a randomised double-blind placebo-controlled study. Diabetologia 52:385–393

Koulajian K, Desai T, Liu GC, Ivovic A, Patterson JN, Tang C, El-Benna J, Joseph JW, Scholey JW, Giacca A (2013) NADPH oxidase inhibition prevents β cell dysfunction induced by prolonged elevation of oleate in rodents. Diabetologia 56:1078–1087

Kriegel MA, Sefik E, Hill JA, Wu HJ, Benoist C, Mathis D (2011) Naturally transmitted segmented filamentous bacteria segregate with diabetes protection in nonobese diabetic mice. Proc Natl Acad Sci USA 108:11548–11553

Kriegel MA, Rathinam C, Flavell RA (2012) Pancreatic islet expression of chemokine CCL2 suppresses autoimmune diabetes via tolerogenic CD11c + CD11b + dendritic cells. Proc Natl Acad Sci USA 109:3457–3462

Lamb RE, Goldstein BJ (2008) Modulating an oxidative-inflammatory cascade: potential new treatment strategy for improving glucose metabolism, insulin resistance, and vascular function. Int J Clin Pract 62:1087–1095

Lamers D, Famulla S, Wronkowitz N, Hartwig S, Lehr S, Ouwens DM, Eckardt K, Kaufman JM, Ryden M, Muller S, Hanisch FG, Ruige J, Arner P, Sell H, Eckel J (2011) Dipeptidyl peptidase 4 is a novel adipokine potentially linking obesity to the metabolic syndrome. Diabetes 60:1917–1925

Larsen CM, Faulenbach M, Vaag A, Volund A, Ehses JA, Seifert B, Mandrup-Poulsen T, Donath MY (2007) Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N Engl J Med 356:1517–1526

Larsen CM, Faulenbach M, Vaag A, Ehses JA, Donath MY, Mandrup-Poulsen T (2009) Sustained effects of interleukin-1 receptor antagonist treatment in type 2 diabetes. Diabetes Care 32:1663–1668

Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sorensen SJ, Hansen LH, Jakobsen M (2010) Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS One 5:e9085

Lee AS, Gibson DL, Zhang Y, Sham HP, Vallance BA, Dutz JP (2010) Gut barrier disruption by an enteric bacterial pathogen accelerates insulitis in NOD mice. Diabetologia 53:741–748

Lee YS, Park MS, Choung JS, Kim SS, Oh HH, Choi CS, Ha SY, Kang Y, Kim Y, Jun HS (2012) Glucagon-like peptide-1 inhibits adipose tissue macrophage infiltration and inflammation in an obese mouse model of diabetes. Diabetologia 55:2456–2468

Lenzen S (2008) Oxidative stress: the vulnerable β-cell. Biochem Soc Trans 36:343–347

Lerner AG, Upton JP, Praveen PV, Ghosh R, Nakagawa Y, Igbaria A, Shen S, Nguyen V, Backes BJ, Heiman M, Heintz N, Greengard P, Hui S, Tang Q, Trusina A, Oakes SA, Papa FR (2012) IRE1α induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. Cell Metab 16:250–264

Levine B, Mizushima N, Virgin HW (2011) Autophagy in immunity and inflammation. Nature 469:323–335

Lightfoot YL, Chen J, Mathews CE (2012) Oxidative stress and β cell dysfunction. Methods Mol Biol 900:347–362

Lindley S, Dayan CM, Bishop A, Roep BO, Peakman M, Tree TI (2005) Defective suppressor function in $CD4^+CD25^+$ T-cells from patients with type 1 diabetes. Diabetes 54:92–99

Lohmann T, Kellner K, Verlohren HJ, Krug J, Steindorf J, Scherbaum WA, Seissler J (2001) Titre and combination of ICA and autoantibodies to glutamic acid decarboxylase discriminate two clinically distinct types of latent autoimmune diabetes in adults (LADA). Diabetologia 44:1005–1010

Long SA, Rieck M, Sanda S, Bollyky JB, Samuels PL, Goland R, Ahmann A, Rabinovitch A, Aggarwal S, Phippard D, Turka LA, Ehlers MR, Bianchine PJ, Boyle KD, Adah SA, Bluestone JA, Buckner JH, Greenbaum CJ (2012) Rapamycin/IL-2 combination therapy in patients with type 1 diabetes augments Tregs yet transiently impairs β-cell function. Diabetes 61:2340–2348

Lumeng CN, Saltiel AR (2011) Inflammatory links between obesity and metabolic disease. J Clin Invest 121:2111–2117

Ma K, Nunemaker CS, Wu R, Chakrabarti SK, Taylor-Fishwick DA, Nadler JL (2010) 12-Lipoxygenase Products Reduce Insulin Secretion and β-Cell Viability in Human Islets. J Clin Endocrinol Metab 95:887–893

Mahdi T, Hanzelmann S, Salehi A, Muhammed SJ, Reinbothe TM, Tang Y, Axelsson AS, Zhou Y, Jing X, Almgren P, Krus U, Taneera J, Blom AM, Lyssenko V, Esguerra JL, Hansson O, Eliasson L, Derry J, Zhang E, Wollheim CB, Groop L, Renstrom E, Rosengren AH (2012) Secreted frizzled-related protein 4 reduces insulin secretion and is overexpressed in type 2 diabetes. Cell Metab 16:625–633

Marhfour I, Lopez XM, Lefkaditis D, Salmon I, Allagnat F, Richardson SJ, Morgan NG, Eizirik DL (2012) Expression of endoplasmic reticulum stress markers in the islets of patients with type 1 diabetes. Diabetologia 55:2417–2420

Markle JG, Frank DN, Mortin-Toth S, Robertson CE, Feazel LM, Rolle-Kampczyk U, von Bergen M, McCoy KD, Macpherson AJ, Danska JS (2013a) Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science 339:1084–1088

Markle JG, Mortin-Toth S, Wong AS, Geng L, Hayday A, Danska JS (2013b) Gammadelta T cells are essential effectors of type 1 diabetes in the nonobese diabetic mouse model. J Immunol 190:5392–5401

Marroqui L, Gonzalez A, Neco P, Caballero-Garrido E, Vieira E, Ripoll C, Nadal A, Quesada I (2012) Role of leptin in the pancreatic β-cell: effects and signaling pathways. J Mol Endocrinol 49:R9–R17

Marselli L, Thorne J, Dahiya S, Sgroi DC, Sharma A, Bonner-Weir S, Marchetti P, Weir GC (2010) Gene expression profiles of β-cell enriched tissue obtained by laser capture microdissection from subjects with type 2 diabetes. PloS One 5:e11499

Martin AP, Rankin S, Pitchford S, Charo IF, Furtado GC, Lira SA (2008) Increased expression of CCL2 in insulin-producing cells of transgenic mice promotes mobilization of myeloid cells from the bone marrow, marked insulitis, and diabetes. Diabetes 57:3025–3033

Martinez J, Verbist K, Wang R, Green DR (2013) The relationship between metabolism and the autophagy machinery during the innate immune response. Cell Metab 17:895–900

Masters SL, Dunne A, Subramanian SL, Hull RL, Tannahill GM, Sharp FA, Becker C, Franchi L, Yoshihara E, Chen Z, Mullooly N, Mielke LA, Harris J, Coll RC, Mills KH, Mok KH, Newsholme P, Nunez G, Yodoi J, Kahn SE, Lavelle EC, O'Neill LA (2010) Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1β in type 2 diabetes. Nat Immunol 11:897–904

Mathis D (2013) Immunological goings-on in visceral adipose tissue. Cell Metab 17:851–859

Matveyenko AV, Liuwantara D, Gurlo T, Kirakossian D, Dalla Man C, Cobelli C, White MF, Copps KD, Volpi E, Fujita S, Butler PC (2012) Pulsatile portal vein insulin delivery enhances hepatic insulin action and signaling. Diabetes 61:2269–2279

McDuffie M, Maybee NA, Keller SR, Stevens BK, Garmey JC, Morris MA, Kropf E, Rival C, Ma K, Carter JD, Tersey SA, Nunemaker CS, Nadler JL (2008) Nonobese diabetic (NOD) mice congenic for a targeted deletion of 12/15-lipoxygenase are protected from autoimmune diabetes. Diabetes 57:199–208

Mehers KL, Long AE, van der Slik AR, Aitken RJ, Nathwani V, Wong FS, Bain S, Gill G, Roep BO, Bingley PJ, Gillespie KM (2011) An increased frequency of NK cell receptor and HLA-C group 1 combinations in early-onset type 1 diabetes. Diabetologia 54:3062–3070

Meier JJ, Veldhuis JD, Butler PC (2005) Pulsatile insulin secretion dictates systemic insulin delivery by regulating hepatic insulin extraction in humans. Diabetes 54:1649–1656

Meyers AJ, Shah RR, Gottlieb PA, Zipris D (2010) Altered Toll-like receptor signaling pathways in human type 1 diabetes. J Mol Med (Berl) 88:1221–1231

Michalska M, Wolf G, Walther R, Newsholme P (2010) The effects of pharmacologic inhibition of NADPH oxidase or iNOS on pro-inflammatory cytokine, palmitic acid or H2O2 -induced mouse islet or clonal pancreatic β cell dysfunction. Biosci Rep 30:445–453

Miller MR, Seifert J, Szabo NJ, Clare-Salzler M, Rewers M, Norris JM (2010) Erythrocyte membrane fatty acid content in infants consuming formulas supplemented with docosahexaenoic acid (DHA) and arachidonic acid (ARA): an observational study. Matern Child Nutr 6:338–346

Miller MR, Yin X, Seifert J, Clare-Salzler M, Eisenbarth GS, Rewers M, Norris JM (2011) Erythrocyte membrane omega-3 fatty acid levels and omega-3 fatty acid intake are not associated with conversion to type 1 diabetes in children with islet autoimmunity: the Diabetes Autoimmunity Study in the Young (DAISY). Pediatr Diabetes 12:669–675

Montane J, Klimek-Abercrombie A, Potter KJ, Westwell-Roper C, Verchere CB (2012) Metabolic stress, IAPP and islet amyloid. Diabetes Obes Metab 14:68–77

Moran A, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Greenbaum CJ, Herold KC, Marks JB, Raskin P, Sanda S, Schatz D, Wherrett DK, Wilson DM, Krischer JP, Skyler JS, Pickersgill L, de Koning E, Ziegler AG, Boehm B, Badenhoop K, Schloot N, Bak JF, Pozzilli P, Mauricio D, Donath MY, Castano L, Wagner A, Lervang HH, Perrild H, Mandrup-Poulsen T, Pociot F, Dinarello CA (2013) Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. Lancet 381:1905–1915

Morgan D, Oliveira-Emilio HR, Keane D, Hirata AE, Santos da Rocha M, Bordin S, Curi R, Newsholme P, Carpinelli AR (2007) Glucose, palmitate and pro-inflammatory cytokines modulate production and activity of a phagocyte-like NADPH oxidase in rat pancreatic islets and a clonal β cell line. Diabetologia 50:359–369

Morioka T, Asilmaz E, Hu J, Dishinger JF, Kurpad AJ, Elias CF, Li H, Elmquist JK, Kennedy RT, Kulkarni RN (2007) Disruption of leptin receptor expression in the pancreas directly affects β cell growth and function in mice. J Clin Invest 117:2860–2868

Murri M, Leiva I, Gomez-Zumaquero JM, Tinahones FJ, Cardona F, Soriguer F, Queipo-Ortuno MI (2013) Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. BMC Med 11:46

Myers MG Jr, Leibel RL, Seeley RJ, Schwartz MW (2010) Obesity and leptin resistance: distinguishing cause from effect. Trends Endocrinol Metab: TEM 21:643–651

Myhr CB, Hulme MA, Wasserfall CH, Hong PJ, Lakshmi PS, Schatz DA, Haller MJ, Brusko TM, Atkinson MA (2013) The autoimmune disease-associated SNP rs917997 of IL18RAP controls IFNgamma production by PBMC. J Autoimmun 44:8–12

Nakata M, Okada T, Ozawa K, Yada T (2007) Resistin induces insulin resistance in pancreatic islets to impair glucose-induced insulin release. Biochem Biophys Res Commun 353:1046–1051

Nakayama M, Inoguchi T, Sonta T, Maeda Y, Sasaki S, Sawada F, Tsubouchi H, Sonoda N, Kobayashi K, Sumimoto H, Nawata H (2005) Increased expression of NAD(P)H oxidase in islets of animal models of type 2 diabetes and its improvement by an AT1 receptor antagonist. Biochem Biophys Res Commun 332:927–933

Nguyen MT, Favelyukis S, Nguyen AK, Reichart D, Scott PA, Jenn A, Liu-Bryan R, Glass CK, Neels JG, Olefsky JM (2007) A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. J Biol Chem 282:35279–35292

Nicoloff G, Blazhev A, Petrova C, Christova P (2004) Circulating immune complexes among diabetic children. Clin Dev Immunol 11:61–66

Nie Y, Ma RC, Chan JC, Xu H, Xu G (2012) Glucose-dependent insulinotropic peptide impairs insulin signaling via inducing adipocyte inflammation in glucose-dependent insulinotropic peptide receptor-overexpressing adipocytes. Faseb J 26:2383–2393

Nikolajczyk BS, Jagannathan-Bogdan M, Denis GV (2012) The outliers become a stampede as immunometabolism reaches a tipping point. Immunol Rev 249:253–275

Nikolic T, Bouma G, Drexhage HA, Leenen PJ (2005) Diabetes-prone NOD mice show an expanded subpopulation of mature circulating monocytes, which preferentially develop into macrophage-like cells in vitro. J Leukoc Biol 78:70–79

Nixon M, Wake DJ, Livingstone DE, Stimson RH, Esteves CL, Seckl JR, Chapman KE, Andrew R, Walker BR (2012) Salicylate downregulates 11β-HSD1 expression in adipose tissue in obese mice and in humans, mediating insulin sensitization. Diabetes 61:790–796

Nogueira TC, Paula FM, Villate O, Colli ML, Moura RF, Cunha DA, Marselli L, Marchetti P, Cnop M, Julier C, Eizirik DL (2013) GLIS3, a susceptibility gene for type 1 and type 2 diabetes, modulates pancreatic β cell apoptosis via regulation of a splice variant of the BH3-only protein Bim. PLoS Genet 9:e1003532

Oliveira HR, Verlengia R, Carvalho CR, Britto LR, Curi R, Carpinelli AR (2003) Pancreatic β-cells express phagocyte-like NAD(P)H oxidase. Diabetes 52:1457–1463

Omar BA, Vikman J, Winzell MS, Voss U, Ekblad E, Foley JE, Ahren B (2013) Enhanced β cell function and anti-inflammatory effect after chronic treatment with the dipeptidyl peptidase-4 inhibitor vildagliptin in an advanced-aged diet-induced obesity mouse model. Diabetologia 56:1752–1760

Orban T, Sosenko JM, Cuthbertson D, Krischer JP, Skyler JS, Jackson R, Yu L, Palmer JP, Schatz D, Eisenbarth G (2009) Pancreatic islet autoantibodies as predictors of type 1 diabetes in the Diabetes Prevention Trial-Type 1. Diabetes Care 32:2269–2274

Ortis F, Miani M, Colli ML, Cunha DA, Gurzov EN, Allagnat F, Chariot A, Eizirik DL (2012) Differential usage of NF-kappaB activating signals by IL-1β and TNF-α in pancreatic β cells. FEBS Lett 586:984–989

Osborn O, Olefsky JM (2012) The cellular and signaling networks linking the immune system and metabolism in disease. Nat Med 18:363–374

Oslowski CM, Urano F (2010) The binary switch between life and death of endoplasmic reticulum-stressed β cells. Curr Opin Endocrinol Diabetes Obes 17:107–112

Oslowski CM, Hara T, O'Sullivan-Murphy B, Kanekura K, Lu S, Hara M, Ishigaki S, Zhu LJ, Hayashi E, Hui ST, Greiner D, Kaufman RJ, Bortell R, Urano F (2012) Thioredoxin-interacting protein mediates ER stress-induced β cell death through initiation of the inflammasome. Cell Metab 16:265–273

Ouchi N, Parker JL, Lugus JJ, Walsh K (2011) Adipokines in inflammation and metabolic disease. Nat Rev Immunol 11:85–97

Padgett LE, Broniowska KA, Hansen PA, Corbett JA, Tse HM (2013) The role of reactive oxygen species and proinflammatory cytokines in type 1 diabetes pathogenesis. Ann N Y Acad Sci 1281:16–35

Patel SB, Reams GP, Spear RM, Freeman RH, Villarreal D (2008) Leptin: linking obesity, the metabolic syndrome, and cardiovascular disease. Curr Hypertens Rep 10:131–137

Pavlatou MG, Mastorakos G, Margeli A, Kouskouni E, Tentolouris N, Katsilambros N, Chrousos GP, Papassotiriou I (2011) Angiotensin blockade in diabetic patients decreases insulin resistance-associated low-grade inflammation. Eur J Clin Invest 41:652–658

Petrie JR, Pearson ER, Sutherland C (2011) Implications of genome wide association studies for the understanding of type 2 diabetes pathophysiology. Biochem Pharmacol 81:471–477
Pi J, Collins S (2010) Reactive oxygen species and uncoupling protein 2 in pancreatic β-cell function. Diabetes Obes Metab 12(Suppl 2):141–148
Piya MK, McTernan PG, Kumar S (2013) Adipokine inflammation and insulin resistance: the role of glucose, lipids and endotoxin. J Endocrinol 216:T1–T15
Poitout V, Robertson RP (2008) Glucolipotoxicity: fuel excess and β-cell dysfunction. Endocr Rev 29:351–366
Prentki M, Nolan CJ (2006) Islet β cell failure in type 2 diabetes. J Clin Invest 116:1802–1812
Prieur X, Mok CY, Velagapudi VR, Nunez V, Fuentes L, Montaner D, Ishikawa K, Camacho A, Barbarroja N, O'Rahilly S, Sethi JK, Dopazo J, Oresic M, Ricote M, Vidal-Puig A (2011) Differential lipid partitioning between adipocytes and tissue macrophages modulates macrophage lipotoxicity and M2/M1 polarization in obese mice. Diabetes 60:797–809
Putnam AL, Brusko TM, Lee MR, Liu W, Szot GL, Ghosh T, Atkinson MA, Bluestone JA (2009) Expansion of human regulatory T-cells from patients with type 1 diabetes. Diabetes 58:652–662
Quagliarini F, Wang Y, Kozlitina J, Grishin NV, Hyde R, Boerwinkle E, Valenzuela DM, Murphy AJ, Cohen JC, Hobbs HH (2012) Atypical angiopoietin-like protein that regulates ANGPTL3. Proc Natl Acad Sci USA 109:19751–19756
Rabinovitch A, Sumoski W, Rajotte RV, Warnock GL (1990) Cytotoxic effects of cytokines on human pancreatic islet cells in monolayer culture. J Clin Endocrinol Metab 71:152–156
Ramos-Lopez E, Scholten F, Aminkeng F, Wild C, Kalhes H, Seidl C, Tonn T, Van der Auwera B, Badenhoop K (2009) Association of KIR2DL2 polymorphism rs2756923 with type 1 diabetes and preliminary evidence for lack of inhibition through HLA-C1 ligand binding. Tissue Antigens 73:599–603
Ramos-Zavala MG, Gonzalez-Ortiz M, Martinez-Abundis E, Robles-Cervantes JA, Gonzalez-Lopez R, Santiago-Hernandez NJ (2011) Effect of diacerein on insulin secretion and metabolic control in drug-naive patients with type 2 diabetes: a randomized clinical trial. Diabetes Care 34:1591–1594
Rao JR, Keating DJ, Chen C, Parkington HC (2012) Adiponectin increases insulin content and cell proliferation in MIN6 cells via PPARgamma-dependent and PPARgamma-independent mechanisms. Diabetes Obes Metab 14:983–989
Redondo MJ, Rewers M, Yu L, Garg S, Pilcher CC, Elliott RB, Eisenbarth GS (1999) Genetic determination of islet cell autoimmunity in monozygotic twin, dizygotic twin, and non-twin siblings of patients with type 1 diabetes: prospective twin study. BMJ 318:698–702
Revollo JR, Korner A, Mills KF, Satoh A, Wang T, Garten A, Dasgupta B, Sasaki Y, Wolberger C, Townsend RR, Milbrandt J, Kiess W, Imai S (2007) Nampt/PBEF/Visfatin regulates insulin secretion in β cells as a systemic NAD biosynthetic enzyme. Cell Metab 6:363–375
Richardson SJ, Willcox A, Bone AJ, Foulis AK, Morgan NG (2009) Islet-associated macrophages in type 2 diabetes. Diabetologia 52:1686–1688
Ridker PM, Thuren T, Zalewski A, Libby P (2011) Interleukin-1β inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). Am Heart J 162:597–605
Ridker PM, Howard CP, Walter V, Everett B, Libby P, Hensen J, Thuren T (2012) Effects of interleukin-1β inhibition with canakinumab on hemoglobin A1c, lipids, C-reactive protein, interleukin-6, and fibrinogen: a phase IIb randomized, placebo-controlled trial. Circulation 126:2739–2748
Rissanen A, Howard CP, Botha J, Thuren T (2012) Effect of anti-IL-1β antibody (canakinumab) on insulin secretion rates in impaired glucose tolerance or type 2 diabetes: results of a randomized, placebo-controlled trial. Diabetes Obes Metab 14:1088–1096

Rodacki M, Svoren B, Butty V, Besse W, Laffel L, Benoist C, Mathis D (2007) Altered natural killer cells in type 1 diabetic patients. Diabetes 56:177–185

Roggli E, Gattesco S, Pautz A, Regazzi R (2012) Involvement of the RNA-binding protein ARE/poly(U)-binding factor 1 (AUF1) in the cytotoxic effects of proinflammatory cytokines on pancreatic β cells. Diabetologia 55:1699–1708

Roivainen M, Knip M, Hyoty H, Kulmala P, Hiltunen M, Vahasalo P, Hovi T, Akerblom HK (1998) Several different enterovirus serotypes can be associated with prediabetic autoimmune episodes and onset of overt IDDM. Childhood Diabetes in Finland (DiMe) Study Group. J Med Virol 56:74–78

Rolandsson O, Palmer JP (2010) Latent autoimmune diabetes in adults (LADA) is dead: long live autoimmune diabetes! Diabetologia 53:1250–1253

Rolo AP, Palmeira CM (2006) Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. Toxicol Appl Pharmacol 212:167–178

Rosmalen JG, van Ewijk W, Leenen PJ (2002) T-cell education in autoimmune diabetes: teachers and students. Trends Immunol 23:40–46

Ryan EA, Lakey JR, Paty BW, Imes S, Korbutt GS, Kneteman NM, Bigam D, Rajotte RV, Shapiro AM (2002) Successful islet transplantation: continued insulin reserve provides long-term glycemic control. Diabetes 51:2148–2157

Saha SS, Ghosh M (2012) Antioxidant and anti-inflammatory effect of conjugated linolenic acid isomers against streptozotocin-induced diabetes. Br J Nutr 108:974–983

Sakai T, Taki T, Nakamoto A, Shuto E, Tsutsumi R, Toshimitsu T, Makino S, Ikegami S (2013) Lactobacillus plantarum OLL2712 regulates glucose metabolism in C57BL/6 mice fed a high-fat diet. J Nutr Sci Vitaminol 59:144–147

Saxena G, Chen J, Shalev A (2010) Intracellular shuttling and mitochondrial function of thioredoxin-interacting protein. J Biol Chem 285:3997–4005

Saxena A, Desbois S, Carrie N, Lawand M, Mars LT, Liblau RS (2012) Tc17 $CD8^+$ T cells potentiate Th1-mediated autoimmune diabetes in a mouse model. J Immunol 189:3140–3149

Scheen AJ (2004) Pathophysiology of insulin secretion. Ann Endocrinol (Paris) 65:29–36

Scheuner D, Kaufman RJ (2008) The unfolded protein response: a pathway that links insulin demand with β-cell failure and diabetes. Endocr Rev 29:317–333

Schulthess FT, Paroni F, Sauter NS, Shu L, Ribaux P, Haataja L, Strieter RM, Oberholzer J, King CC, Maedler K (2009) CXCL10 impairs β cell function and viability in diabetes through TLR4 signaling. Cell Metab 9:125–139

Scott GS, Fishman S, Khai Siew L, Margalit A, Chapman S, Chervonsky AV, Wen L, Gross G, Wong FS (2010) Immunotargeting of insulin reactive CD8 T cells to prevent diabetes. J Autoimmun 35:390–397

Secondulfo M, Iafusco D, Carratu R, deMagistris L, Sapone A, Generoso M, Mezzogiomo A, Sasso FC, Carteni M, De Rosa R, Prisco F, Esposito V (2004) Ultrastructural mucosal alterations and increased intestinal permeability in non-celiac, type I diabetic patients. Dig Liver Dis: Off J Ital Soc Gastroenterol Ital Assoc Study Liver 36:35–45

Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 343:230–238

Shoda LK, Young DL, Ramanujan S, Whiting CC, Atkinson MA, Bluestone JA, Eisenbarth GS, Mathis D, Rossini AA, Campbell SE, Kahn R, Kreuwel HT (2005) A comprehensive review of interventions in the NOD mouse and implications for translation. Immunity 23:115–126

Skurk T, Herder C, Kraft I, Muller-Scholze S, Hauner H, Kolb H (2005a) Production and release of macrophage migration inhibitory factor from human adipocytes. Endocrinology 146:1006–1011

Skurk T, Kolb H, Muller-Scholze S, Rohrig K, Hauner H, Herder C (2005b) The proatherogenic cytokine interleukin-18 is secreted by human adipocytes. Eur J Endocrinol 152:863–868

Skurk T, Alberti-Huber C, Herder C, Hauner H (2007) Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab 92:1023–1033

Skurk T, Mack I, Kempf K, Kolb H, Hauner H, Herder C (2009) Expression and secretion of RANTES (CCL5) in human adipocytes in response to immunological stimuli and hypoxia. Horm Metab Res 41:183–189

Skyler JS, Sosenko JM (2013) The evolution of type 1 diabetes. JAMA: J Am Med Assoc 309:2491–2492

Sloan-Lancaster J, Abu-Raddad E, Polzer J, Miller JW, Scherer JC, De Gaetano A, Berg JK, Landschulz WH (2013) Double-blind, randomized study evaluating the glycemic and anti-inflammatory effects of subcutaneous LY2189102, a neutralizing IL-1β antibody, in patients with type 2 diabetes. Diabetes Care 36(8):2239–2246

Spindel ON, World C, Berk BC (2012) Thioredoxin interacting protein: redox dependent and independent regulatory mechanisms. Antioxid Redox Signal 16:587–596

Staeva TP, Chatenoud L, Insel R, Atkinson MA (2013) Recent lessons learned from prevention and recent-onset type 1 diabetes immunotherapy trials. Diabetes 62:9–17

Steinberg GR (2007) Inflammation in obesity is the common link between defects in fatty acid metabolism and insulin resistance. Cell Cycle 6:888–894

Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA (2001) The hormone resistin links obesity to diabetes. Nature 409:307–312

Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR (2000) Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 321:405–412

Su SC, Pei D, Hsieh CH, Hsiao FC, Wu CZ, Hung YJ (2010) Circulating pro-inflammatory cytokines and adiponectin in young men with type 2 diabetes. Acta Diabetol 48:113–119

Subramanian SL, Hull RL, Zraika S, Aston-Mourney K, Udayasankar J, Kahn SE (2012) cJUN N-terminal kinase (JNK) activation mediates islet amyloid-induced β cell apoptosis in cultured human islet amyloid polypeptide transgenic mouse islets. Diabetologia 55:166–174

Supale S, Li N, Brun T, Maechler P (2012) Mitochondrial dysfunction in pancreatic β cells. Trends Endocrinol Metab: TEM 23:477–487

Tanaka S, Maeda S, Hashimoto M, Fujimori C, Ito Y, Teradaira S, Hirota K, Yoshitomi H, Katakai T, Shimizu A, Nomura T, Sakaguchi N, Sakaguchi S (2010) Graded attenuation of TCR signaling elicits distinct autoimmune diseases by altering thymic T cell selection and regulatory T cell function. J Immunol 185:2295–2305

Tang C, Naassan AE, Chamson-Reig A, Koulajian K, Goh TT, Yoon F, Oprescu AI, Ghanim H, Lewis GF, Dandona P, Donath MY, Ehses JA, Arany E, Giacca A (2013) Susceptibility to fatty acid-induced β-cell dysfunction is enhanced in prediabetic diabetes-prone biobreeding rats: a potential link between β-cell lipotoxicity and islet inflammation. Endocrinology 154: 89–101

Taylor-Fishwick D, Pittenger GL (2010) Harnessing the pancreatic stem cell. Endocrinol Metab Clin North Am 39:763

Taylor-Fishwick D, Pittenger GL, Vinik AI (2008) Transplantation and beyond. Drug Dev Res 69:165–176

Taylor-Fishwick DA, Weaver JR, Grzesik W, Chakrabarti S, Green-Mitchell S, Imai Y, Kuhn N, Nadler JL (2013) Production and function of IL-12 in islets and β cells. Diabetologia 56:126–135

Tersey SA, Carter JD, Rosenberg L, Taylor-Fishwick DA, Mirmira RG, Nadler JL (2012a) Amelioration of type 1 diabetes following treatment of non-obese diabetic mice with INGAP and lisofylline. J Diabetes Mellitus 2:251

Tersey SA, Nishiki Y, Templin AT, Cabrera SM, Stull ND, Colvin SC, Evans-Molina C, Rickus JL, Maier B, Mirmira RG (2012b) Islet β-cell endoplasmic reticulum stress precedes the onset of type 1 diabetes in the nonobese diabetic mouse model. Diabetes 61:818–827

Thomas HE, Irawaty W, Darwiche R, Brodnicki TC, Santamaria P, Allison J, Kay TW (2004) IL-1 receptor deficiency slows progression to diabetes in the NOD mouse. Diabetes 53:113–121

Thorsby E, Ronningen KS (1993) Particular HLA-DQ molecules play a dominant role in determining susceptibility or resistance to type 1 (insulin-dependent) diabetes mellitus. Diabetologia 36:371–377

Tilg H, Moschen AR (2008) Inflammatory mechanisms in the regulation of insulin resistance. Mol Med 14:222–231

Timper K, Grisouard J, Sauter NS, Herzog-Radimerski T, Dembinski K, Peterli R, Frey DM, Zulewski H, Keller U, Muller B, Christ-Crain M (2013) Glucose-dependent insulinotropic polypeptide induces cytokine expression, lipolysis, and insulin resistance in human adipocytes. Am J Physiol Endocrinol Metab 304:E1–E13

Todd JA (2010) Etiology of type 1 diabetes. Immunity 32:457–467

Tree TI, Lawson J, Edwards H, Skowera A, Arif S, Roep BO, Peakman M (2010) Naturally arising human CD4 T-cells that recognize islet autoantigens and secrete interleukin-10 regulate proinflammatory T-cell responses via linked suppression. Diabetes 59:1451–1460

Uchizono Y, Takeya R, Iwase M, Sasaki N, Oku M, Imoto H, Iida M, Sumimoto H (2006) Expression of isoforms of NADPH oxidase components in rat pancreatic islets. Life Sci 80:133–139

Vaarala O, Ilonen J, Ruohtula T, Pesola J, Virtanen SM, Harkonen T, Koski M, Kallioinen H, Tossavainen O, Poussa T, Jarvenpaa AL, Komulainen J, Lounamaa R, Akerblom HK, Knip M (2012) Removal of bovine insulin from cow's milk formula and early initiation of β-cell autoimmunity in the FINDIA Pilot Study. Arch Pediatr Adolesc Med 166:608–614

Valladares R, Sankar D, Li N, Williams E, Lai KK, Abdelgeliel AS, Gonzalez CF, Wasserfall CH, Larkin J, Schatz D, Atkinson MA, Triplett EW, Neu J, Lorca GL (2010) Lactobacillus johnsonii N6.2 mitigates the development of type 1 diabetes in BB-DP rats. PLoS One 5: e10507

van Asseldonk EJ, Stienstra R, Koenen TB, Joosten LA, Netea MG, Tack CJ (2011) Treatment with Anakinra improves disposition index but not insulin sensitivity in nondiabetic subjects with the metabolic syndrome: a randomized, double-blind, placebo-controlled study. J Clin Endocrinol Metab 96:2119–2126

van der Zijl NJ, Moors CC, Goossens GH, Blaak EE, Diamant M (2012) Does interference with the renin-angiotensin system protect against diabetes? Evidence and mechanisms. Diabetes Obes Metab 14:586–595

van Raalte DH, Diamant M (2011) Glucolipotoxicity and β cells in type 2 diabetes mellitus: target for durable therapy? Diabetes Res Clin Pract 93(Suppl 1):S37–S46

Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, Ravussin E, Stephens JM, Dixit VD (2011) The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 17:179–188

Varanasi V, Avanesyan L, Schumann DM, Chervonsky AV (2012) Cytotoxic mechanisms employed by mouse T cells to destroy pancreatic β-cells. Diabetes 61:2862–2870

Velloso LA, Eizirik DL, Cnop M (2013) Type 2 diabetes mellitus-an autoimmune disease? Nat Rev Endocrinol

Verbeeten KC, Elks CE, Daneman D, Ong KK (2011) Association between childhood obesity and subsequent Type 1 diabetes: a systematic review and meta-analysis. Diabet Med: J British Diabet Assoc 28:10–18

von Herrath M, Peakman M, Roep B (2013) Progress in immune-based therapies for type 1 diabetes. Clin Exp Immunol 172:186–202

Wallace C, Rotival M, Cooper JD, Rice CM, Yang JH, McNeill M, Smyth DJ, Niblett D, Cambien F, Tiret L, Todd JA, Clayton DG, Blankenberg S (2012) Statistical colocalization of monocyte gene expression and genetic risk variants for type 1 diabetes. Hum Mol Genet 21:2815–2824

Wang L, Liu Y, Yan Lu S, Nguyen KT, Schroer SA, Suzuki A, Mak TW, Gaisano H, Woo M (2010) Deletion of Pten in pancreatic β-cells protects against deficient β-cell mass and function in mouse models of type 2 diabetes. Diabetes 59:3117–3126

Weaver JR, Holman TR, Imai Y, Jadhav A, Kenyon V, Maloney DJ, Nadler JL, Rai G, Simeonov A, Taylor-Fishwick DA (2012) Integration of pro-inflammatory cytokines, 12-lipoxygenase and NOX-1 in pancreatic islet β cell dysfunction. Mol Cell Endocrinol 358:88–95

Wei FY, Suzuki T, Watanabe S, Kimura S, Kaitsuka T, Fujimura A, Matsui H, Atta M, Michiue H, Fontecave M, Yamagata K, Suzuki T, Tomizawa K (2011) Deficit of tRNA(Lys) modification by Cdkal1 causes the development of type 2 diabetes in mice. J Clin Invest 121:3598–3608

Weir GC, Bonner-Weir S (2013) Islet β cell mass in diabetes and how it relates to function, birth, and death. Ann N Y Acad Sci 1281:92–105

Wellen KE, Hotamisligil GS (2003) Obesity-induced inflammatory changes in adipose tissue. J Clin Invest 112:1785–1788

Wellen KE, Hotamisligil GS (2005) Inflammation, stress, and diabetes. J Clin Invest 115:1111–1119

Westermark P, Andersson A, Westermark GT (2011) Islet amyloid polypeptide, islet amyloid, and diabetes mellitus. Physiol Rev 91:795–826

Westwell-Roper C, Dai DL, Soukhatcheva G, Potter KJ, van Rooijen N, Ehses JA, Verchere CB (2011) IL-1 blockade attenuates islet amyloid polypeptide-induced proinflammatory cytokine release and pancreatic islet graft dysfunction. J Immunol 187:2755–2765

Wijesekara N, Krishnamurthy M, Bhattacharjee A, Suhail A, Sweeney G, Wheeler MB (2010) Adiponectin-induced ERK and Akt phosphorylation protects against pancreatic β cell apoptosis and increases insulin gene expression and secretion. J Biol Chem 285:33623–33631

Wilkin TJ (2009) The accelerator hypothesis: a review of the evidence for insulin resistance as the basis for type I as well as type II diabetes. Int J Obes (Lond) 33:716–726

Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG (2009) Analysis of islet inflammation in human type 1 diabetes. Clin Exp Immunol 155:173–181

Winkler C, Raab J, Grallert H, Ziegler AG (2012) Lack of association of type 2 diabetes susceptibility genotypes and body weight on the development of islet autoimmunity and type 1 diabetes. PLoS One 7:e35410

Xu G, Chen J, Jing G, Shalev A (2013) Thioredoxin-interacting protein regulates insulin transcription through microRNA-204. Nat Med 19:1141–1146

Xu X, Zheng S, Yang F, Shi Y, Gu Y, Chen H, Zhang M, Yang T (2014) Increased Th22 cells are independently associated with Th17 cells in type 1 diabetes. Endocrine 46:90–98

Yang ZD, Chen M, Wu R, McDuffie M, Nadler JL (2002) The anti-inflammatory compound lisofylline prevents Type I diabetes in non-obese diabetic mice. Diabetologia 45:1307–1314

Yang Z, Chen M, Fialkow LB, Ellett JD, Wu R, Nadler JL (2003) The novel anti-inflammatory compound, lisofylline, prevents diabetes in multiple low-dose streptozotocin-treated mice. Pancreas 26:e99–e104

Yang Z, Chen M, Ellett JD, Fialkow LB, Carter JD, McDuffie M, Nadler JL (2004) Autoimmune diabetes is blocked in Stat4-deficient mice. J Autoimmun 22:191–200

Yang Z, Chen M, Carter JD, Nunemaker CS, Garmey JC, Kimble SD, Nadler JL (2006) Combined treatment with lisofylline and exendin-4 reverses autoimmune diabetes. Biochem Biophys Res Commun 344:1017–1022

Yi P, Park JS, Melton DA (2013) Betatrophin: a hormone that controls pancreatic β cell proliferation. Cell 153:747–758

Yin N, Xu J, Ginhoux F, Randolph GJ, Merad M, Ding Y, Bromberg JS (2012) Functional specialization of islet dendritic cell subsets. J Immunol 188:4921–4930

Ylipaasto P, Smura T, Gopalacharyulu P, Paananen A, Seppanen-Laakso T, Kaijalainen S, Ahlfors H, Korsgren O, Lakey JR, Lahesmaa R, Piemonti L, Oresic M, Galama J, Roivainen M (2012) Enterovirus-induced gene expression profile is critical for human pancreatic islet destruction. Diabetologia 55:3273–3283

Youssef-Elabd EM, McGee KC, Tripathi G, Aldaghri N, Abdalla MS, Sharada HM, Ashour E, Amin AI, Ceriello A, O'Hare JP, Kumar S, McTernan PG, Harte AL (2012) Acute and chronic saturated fatty acid treatment as a key instigator of the TLR-mediated inflammatory response in human adipose tissue, in vitro. J Nutr Biochem 23:39–50

Zhang K, Kaufman RJ (2008) From endoplasmic-reticulum stress to the inflammatory response. Nature 454:455–462

Zhong J, Rao X, Rajagopalan S (2013) An emerging role of dipeptidyl peptidase 4 (DPP4) beyond glucose control: potential implications in cardiovascular disease. Atherosclerosis 226:305–314

Ziegler AG, Nepom GT (2010) Prediction and pathogenesis in type 1 diabetes. Immunity 32:468–478

Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS (2013) Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA: J Am Med Assoc 309:2473–2479

Zraika S, Hull RL, Udayasankar J, Aston-Mourney K, Subramanian SL, Kisilevsky R, Szarek WA, Kahn SE (2009) Oxidative stress is induced by islet amyloid formation and time-dependently mediates amyloid-induced β cell apoptosis. Diabetologia 52:626–635

Immunology of β-Cell Destruction

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Åke Lernmark and Daria LaTorre

Contents

Abstract

The pancreatic islet β cells are the target for an autoimmune process that eventually results in an inability to control blood glucose due to the lack of insulin. The different steps that eventually lead to the complete loss of the β cells are reviewed to include the very first step of a triggering event that initiates the development of β-cell autoimmunity to the last step of appearance of islet cell autoantibodies, which may mark that insulitis is about to form. The observations that the initial β-cell destruction by virus or other environmental factors triggers islet autoimmunity not in the islets but in the draining pancreatic lymph nodes are reviewed along with possible basic mechanisms of loss of tolerance to islet autoantigens. Once islet autoimmunity is established, the question is how β cells are progressively killed by autoreactive lymphocytes, which eventually results

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_18,

in chronic insulitis. These events have been examined in spontaneously diabetic mice or rats, but controlled clinical trials have shown that rodent observations cannot always be translated into mechanisms in humans. Attempts are therefore needed to clarify the step 1 triggering mechanisms and the step to chronic autoimmune insulitis to develop evidence-based treatment approaches to prevent type 1 diabetes.

Keywords

Antigen-presenting cells • Autoantigen • $CD4^+$ T-cells • $CD8^+$ T-cells • Dendritic cells • Insulitis • Islet autoantibodies • Islet autoimmunity • Prediction • Prevention • T regulatory cells

Abbreviations

APC	Antigen-presenting cells
BB	Biobreeding
BCR	β-cell receptor
CTLA-4	Cytolytic T-lymphocyte-associated antigen
cTreg	Conventional regulatory T
DC	Dendritic cells
Fas-L	Fas-Ligand
FOXP3	Forkhead–winged helix
GABA	Gamma-aminobutyric acid
GAD	Glutamic acid decarboxylase
HLA	Human leukocyte antigens
HSP	Heat-shock protein
IA-2	Insulinoma-associated antigen-2
IAA	Insulin autoantibodies
ICA	Islet cell antibodies
ICAM	Intercellular adhesion molecule
ICSA	Islet cell surface antibodies
IDO	Indoleamine 2 3-dioxygenase
IFN	Interferon
IL	Interleukin
iVEC	Islet vascular endothelial cells
LFA-1	Leukocyte function-associated antigen-1
NF	Nuclear factor
NK	Natural killer lymphocyte
NKT	Natural killer T-cell
NO	Nitric oxide
NOD	Nonobese diabetic
nTreg	Natural regulatory T
PBMC	Peripheral blood mononuclear cells
PD-1	Programmed death-1
pDC	Plasmacytoid dendritic cell
pLN	Pancreatic lymph node

pMHC	Peptide–MHC
PRR	Pattern recognition receptors
TCR	T-cell receptor
TEDDY study	The Environmental Determinants of Diabetes in the Young
TF	Transcription factor
TGF	Transforming growth factor
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	Regulatory T-cell
TSA	Tissue-specific antigen
VNTR	Variable nucleotide tandem repeat
ZnT8	Zinc transporter isoform-8

Background and Historical Perspectives

Immune-mediated selective destruction of the pancreatic islet β-cells is the hallmark of autoimmune (type 1) diabetes mellitus (T1D) formerly known as juvenile diabetes or insulin-dependent diabetes mellitus (Eisenbarth 1986; Atkinson et al. 2014; Bach 1997). The immunogenetic feature of the disease is a polygenic inheritance of susceptibility, which is reflected in a highly polyclonal autoimmune response targeting several β-cell autoantigens. The autoimmune response is associated with progressive β-cell destruction that eventually leads to overt clinical disease. As attested by prospective studies of children at genetic risk for T1D (DIPP, DAISY, and BabyDIAB), the appearance of specific islet autoantibodies marks the initiation of islet autoimmunity and may be detectable for months to years (Ziegler et al. 2013) during which time β-cell dysfunction proceeds asymptomatically (Vehik et al. 2011). T1D may therefore be viewed as a two-step disease. The first step is the initiation of islet autoimmunity. The second step is appearance of diabetes when islet autoimmunity has caused a major β-cell loss (>80 %) and insulin deficiency becomes clinically manifest (Bingley and Gale 2006).

At diagnosis the typical histological finding of affected islets, first described in short-duration diabetes patients at the beginning of the last century (Weichselbaum 1910) and termed “insulitis” (Von Meyenburg 1940; Gepts 1965), consists of an infiltrate of inflammatory cells associated with a loss of the β-cell endocrine subpopulation. The infiltrate consists of mononuclear cells (Gepts and De Mey 1978; Gepts and Lecompte 1981) and T and B lymphocytes (Itoh 1989; Bottazzo et al. 1985). Little is known about insulitis during the first step of the disease when subjects have preclinical islet autoimmunity. Recent studies suggest that the mere presence of a single islet autoantibody does not predict insulitis (In’t Veld et al. 2007; Campbell-Thompson et al. 2013).

The understanding of T1D etiology and pathogenesis is complicated by the lack of epidemiological data on the first step of the disease. In contrast the epidemiological knowledge of T1D is developing rapidly through registers in many different countries. The incidence is different among age groups, highest among children

(EURODIAB ACE Study Group 2000; Patterson et al. 2009; Onkamo et al. 1999; Karvonen et al. 2000), but the disease may occur at any age (Todd and Farrall 1996).

Annual incidence shows geographical variation among different countries and ethnic groups, from 0.1 per 100,000 children in parts of Asia and South America to the highest rate in Finland (64.2 per 100,000) (Onkamo et al. 1999; Karvonen et al. 2000). The mode of inheritance is complex as 80–85 % of T1D is occurring sporadically (Dahlquist et al. 1989), and the risk of becoming diabetic is approximately 7 % for a sibling and 6 % for the children of T1D parents (Dahlquist et al. 1989; Akesson et al. 2005).

An autoimmune etiology for T1D was suspected approximately 40 years ago from the association between diabetes and other autoimmune diseases (Ungar et al. 1968; Nerup and Binder 1973; Barker 2006). The first attempt to identify an autoimmune reaction toward the endocrine pancreas dates back to 1973 when testing for leukocyte migration inhibition to islet antigens suggested that T1D patients might be sensitized to pancreatic antigens (Nerup et al. 1973). Nearly concomitantly T1D was reported to be correlated to histocompatibility antigens (HLA), previously shown to be associated with other autoimmune diseases (Nerup et al. 1974; 1979). Association studies have proved that the greatest contribution to genetic susceptibility to T1D is exerted by HLA class II alleles on chromosome 6 where the HLA-DQ haplotypes DQ2 and DQ8 confer the highest risk and DQ6.2 the highest protection (Owerbach et al. 1983; Todd et al. 1987; Todd 2010). The detailed mechanisms by which different HLA molecules provide either risk or resistance to T1D are not fully understood (Todd 2010; Thorsby and Lie 2005). It is possible that different conformations of the MHC molecules pocket yield different peptide-binding properties and influence antigen presentation by antigen-presenting cells (APC) to effector T-cells (Todd 2010; Thorsby and Lie 2005; Delli et al. 2012).

The HLA genetic factors are necessary but not sufficient for islet autoimmunity and T1D. Environmental factors will therefore play a major role in the penetrance of a susceptible genotype. Virus infections have figured prominently in T1D epidemiological investigations (Yoon et al. 1989; Jun and Yoon 1994). Recent studies indicate that virus infections may have multiple effects on β-cell destruction and T1D development. The first is that gestational infections may sensitize the offspring to the eventual development of T1D (Dahlquist et al. 1999a, b; Lindehammer et al. 2011). The second is that virus may trigger islet cell autoimmunity (Lee et al. 2013; Parikka et al. 2012). The third is that the progression in islet autoantibody-positive subjects may be accelerated by virus infection (Stene et al. 2010). The possible contribution of a virus infection to trigger islet autoimmunity (step 1 of the disease) or to affect the progression to clinical onset therefore needs to be sorted out. The contribution of dietary factors is equally controversial (Blom et al. 1991, 1989; Lamb et al. 2008; Virtanen et al. 2012; Landin-Olsson et al. 2013). Maternal factors (Blom et al. 1991; Dahlquist et al. 1990), vaccinations (Blom et al. 1991; Helmke et al. 1986), or toxins (Myers et al. 2003) have also been considered.

Table 1 β-cell autoantigens

Antigen	Mol weight (Da)	Autoantibody abbreviation	References
Glutamic acid decarboxylase	65,000	GAD65A; GADA	Uibo and Lernmark 2008
Insulin	6,000	IAA	Palmer et al. 1983
IA-2	40,000	IA-2A	Lan et al. 1996
IA-2-β (Phogrin)	37,000	IA-2βA	Kawasaki et al. 1996
Zinc transporter ZnT8 R/W/Q variants	41,000	ZnT8A	Wenzlau et al. 2007

Although the event that initiates the autoimmune process (step 1) is not yet understood, the fact that it specifically targets the β cells promoted the attempts to find β-cell-specific autoantigens. The interest was initially focused on autoantibodies as useful tools in identification of autoantigenic molecules (Table 1) and to clarify the pathological immune response. The first description of pancreatic islet autoantibodies was published in 1974 when indirect immunofluorescence on frozen human pancreas sections revealed circulating islet cell antibodies (ICA) in the serum of T1D patients with polyendocrine disease (Bottazzo et al. 1974). However, no specific attempt was made at the time to identify the autoantigen(s). A few years later islet cell surface antibodies (ICSA) were demonstrated in newly diagnosed T1D patients using dispersed cell preparations of rodent pancreatic islets (Lernmark et al. 1978; Dobersen et al. 1980). The molecular characteristics of islet autoantigens remained unknown until the demonstration in 1982 that sera from new-onset T1D patients had autoantibodies immunoprecipitating a 64 kDa protein in isolated human islets (Baekkeskov et al. 1982). The 64 kDa immunoprecipitate proved in 1990 to have gamma-aminobutyric acid (GABA)-synthesizing enzymatic activity (Baekkeskov et al. 1990). Molecular cloning of human islet glutamic acid decarboxylase (GAD) showed that the β cells expressed the unique human isoenzyme, GAD65 (Karlsen et al. 1991). GAD65 is expressed in several cell types, but, apart from some brain neurons, it is mainly localized to synaptic-like microvesicles in the β cells (Karlsen et al. 1992; Christgau et al. 1991). GAD65 is in part responsible for the β-cell-specific pattern of ICA (Marshall et al. 1994).

In 1983 autoantibodies reacting with insulin (insulin autoantibodies, IAA) were demonstrated in T1D patients uncorrelated to insulin administration (Palmer et al. 1983). In 1994 trypsin digestion of the 64 kDa immunoprecipitate revealed a 37/40 kDa autoantigen pair recognized by sera of T1D patients (Christie 1993). This observation eventually led to the identification of the insulinoma-associated antigen-2 (IA-2) (Lan et al. 1994) and IA-2 β (or phogrin) (Wasmeier and Hutton 1996; Kawasaki et al. 1996). IA-2 is a transmembrane molecule of islet secretory granules and is implicated in insulin secretion (Kubosaki et al. 2005). In 2007 autoantibodies to the zinc transporter isoform-8 (ZnT8) were reported (Wenzlau et al. 2007). The ZnT8 protein mediates Zn^{2+} cation transport into the insulin granules facilitating the formation of insulin crystals (Wenzlau et al. 2008; Lemaire et al. 2009). ZnT8 polymorphic variants (Wenzlau et al. 2008) represent not only

targets of islet autoimmunity but also a genetic marker for type 2 diabetes (Chimienti et al. 2004, 2013).

Continued study of T1D sera has identified additional candidate targets of the humoral immune response. Autoantigens reported so far have different tissue expression patterns and subcellular localization and are referred to as minor autoantigens when the frequency of the respective autoantibodies is below 30 % in newly diagnosed T1D patients (Hirai et al. 2008). Potential minor autoantigens that either need confirmation or further studies include DNA topoisomerase II (Chang et al. 1996, 2004), heat-shock protein 60 (HSP60) (Ozawa et al. 1996), HSP-70 (Abulafia-Lapid et al. 2003), HSP-90 (Qin et al. 2003), vesicle-associated membrane protein-2 (VAMP2)73, neuropeptide Y (NPY)73, (Skarstrand et al. 2013a), carboxypeptidase H (CPH) (Castano et al. 1991), ICA69 (Kerokoski et al. 1999) (Pietropaolo et al. 1993), SOX13 (Torn et al. 2002), Glima38 (Aanstoot et al. 1996), and INS-IGF2 (Kanatsuna et al. 2013). Ganglioside such as the GM-2 ganglioside (Dotta et al. 1997) and sulfatides (Buschard et al. 1993) have also been considered. This wide array of islet autoantigens needs to be better defined. Autoantigens that trigger islet autoimmunity (step 1) needs to be separated from those representing autoantigen spreading during progression to clinical onset (step 2). It is critical that all autoantigen candidates are tested in samples from the past Diabetes Autoantibody Standardization Program (Torn et al. 2008; Yu et al. 2012) or the ongoing Islet Autoantibody Standardization Program (IASP). At this time, the relevance of minor autoantigens for the prediction of T1D is unclear. Despite their attested association with T1D, there is no evidence that islet autoantibodies directly contribute to β-cell damage. The role of B lymphocytes producing islet autoantibodies as APC is understudied (Pihoker et al. 2005; Lernmark and Larsson 2013).

Studies of the cellular arm in human T1D have detected $CD4^+$ and $CD8^+$ T-cells that recognize the same autoantigens as targeted by the humoral arm (Roep and Peakman 2012; Nepom and Buckner 2012). Cellular immunoreactivity to islet autoantigens is less easily assessed than the autoantibody response and is not yet applicable in the clinic. Most of the studies performed in the last decade to identify self-reactive T-cells in the peripheral blood of T1D patients are based on the indirect detection of T-cell presence through antigen-induced proliferation assays, tetramer stimulation, or cytokine release (ELISPOT) analysis (Nepom and Buckner 2012; Ziegler and Nepom 2010). The tetramer technique is highly specific for the HLA type and the peptide lodged in the MHC peptide-binding groove. As pointed out in a recent T-cell workshop, traditional in vitro proliferation assays suffer from methodological limitations (Mallone et al. 2011a; Nagata et al. 2004). As will be discussed later, these studies have produced inconsistent results and have globally failed to detect marked differences between T1D patients and controls.

The understanding of T1D has improved over the years since the rediscovery of insulitis in 1965, the realization that islet autoantibody -positive subjects may not have insulitis14 and that newly diagnosed T1D patients have β cells left and that these apparently dysfunctional cells tend to hyperexpress HLA class I heterodimers 15,

(Atkinson and Gianani 2009; Campbell-Thompson et al. 2012; Pociot et al. 2010). The recognition that T1D is a two-step disease characterized by a long prodrome of islet autoimmunity prior to clinical onset has allowed new hypotheses to be developed as to the initiation of the β-cell destructive process. The transition from islet autoimmunity to clinical T1D will also require a redefinition of the role of environmental factors triggering the disease. In this chapter we will review possible mechanisms of induction of β-cell autoimmunity and the role of environmental factors in this process.

Autoimmune β-Cell Destruction

Genetic Etiology

The major genetic factor for T1D is HLA-DQ on chromosome 620,27,32. Both sib-pair analyses and association studies in Caucasians have indicated that the HLA-DQ A1-B1 haplotypes A1*0301-B1*0302 (DQ8) and A1*0501-B1*0201 (DQ2), alone or in combination (DQ2/8), confer the highest risk for T1D. Nearly 90 % of newly diagnosed children carry DQ2/8 (about 30 %), DQ8, or DQ2 in combination with other haplotypes 33, (Sanjeevi et al. 1995). Among the many haplotypes, there are combinations, in particular with the DQA1*0201-B1*0602 (DQ6.2) haplotype, which is negatively associated (protective) with T1D. The effect is attenuated with increasing age (Graham et al. 2002). The rising incidence of T1D is puzzling as it is associated with a reduced overall contribution of high-risk HLA types in parallel with an increase in DQ8 and DQ2 combinations which did not confer risk 20 years ago (Resic-Lindehammer et al. 2008; Fourlanos et al. 2008). The mechanisms by which DQ8, DQ2, or both increase the risk for T1D are not fully clarified. The function of the DQ heterodimers to present antigenic peptides to the immune system is well understood. It remains to be determined why the DQ2/8 heterozygosity is associated with a young age at onset (Badenhoop et al. 2009). It has been speculated that the DQ2 and DQ8 molecules are important to maintain central or peripheral tolerance to the β-cell autoantigens GAD65, IA-2, insulin, or ZnT8. This possibility needs further exploration as it cannot be excluded that the primary association between T1D and HLA is the "step 1" part of the disease rather than the progression to clinical onset. This hypothesis is supported by the observation that the presence in healthy subjects of GAD65 autoantibodies is associated with DQ2 and IA-2 autoantibodies with DQ8 (Rolandsson et al. 1999).

Several investigations suggest that HLA contributes to about 60 % to the genetic risk of T1D (Todd et al. 1988; Concannon et al. 2005). Major efforts have therefore been made to identify non-HLA genetic risk factors for type 1 diabetes (Concannon et al. 2009). These studies have been highly rewarding as more than 40 genetic factors (see examples in Table 2) have been found to contribute (Pociot et al. 2010; Rich et al. 2009). Interestingly enough many of the genetic factors are important to the function of the immune system. PTPN22 is a regulator of T-cell function, and a

Table 2 Non-HLA genetic factors in type 1 diabetes

Gene (Syno.)	Name	Chromosome	Function	Association with other autoimmune diseases
PTPN22 (PEP, Lypl, Lyp2, LYP, PTPN8)	Protein tyrosine phosphatase, non-receptor type 22 (lymphoid)	1p13	Encodes tyrosine phosphatase and may be involved in regulating CBL function in the T-cell receptor signaling pathway	T1D and 22 other diseases
CTLA-4 (DDM12, CELIAC3)	Cytotoxic T-lymphocyte-associated protein 4	2q33	Possible involvement in regulating T-cell activation	T1DM and 99 other diseases
IFH1 (MDA5)	Interferon induced with helicase C domain 1	Chr.2q24	Proposed involvement in innate immune defense against viruses through interferon response	T1DM association
IL2 (lymphokine. TCGF)	Interleukin 2	Chr.4q27	Encodes a cytokine important for T- and β-cells proliferation. Stimulate β-cells, monocytes, and NK cells	T1DM and 39 other diseases
ITPR3 (IP3R3)	Inositol 1, 4, 5-triphosphate receptor 3	Chr.6p21.3	A second messenger that mediates the release of intracellular calcium	Strong T1DM association
BACH2 (BTB and CNC homology 1)	Basic leucine zipper transcription factor 2	Chr.6q15	Important roles in coordinating transcription activation and repression by MAFK (by similarity)	T1DM association
IL2RA (IDDM 10, CD25)	Interleukin-2 receptor α (chain)	Chr.10p15	Receptor for interleukin-2	Strong association with T1DM
INS VNTR (proinsulin, ILPR, MODY)	Insulin II; insulin 2; insulin	Chr.11p15	Regulating glucose metabolism through adjusting central tolerance to insulin	T1DM and 38 other diseases
TH (TYH, The)	Tyrosine hydroxylase	Chr.11p15	Encodes a protein that converts tyrosine to dopamine. Plays a key role in adrenergic neurons physiology	T1DM and 35 other diseases
ERBB3 (c-erbB3, HER3, LCCS2)	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3	Chr.12p13	Encodes a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases binds and is activated by neuregulins and NTAK	T1DM and multiple sclerosis
C12orf30 (C12orf51, KIAA0614)	Similar to KIAA0614 protein	Chr.12q24	Not yet determined	T1DM association

(*continued*)

Table 2 (continued)

Gene (Syno.)	Name	Chromosome	Function	Association with other autoimmune diseases
CLEC16A/ KIAA0350 (GoP-1)	C-type lectin domain family 16, member A	Chr.16p13	Unknown. Proposed to be related to immune modulation mechanisms	Strong association with T1DM
PTPN2	Protein tyrosine phosphatase, non-receptor type 2	Chr.18p11	Encode a PTP family protein and may be related to growth factor-mediated cell signaling	T1DM association
BASH3A (TULA, CLIP4)	Ubiquitin-associated and SH3 domain-containing protein A	Chr.21q22	Promotes accumulation of activated target receptors, such as T-cell receptors. EGFR and PDGFRB	T1DM association

genetic polymorphism results in a phosphatase variant that increases the risk not only for T1D but also for rheumatoid arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus, Graves' disease, generalized vitiligo, and other human autoimmune diseases (Gregersen 2005; Vang et al. 2007). The PTPN22 polymorphism seems in particular to affect progression from prediabetes to clinical disease (Hermann et al. 2006) also in individuals with lower-risk HLA genotypes (Maziarz et al. 2010).

The variable nucleotide tandem repeat in the promoter region of the insulin gene INS VNTR seems to contribute to T1D by the mechanisms of central tolerance (Pugliese et al. 1997). In newly diagnosed T1D patients, the presence of insulin autoantibodies was associated with the INS VNTR polymorphism 101. The many other genetic factors listed in Table 2 are all shown to be significantly associated with T1D 108. The function of these genes is understood individually, but it is not clear how these factors interact to increase the risk for the development of islet autoimmunity (step 1), T1D (step 2), or both. Since the majority of the genetic factors seem to be associated with the immune system, it is attractive to speculate that their contribution is related to the ability of the immune system to mount an autoimmune reaction specifically directed toward the islet β-cells.

Immune Cells in Tolerance

Epitope presentation to T- and β-cells is the key step in the generation of tolerance, in its early failure and during the maintenance of autoimmunity. The capacity to distinguish between self and nonself, which is the hallmark of a functional immune system, is lost when central and peripheral tolerization fail, leading to the development and expansion of autoreactive pathogenic effector cells. Central tolerance is induced at the site of lymphocyte development (the thymus and bone marrow,

respectively, for T- and β-cells), while peripheral tolerance occurs at sites of antigen recognition, namely, in lymphoid and nonlymphoid tissues. Central to the function of tolerance is APC.

APC

The recognition by T and B lymphocytes of antigens presented in the context of MHC surface of an APC is the first step of the adaptive immune response. Macrophages and particularly DC are the most efficient APC as they show constitutive expression of MHC class II molecules, cytokine secretion, and migrating capacity. APC have a dual role: uptake, processing, and presentation of antigens to T-cells and regulating T-cell-driven responses through cytokine release. APC are involved in T-cell tolerance mechanisms at both central (clonal deletion) and peripheral level (clonal anergy). Negative selection of autoreactive clonotypes derived by random T-cell receptor (TCR) rearrangement is guided by T-cell affinity for self-peptide–MHC (pMHC) complexes presented in the thymus (Geenen et al. 2010). An inadequate binding affinity spares self-reactive T-cells from apoptosis. The thymic expression of tissue-specific antigens (TSA) is regulated by the autoimmune regulatory (AIRE) transcription factor. Insufficient level of expression and presentation of TSA-derived peptides is observed in subjects with a mutated AIRE gene. It is possible that some β-cell autoantigens are not present in the thymus at sufficient concentrations to induce negative selection. This mechanism may explain the correlation of T1D protection with the "long form" of INS VNTR (Bennett and Todd 1996; Walter et al. 2003; Nielsen et al. 2006). The number of "tandem repeats" modulate thymic expression of this autoantigen, and the "long variant" results in increased insulin mRNA within the thymus. This higher thymic insulin expression is thought to enhance the deletion of insulin-specific thymocytes and may account for the protective phenotype.

Transcriptional modifications due to alternative splicing have been proposed to explain IA-2 immunogenicity as IA-2 is not expressed full length in thymus but in an alternatively spliced transcript derived from the deletion of exon 13 (Pugliese et al. 2001). This may account for the escape of a subset of IA-2-reactive T-cells. Interestingly, several B- and T-cell epitopes map to IA-2 exon 13 (Diez et al. 2001). So far it is not clear to what extent central tolerance and thymic expression are important to antigen presentation of the other two major autoantigens.

Among APC, DC are peculiar, highly specialized effectors with ontogenic, morphologic, and functional heterogeneity and can be mainly divided into conventional or myeloid DC (mDC) and plasmacytoid DC (pDC), depending on superficial clusters of differentiation and secretive function (Liu 2005). pDC are potent productors of IFN-α and are connected to the innate immune system through the expression of toll-like receptors (TLR) specific for the detection of viral infections. Emerging evidence suggests a close relationship between pDC and autoimmune conditions. In healthy subjects, autoantigen-bearing DC are physiologically found in blood, peripheral lymphoid organs, and thymus where they are an important source of TSA (Hernandez et al. 2001). DC were also reported to display proinsulin epitopes through direct transcriptional events in a capture-independent

way (Garcia et al. 2005). After the activation by an antigen, DC undergo maturation, express pMHC complexes, and promote antigen-specific T-cell clonal expansion. At this mature stage, DC are generally immunogenic and produce costimulatory molecules and several cytokines.

The physiology of β-cells as APC indicates that these cells are able to take up antigen at very low concentrations through their antigen-specific membrane-bound immunoglobulin and to present it to T-cells. The antigen presentation is enhanced in the presence of specific autoantibodies (Amigorena and Bonnerot 1998). β-cells may be important for the spreading of T- and β-cell determinants during the progression of the disease (Jaume et al. 2002; Steed et al. 2008). The minute amounts of antigen presented by β-cells may be important for the maintenance of autoimmune reactivity in the later phase once most of the target tissue has been destroyed (Uibo and Lernmark 2008; Steed et al. 2008). HLA-restricted B- and T-cell epitopes are in close proximity within the GAD65 molecule (Fenalti et al. 2008), and recently an overlap within T and B IA-2 epitopes has been described (Weenink et al. 2009). These observations suggest that antigen–antibody complexes may influence antigen presentation by APC and thereby T-cell reactivity. There are major gaps in our understanding of the possible importance of the T-β-cell synapse within the human islets of Langerhans.

There is wide evidence from studies on T1D pancreas with insulitis that MHC class II expression is increased on islet vascular endothelial cells (iVEC) (Hanninen et al. 1992; Greening et al. 2003). More recent data on iVEC suggest that these cells are capable to internalize, process, and present disease-relevant epitopes from GAD65 (Greening et al. 2003) and insulin (Savinov et al. 2003). The in vivo acquisition of these autoantigens by iVEC is not clearly established. Since iVEC are physiologically exposed to very high insulin concentration, it is likely that these cells take up insulin and process it into peptides through endosomal degradation rather than acquire peptides or pMHC complexes produced by β-cells (Savinov et al. 2003). The mechanism is even more unclear for non-secreted antigens. Although it is uncertain whether islet vascular endothelium has any prominent role in the priming of autoreactive T-cells, given the recognized importance of professional APC, it has been suggested that iVEC may be important for the trafficking of activated T-cells providing antigen-driven homing specificity (Savinov et al. 2003).

T-Cells

Recent progress in studying peripheral tolerance has highlighted the importance of immunoregulation by Treg, co-expressing CD4 and the α chain of the IL-2 receptor complex (CD 25) (Sakaguchi et al. 2008). Treg are potent suppressors of organ-specific autoimmunity. Natural Treg (nTreg) originate from intrathymic recognition of self-pMHC complexes [177, 178] and are functionally marked by the constitutive expression of forkhead–winged helix transcription factor (FOXP3) (Tang and Bluestone 2008), while conventional Treg (cTreg) differentiate from naïve $CD4^+$ T-cells in the periphery (Sakaguchi et al. 2008). Although FOXP3 plays a major role in Treg development and activity, as mutations in FOXP3 gene in

humans determine severe multiorgan autoimmunity (IPEX syndrome) (Gambineri et al. 2003), Treg function is complex and involves other transcriptional signaling as TGF-β, IL-2, and possibly others. The possible dysregulation of IL-2 signaling in Treg suppressor activity is supported by the association of T1D and polymorphisms within the IL-2 receptor α gene region in humans (Bach 2003). Immunoregulation by Treg affects T-cells, β-cells, and APC antigen-specific cellular responses in different manners. These include production of anti-inflammatory cytokines (TGF-β, IL-10, and IL-35) and contact-dependent mechanisms possibly involving CTLA-4 and direct cytolysis (Zhou et al. 2009). Further studies in humans are complicated by the difficulties to obtain T-cells from the pancreatic islets let alone the pancreatic draining lymph nodes.

B-Cells

Little is known about self-tolerance mechanisms for β-cells (Shlomchik 2008). Immature β-cells in the bone marrow express a potentially polyreactive β-cell receptor (BCR), which results from stochastic gene recombination. It is thought that 20–50 % of autoreactive immature β-cells undergo rearrangement of immunoglobulin light chain genes. The remaining self-reactive β-cells undergo peripheral deletion or peripheral anergy. The extent to which deletion and anergy contribute to β-cell tolerance has not yet been determined. Although evidence of aberrant receptor editing has been associated with autoimmunity in mouse and human diseases (Wardemann and Nussenzweig 2007), it is still unclear to what extent these defects participate in the establishment of autoimmunity.

What Happens in the Islet?

It is presently unclear whether the initiation of autoimmune β-cell destruction in humans requires a set of autoreactive T-cells recognizing β-cell antigen peptides presented on HLA class I heterodimers. The best current evidence in a case report (Bottazzo et al. 1985) or in the nPOD pancreata (Atkinson and Gianani 2009; Campbell-Thompson et al. 2012) is the presence of $CD8^+$ T-cells that are thought to recognize specific autoantigens. The variability in reactivity to individual autoantigens may in turn be due to "epitope spreading" which consists of intramolecular shifting of the recognized epitopes with the progression of the autoimmune attack. In addition, autoantigen spreading may occur as the risk for clinical diagnosis is increasing with an increased number of islet autoantibodies (Sosenko et al. 2013) preceded by activation of new T-cell clonotypes. These events may provide an explanation for the widely diversified anti-islet immune response in T1D.

Priming of naïve $CD4^+$ T-cells by islet antigen-presenting APC would be the first event in initiating islet autoimmunity (step 1) and diabetogenesis (step 2) (Fig. 1). This event most likely takes place in the pancreatic lymph nodes (pLN). Islet antigen presentation in pLN in humans is unclear. What promotes the earliest event, namely, uptake of antigen by APC in the islets, is still a matter of debate.

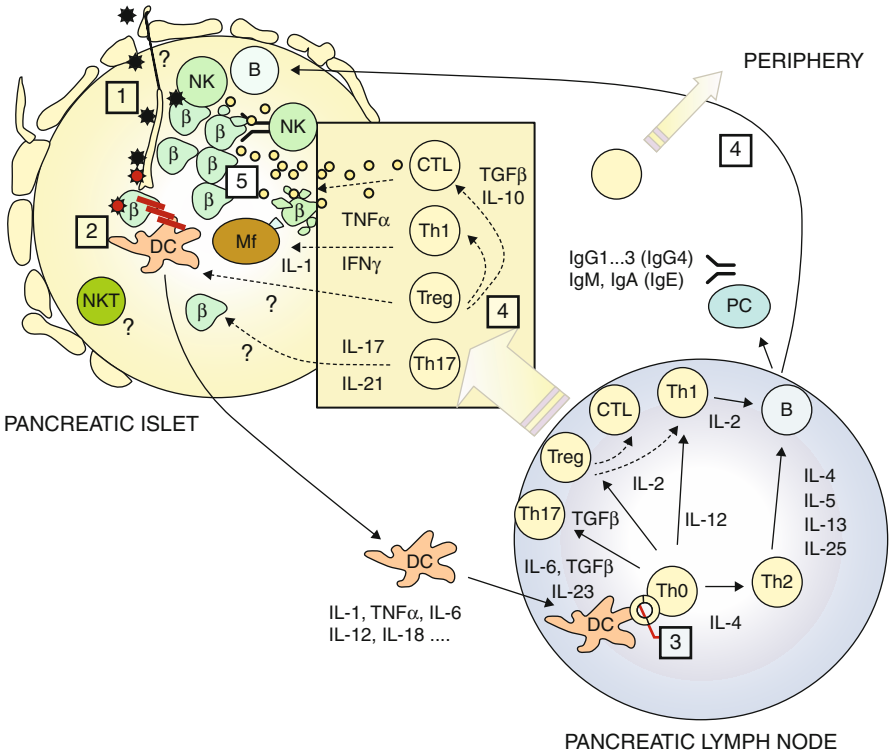

Fig. 1 Schematic view on possible immunopathogenesis of β-cell destruction. Steps of events: (Eisenbarth 1986) environmental factors are conditioning the relevant milieu by activation of dendritic cells (DC), macrophages (Mf), natural killer (NK) cells, and natural killer T-cells (NKT); (Atkinson et al. 2013) intake of antigens or cross-reactive peptides by dendritic cells (DC); (Bach 1997) presentation of peptides to naive T helper (Th0) cells and subsequent activation and proliferation of type 1 (Th1) and type 2 (Th2) helper cells, IL-17-producing helper cells (Th17), regulatory T (Treg)-cells, cytotoxic T-cells (CTL), β-cells and plasma cells (PC) and activation of different cell subsets by cytokines (Ziegler et al. 2013); migration of activated cells from pancreatic lymph node to the islets, cross talk with periphery; and β-cell destruction by cytokine- and perforin-/granzyme-mediated mechanisms. – environmental factor (virus, etc.); ✸ – islet autoantigens; — – islet autoantigens or cross-reactive peptides; ○ – islet-specific T-cell; Ab – autoantibodies

Initial, still not fully characterized insults (virus infection or other external damage, e.g., environmental toxins) may elicit an innate immune response through the generation of exogenous or endogenous ligands for the pattern recognition receptors (PRR) on the β-cell surface. The activation of these receptors triggers intracellular responses including cytokine production, endoplasmic reticulum stress, and accumulation of misfolded proteins. β-cell apoptosis and local inflammation may ensue. Dying β-cells may release immunostimulatory "danger" signals physiologically aimed at eliminating the initial harmful factor. This requires a transfer to adaptive immune response mediated by the enrollment of APC and the

establishment of a pro-inflammatory local environment (IFN, IL1β, and chemokines) to attract other immune cells. A defective resolution of the early inflammation results in a chronic destructive autoimmune reaction and may be dependent on the individual genetic background. For example, the DR3-DQ2 haplotype seems to be permissive of organ-specific autoimmunity (Lio et al. 1997).

DC have been demonstrated in human insulitis (Lernmark et al. 1995), but further studies of human pancreatic specimens from islet autoantibody-positive subjects or newly diagnosed T1D patients will be needed. DC may cross-present peptides derived from apoptotic cells directly onto MHC class I molecules without processing in the cytosol. Taken together, it is likely that antigens derived from β-cells dying upon external damage may be taken up by APC in the pancreatic islets and transported to pLN (Fig. 1). In humans this has not been possible to be fully demonstrated though expression of the β-cell autoantigens proinsulin, GAD65, and IA-2 has been detected in human peripheral DC. Currently our understanding of possible mechanisms of the very early events in islet autoimmunity relies on studies in animals.

Virus-Induced β-Cell Killing

Regardless of the numerous reports of TD1 onset following viral diseases, no conclusive pathogenic connection has been found between viral infection and human islet autoimmunity. The studies are complicated by the lack of data that distinguished triggering of islet autoimmunity (step 1) from virus infection affecting islets in subjects with islet autoantibodies or studied at the time of clinical diagnosis. Nevertheless, pancreatic islets showed the expression of innate PRR when infected by virus or exposed to virus-related cytokines as IFN and IL-1β (Hultcrantz et al. 2007). Virus antigen peptides need to be presented on MHC class I on the β-cell surface to be recognized by CTL. The critical question is to what extent a virus-infected β cell is copresenting viral and β-cell antigens on MHC class I molecules. Some viral antigen sequences are similar to self-peptides and may mislead T-cell responses. This phenomenon of "molecular mimicry" has been proposed between PC-2 antigen from Coxsackie B and GAD65 (Atkinson et al. 1994), between Rotavirus and IA-2 and for rubella (Honeyman et al. 1998). It is possible that these events are more relevant to the amplification of the autoimmune process and its maintenance after the resolution of the viral infection, than to the initial triggering of autoimmunity. As previously described, virus may activate β-cell intracellular signaling that induces altered expression of self-antigens on the β-cell surface ("neoantigens" or "cryptic antigens") and participates in the cascade leading to β-cell apoptosis and insulitis. Virus replication in the β cell may result in its necrosis and in release of previously sequestered cellular constituents ("hidden antigens") lacking induced thymic tolerance. The uptake and presentation of these self-antigens by APC to $CD4^+$ T-cells may eventually lead to the formation of specific autoantibodies. Coxsackie B4 has been isolated from the β cells of T1D new-onset patients (Dotta et al. 2007). In summary, there is wide evidence that virus infections may accelerate islet

autoimmunity (step 2) leading to clinical onset of T1D. The mechanism may be an increase in insulin resistance or a boost in β-cell killing induced by the virus infection. The major question to be answered is whether a virus infecting and replicating in human β-cells induces islet autoimmunity. The DIPP study (Oikarinen et al. 2012) and the ongoing TEDDY (the Environmental Determinants of Diabetes in the Young) study may be able to answer this question (TEDDY Study Group 2008; Hagopian et al. 2011).

Cytotoxin-Induced β-Cell Killing

Alloxan, streptozotocin, and the rodenticide (Vacor) are well-known β-cell cytotoxic agents. It is important to note that both alloxan and streptozotocin are more toxic to rodent than human β cells. Other chemicals that may be potential human β-cell cytotoxins are nitrosamine derivatives as well as dietary microbial toxins. Epidemiological data suggest that an increase in nitrate-treated food items increases the risk for children to develop T1D (Dahlquist et al. 1990). Other compounds structurally related to streptozotocin or alloxan have been implicated as possible environmental agents contributing to human T1D. Most prominently these compounds include the rodenticide pyriminil (Vacor) that induces islet cell surface antibodies and confirms that β-cell destruction in humans may cause islet autoimmunity (Karam et al. 1980; Esposti et al. 1996).

In summary, several virus and chemical agents directly affecting islet cells may be causative in the initiation of the autoimmune β-cell destructive process. Alternatively these factors may potentiate a process initiated by other environmental factors, which are currently under scrutiny in the TEDDY study (TEDDY Study Group 2008; Hagopian et al. 2011). In individuals prone to develop T1D, environmental chemicals may play a detrimental role by repeat injuries to the pancreatic β cells over several years of life. This in combination with a poor regenerative capacity of the β cell and islet autoimmunity may eventually induce diabetes.

Antigen Presentation in Pancreatic Lymph Nodes

Although specific mechanisms in humans remain unclear, APC loaded with β-cell antigens migrate from the islets to the pLN, where the processed antigens are presented to naïve $CD4^+$ T-cells (Th0) (Fig. 1). In the pLN primed $CD4^+$ T-cells proliferate and differentiate into several subsets, as type 1 $CD4^+$ T-cells (Th1), IL-17-producing $CD4^+$ T-cells (Th17), and Treg cells, and activate naïve $CD8^+$ T- and β-cells into CTL and plasma cells, respectively. The expansion of $CD4^+$ T-cells toward lineages of pro-inflammatory subtype (Th1 and Th17) is mainly promoted by the cytokine milieu, through IL-6, IL-12, and IL-23, whereas a balance toward IL-4, IL-5, IL-13, and IL-25 would decrease the inflammation. Th1 cells release IFN-γ, which activates macrophages, TNF-α, IL-12, and IL-18. The recent discovery of Th17 cells that are potent inducers of tissue inflammation and autoimmunity is of interest as they may have a role in islet destruction.

Activation and differentiation of naïve $CD8^+$ T-cells to antigen-specific CTL are dependent on "cross-priming." This is the cognate recognition of the same antigen by the $CD8^+$ and the $CD4^+$ T-cells on the same APC. The interaction between CD40 on APC and CD154 on $CD4^+$ T-cells induces upregulation of costimulatory molecules for the activation of the $CD8^+$ T-cells and increases the local production of pro-inflammatory cytokines such as TNF-α and IL-12. Alternatively the IFN-γ produced by $CD8^+$ T-cells could enhance $CD4^+$ T-cell action (Viglietta et al. 2002). When cognate interaction occurs between β-cells and activated $CD4^+$ T-cells, the β-cells differentiate into plasma cells. They start to secrete immunoglobulins with the same specificity as the previous membrane-bound immunoglobulin upon stimulation of T-cell-released "Th2" cytokines IL-4 and IL-5.

Homing of T-Cells to Islets

Primed β-cell-specific effector T-cells gain access to peripheral nonlymphoid tissues, migrate to the pancreas, and reach the β cells (Fig. 1). The molecular basis for this directed migration (homing) of autoreactive T-cells to the islets and for endothelial transmigration is not clarified. The processes guiding islet autoantigen-specific T-cells into islets are not known. In pancreas transplantations between monozygotic twins without immunosuppression islets in the donor pancreas were infiltrated by $CD8^+$ T-cells in association with the loss of β-cell function (Sibley et al. 1985). These experiments demonstrate the immunologic memory of the recipient, as well as β-cell killing by CTL, indicating that autoreactive CTL are reactivated. The mechanism of reactivation is unclear. It has been proposed that T-cells can be programmed to a specific tissue tropism through a distinct "homing receptor pattern" acquired at the site of priming (Dudda and Martin 2004). Upon second contact with cognate antigen in the islet, CTL are retained inside the islet tissue and may initiate insulitis (Fig. 1). Any β-cell-specific CTL may recognize antigens expressed on MHC class I molecules. MHC class I overexpression on islet cells, previously described in pancreas with insulitis (Atkinson and Gianani 2009; Foulis et al. 1991), is likely to be involved. Although routine investigation of the early phase (step 1) is not feasible in humans, immunocytochemistry on pancreas biopsy specimens from new-onset T1D patients in Japan indicates the presence of $CD8^+$ T-cells and activated macrophages secreting inflammatory cytokines (Imagawa et al. 2001). The ongoing inflammatory islet milieu expands the recruitment of autoreactive CTL through the expression of chemokines and homing ligands from the β-cells. As physiological response to the inflammation, islet endothelium upregulates the expression of surface adhesion molecules that increase vascular permeability and facilitate the recruitment of effector cells. Adhesion and diapedesis of T-cells are possible through the interactions of T-cell surface molecules (integrins) such as leukocyte function-associated antigen-1 (LFA-1) and very late activation antigen-4 (VLA-4) with their counter ligands on VEC. These include intercellular adhesion molecules (ICAM) and junctional adhesion molecules (JAM-1). This hyperexpression of adhesion molecules is documented in

new-onset diabetes pancreas (Uno et al. 2007). It is now proposed that after migration from pLN, activated T-cells require an additional upregulation of LFA-1 functional activity for the successful adhesion to VEC (Somoza et al. 1994). The hypothesis that iVEC may participate in T-cell-selective recruitment and adhesion in an antigen-specific fashion is intriguing. A recent study reported that GAD65 presentation by iVEC markedly promotes the in vitro transmigration of GAD65-autoreactive T-cells across iVEC monolayers in an LFA-1-dependent fashion (Somoza et al. 1994). In this process, $CD4^+$ T-cells may also intervene by secreting various lymphokines that attract and activate other cell types such as monocytes, eosinophils, and natural killer lymphocytes (NK). Whether islet-specific autoantibodies secreted by plasma cells take part in the islet destruction or are merely recruited upon the ongoing discharge of autoantigens is still a matter of debate since a defined pathogenetic effect has not been proven. Clinical evidence in humans does not support this hypothesis. It is still a matter of debate whether autoantibodies reacting to antigen-binding areas of autoantibodies (anti-idiotype) may be of relevance within the autoimmune process through the blockade of circulating self-autoantibodies (Oak et al. 2008; Ortqvist et al. 2010; Wang et al. 2012).

Insulitis and β-Cell Destruction

The progression from the initiating phase to an adaptive immune response is thought to take place very early during insulitis and determine the final outcome toward the generation of a prolonged devastating autoimmune reaction or the resolution of inflammation and preservation of islet integrity. Target-cell death further activates PRR that in turn promote the progression of insulitis perhaps through IFN-α-mediated upregulation of MHC class I molecules on pancreatic islet cells. IFN and other macrophage-derived cytokines prompt NK activation. These cells exert nonantigen-specific cytotoxicity through the release of perforin, after the activation of surface receptors, as NKG2D that recognizes viral products and other specific ligands. NKT cells on the other hand may be considered as innate-like lymphocytes as they may co-express NK cell surface markers including NK1.1 (human CD161) and TCR. Most NKT cells recognize glycolipid antigens presented on the MHC class I-like molecule CD1d (Kronenberg 2005). The possible role of NK in β-cell damage has not yet been clarified.

As the islet invasion progresses, chemokine-attracted macrophages contribute to the recruitment of other immune cells that also release multiple chemokines and pro-inflammatory cytokines. These inflammatory signals create an overall immunoactivatory environment that modifies DC phenotype and shifts $CD4^+$ T-cells toward "Th1-like" responses which promote the expansion of CTL and shelters them from peripheral tolerance. If this vicious circle is not interrupted, the maintenance and amplification of insulitis result in accumulation of immune cells and their cytotoxic mediators that may act synergistically to destroy the β cells (Fig. 1). In the later stages, the destructive process may be worsened in the course of β-cell failure as the

hyperglycemic environment may locally enhance insulin or GAD65 epitope presentation (Skowera et al. 2008).

Further studies of early insulitis (step 2) in humans will be needed to fully appreciate the initiating mechanisms of infiltration of immune cells. Effective prevention of T1D may require a better understanding of the events of chronic insulitis. We speculate that a chronic insulitis, which includes APC-presenting islet autoantigens within the islets as opposed to the pLN, represents a refractory state to immunosuppression. This may explain why immunosuppression at the time of clinical diagnosis of T1D is ineffective. It cannot be excluded that immunosuppression therapy may be successful provided that the treatment is started prior to chronic insulitis.

Is β-Cell Destruction Reflected in the Blood?

Assaying the cells involved in β-cell damage may give insights about the induction and maintenance of islet autoimmune destruction. Several possible immunological alterations have been investigated in the peripheral blood of T1D patients and at-risk subjects to differentiate them from healthy subjects.

APC

An abnormal cytokine response by DC from T1D patients upon antigenic (Mollah et al. 2008) or nonantigenic stimulation was proposed (Allen et al. 2009). More robustly, phenotypic characterization suggests that DC from recent-onset T1D patients exhibit an immature phenotype and may have a decreased T-cell stimulatory capacity compared to controls (Vidard et al. 1992). DC may therefore indirectly participate to T1D autoimmunity through a reduced efficacy in stimulating Treg. This immature phenotype of T1D human DC may result from abnormal activation of the NF-kB pathway (Vidard et al. 1992). This is consistent with the strong involvement of this transcription factor in the induction of self-tolerance (Osugi et al. 2002). Studies investigating the peripheral DC count reported a reduction in absolute number of blood DC in T1D children and more recently a modest but significant increase in the relative frequency of pDC subset, strictly time related with disease onset (Allen et al. 2009). However, the present observations about DC in human diabetes rely upon studies on in vitro monocyte-generated DC that may not reflect the true in vivo situation.

T-Cells

Many studies on peripheral blood mononuclear cells (PBMC) of T1D patients are aimed at detecting the presence of islet-specific $CD4^+$ and $CD8^+$ T-cells upon stimulation with synthetic peptides from islet antigens. The immunogenic epitopes are selected among putative immunodominant regions within the multiple islet autoantigens. Many of these studies report a higher frequency of islet-specific self-reactive T-cells in T1D patients than in control subjects when T-cells

are detected by either functional tests of antigen-induced proliferative (Endl et al. 1997), cytokine secretion (Dang et al. 2011; Faresjo et al. 2006; Herold et al. 2009), or tetramer staining (Nepom 2012; Reijonen et al. 2004).

$CD8^+$ and $CD4^+$ T-cells from T1D patients target a wide array of epitopes within GAD65 molecule (Endl et al. 1997; Wicker et al. 1996; Schloot et al. 1997; Harfouch-Hammoud et al. 1999; Peakman et al. 2001; Cernea and Herold 2010; Oling et al. 2012; Hjorth et al. 2011), insulin and proinsulin (Rudy et al. 1995; Geluk et al. 1998; Chen et al. 2001; Mannering et al. 2009; Mallone et al. 2011b; Abreu et al. 2012; Heninger et al. 2013; Tree et al. 2000; Kelemen et al. 2004), IA-2 (Hanninen et al. 2010; Velthuis et al. 2010; Peakman et al. 1999), IGRP (Velthuis et al. 2010; Alkemade et al. 2013; Unger et al. 2007), I-A2β (phogrin) (Tree et al. 2000; Kelemen et al. 2004; Achenbach et al. 2002), islet amyloid polypeptide (IAPP) (Velthuis et al. 2010), and glial fibrillary acidic protein (GFAP) (Standifer et al. 2006) as comprehensively summarized in a review updated in 2006 (Seyfert-Margolis et al. 2006; Roep and Peakman 2011). These investigations, mostly oriented toward epitope identification, provide evidence of multiple immunodominant β-cell regions targeted by CTL in human T1D but do not fully clarify the development of the T-cell-specific responses during the progression of the disease. In fact, no single epitope has proven to be discriminatory between health and disease. A hierarchy of T-cell responsiveness was proposed for proinsulin peptides (Arif et al. 2004). In some ways the choice of the epitope may also be misleading. Candidate sequences are usually selected on the basis of predicted TCR-pMHC-binding motifs (Rammensee et al. 1999) or affinity algorithms (Parker et al. 1994), whereas the strength of the TCR-pMHC complex interaction may inversely correlate with immunogenicity (Baker et al. 2008), in accordance with an insufficient negative thymic selection. This bias can be avoided through the analysis of multiepitope, multiantigen panels (Baker et al. 2008). Moreover, epitopes that have been proved of relevance in mice may guide the search efforts in humans, as recently done with IGRP peptides (Jarchum et al. 2008). At the present time, there is a lack of a precise, reproducible, and standardized method for detection and identification of β-cell specific autoreactive T-cells. Such a method is needed to reliably identify subjects with islet autoimmunity who may progress to clinical onset. Some authors report that the use of multiple epitopes achieves more diagnostic sensitivity and better discrimination of T1D from controls (van Endert et al. 2006). It is therefore still unclear to what extent all the data provided may be translated into evaluation of risk for islet autoimmunity or clinical onset. Moreover, autoreactive T-cells-specific responses for T1D self-antigens have been widely described in healthy individuals in stimulation assays with peptides from GAD65 (Danke et al. 2005; Monti et al. 2007) and insulin (Yang et al. 2008; Monti et al. 2009). Several differences have been proposed between self-reactive T $CD4^+$ T-cells from T1D and controls. Only GAD65-reactive T-cells from T1D subjects seem to be fully autoantigen-experienced in vivo and express the memory T-cell marker CD45RA (Danke et al. 2005) and are capable of activation in the absence of CD28/B7 costimulatorysignals (Viglietta et al. 2002). It was also

recently proposed that $CD4^+$ T-cells from T1D subjects may have a lower threshold of activation as compared to healthy controls (Yang et al. 2008).

In healthy individuals, self-reactive T-cells are probably present but quiescent for the immunosuppressive action of Treg. This is confirmed by the experimental observation that Treg in vitro depletion is followed by amplification of autoreactive T-cells only in samples from healthy individuals (Yang et al. 2008). The Treg pool in human T1D has also been extensively investigated, and a deficiency in Treg peripheral frequency has been reported in patients compared to controls (Tree et al. 2006). Subsequent investigations have failed to uniformly replicate these findings and have suggested that T1D nTreg may rather display an impaired immune suppressor function (Brusko et al. 2007; Lindley et al. 2005). Globally it seems that a simple deficiency in the peripheral Treg repertoire is not confirmed, but a local impairment of Treg activity at the site of inflammation cannot be excluded.

The peripheral blood from T1D patients may display an imbalance toward inflammation. Autoantigen-driven cytokine secretion by $CD4^+$ T-cells from T1D patients may be polarized toward INF-γ, while HLA-matched healthy controls display $IL\text{-}10^+$ c Treg-like responses (Arif et al. 2004). This "regulatory phenotype" skewed toward IL-10 has also been reported in association with later onset of TID (Arif et al. 2004) and better glycemic control (Sanda et al. 2008). Increased levels of "Th1 cell"-derived chemokines CC13, CC14, and CXC110 (Sanda et al. 2008; Nicoletti et al. 2002) and of adhesion molecules ICAM and L-selectin (CD62L) (Lampeter et al. 1992) have been found in serum of T1D patients. The NK population in the peripheral blood of T1D patients may be decreased, but these findings have not been universally replicated (Rodacki et al. 2006). A larger study confirmed a functional impairment of NK cells in T1D patients, i.e., reduced surface expression of activating receptors and low levels of IFN-γ and perforin, and suggested that these alterations may be a consequence of T1D, since they are evident exclusively in long-standing disease (Rodacki et al. 2007). It has also been reported that activated NK cells in T1D patients display a reduced expression of NKG2D receptor (Rodacki et al. 2006). It is possible that a downregulation of NKG2D receptor mediates the increased risk for T1D associated with polymorphisms of MHC class I chain-related (MIC) proteins that are NKG2D natural ligands (Gambelunghe et al. 2000).

B-Cells and Autoantibodies

The assessment of disorders of humoral immunity in T1D relies on the monitoring of circulating islet-reactive autoantibodies (Table 2). Autoantibodies against at least one of the islet cell autoantigens GAD65, IA-2, insulin, and ZnT8 represent in more than 95 % of T1D patients and in only 1–2 % of the general population (Andersson et al. 2011). Radio-binding assay of these autoantibodies has replaced the ICA assay. GAD65 antibodies are found in 70–75 % of T1D patients (Delli et al. 2012) and show a diagnostic sensitivity of 70–80 % and a diagnostic specificity of 98–99 % (Vaziri-Sani et al. 2010). The major antigenic epitopes of

GAD65 are the middle-(Padoa et al. 2003) and C-terminal (Hampe et al. 2000; Richter et al. 1993) region and are in close proximity to T-cell disease-relevant determinants (Fenalti and Buckle 2010). Differential epitope specificities, as identified by monoclonal antibodies to GAD65 epitopes within the C-terminal region, align with distinct autoimmune disease phenotypes, and the binding of N-terminal epitope is associated with slowly progressive β-cell failure (Kobayashi et al. 2003). It was recently suggested that the presence of GAD65 autoantibodies in T1D patients may be the result of an "unmasking' due to the lack of GAD65-anti-idiotypic antibodies (Oak et al. 2008). These anti-idiotypic antibodies are reported to highly discriminate T1D from healthy subjects and may be of some relevance in the pathogenesis of islet autoimmunity.

IAA are found in approximately 50–70 % of T1D patients (Delli et al. 2012; Hagopian et al. 1995) and are the first islet autoantibody to appear (Kukko et al. 2005) suggesting an involvement of insulin as primary autoimmune triggering antigen also in humans (Eisenbarth and Jeffrey 2008). Epitopes targeted by IAA are placed within A and B chains and are shared between insulin and proinsulin (Brooks-Worrell et al. 1999).

IA-2 antibodies are detected in 60–70 % of patients with new-onset T1D and tend to appear closer to the clinical onset (Andersson et al. 2011; 2013). Epitopes for IA-2 autoantibodies are found exclusively within the cytoplasmic region of the molecule and predominantly within the tyrosine phosphatase-like domain (Hatfield et al. 1997; Zhang et al. 1997).

Autoantibodies to ZnT8 are detected in 60–80 % of newly diagnosed T1D (Andersson et al. 2011; Andersson et al. 2013). The polymorphism at position 325 is a major target for ZnT8 autoantibodies demonstrating considerable binding specificity (Skarstrand et al. 2013b).

Prediction of β-Cell Destruction

Standardized methods have made islet autoantibodies the most useful marker for T1D and for enrollment of subjects into clinical preventiontrials (Torn et al. 2008). The number of islet autoantibodies is the best predictor for the risk of clinical onset (Sosenko et al. 2011a; Sosenko et al. 2011b; Elding Larsson et al. 2013). The predictive power is enhanced by the combination of multiple markers. The stepwise appearance of islet autoantibodies may signal autoantigen spreading and a worsening of the pathogenic process that eventually may lead to a major loss of β cells. The ongoing and escalating islet autoimmunity may also signal that the islets may eventually be infiltrated by mononuclear cells. Most critical will be the infiltration of $CD8^+$ T-cells that are recognizing islet autoantigens presented on HLA class I proteins on the β-cell surface.

The prediction power for T1D reaches 100 % in case of multiple positivity (Fig. 2). Importantly, in case of a single autoantibody, the correlation between islet

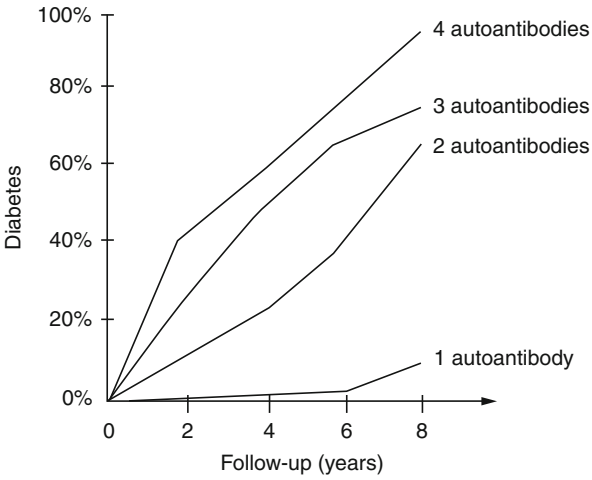

Fig. 2 Diagrammatic presentation of the effect of multiple islet autoantibodies on the risk of developing T1D in the Diabetes Prevention Trial – Type 1(DTP-1) (Courtesy of Jay Skyler)

autoimmunity and histological evidence of insulitis is weak (In't Veld et al. 2007). Longitudinal studies investigating DC and T-cells in at-risk subjects are lacking. Some reports have found poor in vitro maturation and pro-inflammatory cytokine response in DC from children at genetic risk for TID.

Concluding Remarks

In conclusion, the β cell in T1D is the major target for an autoimmune process that takes place in two steps. The first step is the development of an autoimmune reaction directed toward specific β-cell autoantigens. This step results in the appearance of circulating autoantibodies to β-cell autoantigens including GAD65, IA-2, ZnT8, and insulin. While the triggering phase may be short (hours, days), the ensuing islet autoimmunity leading up to step 2 – onset of clinical diabetes – may be months to years. The number of autoantibodies predicts T1D risk. The second step, progression from islet autoimmunity to the clinical onset of T1D, is associated with a major loss of β cells due to insulitis, but recent data indicate that the function of residual β cells may be inhibited. Insulitis appears late in the autoimmune process and can be recapitulated in pancreas and islets transplantation. The immunological memory of β-cell autoantigen is chronic. Efforts are needed both to detect intra-islet events that precede the development of autoantibodies and to disclose when islet autoantibody positivity is marking that the β-cell destructive process of insulitis is about to be established. A better understanding of step one and two events will be necessary for the ultimate prevention of β-cell destruction and of T1D.

Acknowledgement The research in the authors laboratory has been supported by the National Institutes of Health (grant DK63861), Juvenile Diabetes research foundation, the Swedish Research Council, Diabetesfonden, Childhood Diabetes Fund, and Skåne County Council for Research and Development.

Cross-References

- ▶ Apoptosis in Pancreatic β-Islet Cells in Type 1 and Type 2 Diabetes
- ▶ Current Approaches and Future Prospects for the Prevention of β-Cell Destruction in Autoimmune Diabetes
- ▶ Inflammatory Pathways Linked to β Cell Demise in Diabetes
- ▶ Microscopic Anatomy of the Human Islet of Langerhans

References

Aanstoot HJ, Kang SM, Kim J et al (1996) Identification and characterization of glima 38, a glycosylated islet cell membrane antigen, which together with GAD65 and IA2 marks the early phases of autoimmune response in type 1 diabetes. J Clin Invest 97(12):2772–2783

Abreu JR, Martina S, Verrijn Stuart AA et al (2012) CD8 T cell autoreactivity to preproinsulin epitopes with very low human leucocyte antigen class I binding affinity. Clin Exp Immunol 170(1):57–65

Abulafia-Lapid R, Gillis D, Yosef O, Atlan H, Cohen IR (2003) T cells and autoantibodies to human HSP70 in type 1 diabetes in children. J Autoimmun 20(4):313–321

Achenbach P, Kelemen K, Wegmann DR, Hutton JC (2002) Spontaneous peripheral T-cell responses to the IA-2β (phogrin) autoantigen in young nonobese diabetic mice. J Autoimmun 19(3):111–116

Akesson K, Nystrom L, Farnkvist L, Ostman J, Lernmark A, Kockum I (2005) Increased risk of diabetes among relatives of female insulin-treated patients diagnosed at 15–34 years of age. Diabet Med 22(11):1551–1557

Alkemade GM, Clemente-Casares X, Yu Z et al (2013) Local autoantigen expression as essential gatekeeper of memory T-cell recruitment to islet grafts in diabetic hosts. Diabetes 62(3):905–911

Allen JS, Pang K, Skowera A et al (2009) Plasmacytoid dendritic cells are proportionally expanded at diagnosis of type 1 diabetes and enhance islet autoantigen presentation to T-cells through immune complex capture. Diabetes 58(1):138–145

Amigorena S, Bonnerot C (1998) Role of β-cell and Fc receptors in the selection of T-cell epitopes. Curr Opin Immunol 10(1):88–92

Andersson C, Larsson K, Vaziri-Sani F et al (2011) The three ZNT8 autoantibody variants together improve the diagnostic sensitivity of childhood and adolescent type 1 diabetes. Autoimmunity 44(5):394–405

Andersson C, Vaziri-Sani F, Delli A et al (2013) Triple specificity of ZnT8 autoantibodies in relation to HLA and other islet autoantibodies in childhood and adolescent type 1 diabetes. Pediatr Diabetes 14(2):97–105

Arif S, Tree TI, Astill TP et al (2004) Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. J Clin Invest 113(3):451–463

Atkinson MA, Gianani R (2009) The pancreas in human type 1 diabetes: providing new answers to age-old questions. Curr Opin Endocrinol Diabetes Obes 16(4):279–285

Atkinson MA, Bowman MA, Campbell L, Darrow BL, Kaufman DL, Maclaren NK (1994) Cellular immunity to a determinant common to glutamate decarboxylase and Coxsackie virus in insulin-dependent diabetes. J Clin Invest 94(5):2125–2129

Atkinson MA, Eisenbarth GS, Michels AW (2014) Type 1 diabetes. Lancet 383:69-82

Bach JF (1997) Autoimmunity and type I diabetes. Trends Endocrinol Metab 8(2):71–74

Bach JF (2003) Regulatory T, cells under scrutiny. Nat Rev Immunol 3(3):189–198

Badenhoop K, Kahles H, Seidl C et al (2009) MHC-environment interactions leading to type 1 diabetes: feasibility of an analysis of HLA DR-DQ alleles in relation to manifestation periods and dates of birth. Diabetes Obes Metab 11(Suppl 1):88–91

Baekkeskov S, Nielsen JH, Marner B, Bilde T, Ludvigsson J, Lernmark A (1982) Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. Nature 298(5870):167–169

Baekkeskov S, Aanstoot HJ, Christgau S et al (1990) Identification of the 64 K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 347(6289):151–156

Baker C, de Marquesini LGP, Bishop AJ, Hedges AJ, Dayan CM, Wong FS (2008) Human CD8 responses to a complete epitope set from preproinsulin: implications for approaches to epitope discovery. J Clin Immunol 28(4):350–360

Barker JM (2006) Clinical review: type 1 diabetes-associated autoimmunity: natural history, genetic associations, and screening. J Clin Endocrinol Metab 91(4):1210–1217

Bennett ST, Todd JA (1996) Human type 1 diabetes and the insulin gene: principles of mapping polygenes. Annu Rev Genet 30:343–370

Bingley PJ, Gale EA (2006) Progression to type 1 diabetes in islet cell antibody-positive relatives in the European Nicotinamide Diabetes Intervention Trial: the role of additional immune, genetic and metabolic markers of risk. Diabetologia 49(5):881–890

Blom L, Lundmark K, Dahlquist G, Persson LA (1989) Estimating children's eating habits. Validity of a questionnaire measuring food frequency compared to a 7-day record. Acta Paediatr Scand 78(6):858–864

Blom L, Nystrom L, Dahlquist G (1991) The Swedish childhood diabetes study. Vaccinations and infections as risk determinants for diabetes in childhood. Diabetologia 34(3):176–181

Bottazzo GF, Florin-Christensen A, Doniach D (1974) Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 2(7892):1279–1283

Bottazzo GF, Dean BM, McNally JM, MacKay EH, Swift PG, Gamble DR (1985) In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulitis. N Engl J Med 313(6):353–360

Brooks-Worrell BM, Nielson D, Palmer JP (1999) Insulin autoantibodies and insulin antibodies have similar binding characteristics. Proc Assoc Am Physicians 111(1):92–96

Brusko T, Wasserfall C, McGrail K et al (2007) No alterations in the frequency of $FOXP3^+$ regulatory T-cells in type 1 diabetes. Diabetes 56(3):604–612

Buschard K, Josefsen K, Horn T, Fredman P (1993) Sulphatide and sulphatide antibodies in insulin-dependent diabetes mellitus. Lancet 342(8875):840

Campbell-Thompson M, Wasserfall C, Kaddis J et al (2012) Network for Pancreatic Organ Donors with Diabetes (nPOD): developing a tissue biobank for type 1 diabetes. Diabetes Metab Res Rev 28(7):608–617

Campbell-Thompson ML, Atkinson MA, Butler AE et al (2013) The diagnosis of insulitis in human type 1 diabetes. Diabetologia 56(11):2541–2543

Castano L, Russo E, Zhou L, Lipes MA, Eisenbarth GS (1991) Identification and cloning of a granule autoantigen (carboxypeptidase-H) associated with type I diabetes. J Clin Endocrinol Metab 73(6):1197–1201

Cernea S, Herold KC (2010) Monitoring of antigen-specific CD8 T cells in patients with type 1 diabetes treated with antiCD3 monoclonal antibodies. Clin Immunol 134(2):121–129

Chang YH, Hwang J, Shang HF, Tsai ST (1996) Characterization of human DNA topoisomerase II as an autoantigen recognized by patients with IDDM. Diabetes 45(4):408–414

Chang YH, Shiau MY, Tsai ST, Lan MS (2004) Autoantibodies against IA-2, GAD, and topoisomerase II in type 1 diabetic patients. Biochem Biophys Res Commun 320(3):802–809

Chen W, Bergerot I, Elliott JF et al (2001) Evidence that a peptide spanning the B-C junction of proinsulin is an early Autoantigen epitope in the pathogenesis of type 1 diabetes. J Immunol 167(9):4926–4935

Chimienti F (2013) Zinc, pancreatic islet cell function and diabetes: new insights into an old story. Nutr Res Rev 26(1):1–11

Chimienti F, Devergnas S, Favier A, Seve M (2004) Identification and cloning of a β-cell-specific zinc transporter, ZnT-8, localized into insulin secretory granules. Diabetes 53(9):2330–2337

Christgau S, Schierbeck H, Aanstoot HJ et al (1991) Pancreatic β cells express two autoantigenic forms of glutamic acid decarboxylase, a 65-kDa hydrophilic form and a 64-kDa amphiphilic form which can be both membrane-bound and soluble. J Biol Chem 266(34):23516

Christie MR (1993) Characterization of distinct islet protein autoantigens associated with type 1 diabetes. Autoimmunity 15(Suppl):1–3

Concannon P, Erlich HA, Julier C et al (2005) Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1,435 multiplex families. Diabetes 54(10):2995–3001

Concannon P, Rich SS, Nepom GT (2009) Genetics of type 1A diabetes. N Engl J Med 360(16):1646–1654

Dahlquist G, Blom L, Tuvemo T, Nystrom L, Sandstrom A, Wall S (1989) The Swedish childhood diabetes study – results from a nine year case register and a one year case-referent study indicating that type 1 (insulin-dependent) diabetes mellitus is associated with both type 2 (non-insulin-dependent) diabetes mellitus and autoimmune disorders. Diabetologia 32(1):2–6

Dahlquist GG, Blom LG, Persson LA, Sandstrom AI, Wall SG (1990) Dietary factors and the risk of developing insulin dependent diabetes in childhood. BMJ 300(6735):1302–1306

Dahlquist GG, Patterson C, Soltesz G (1999a) Perinatal risk factors for childhood type 1 diabetes in Europe. The EURODIAB Substudy 2 Study Group. Diabetes Care 22(10):1698–1702

Dahlquist GG, Boman JE, Juto P (1999b) Enteroviral RNA and IgM antibodies in early pregnancy and risk for childhood-onset IDDM in offspring. Diabetes Care 22(2):364–365

Dang M, Rockell J, Wagner R et al (2011) Human type 1 diabetes is associated with T cell autoimmunity to zinc transporter 8. J Immunol 186(10):6056–6063

Danke NA, Yang J, Greenbaum C, Kwok WW (2005) Comparative study of GAD65-specific $CD4^+$ T cells in healthy and type 1 diabetic subjects. J Autoimmun 25(4):303–311

Delli AJ, Vaziri-Sani F, Lindblad B et al (2012) Zinc transporter 8 autoantibodies and their association with SLC30A8 and HLA-DQ genes differ between immigrant and Swedish patients with newly diagnosed type 1 diabetes in the better diabetes diagnosis study. Diabetes 61(10):2556–2564

Diez J, Park Y, Zeller M et al (2001) Differential splicing of the IA-2 mRNA in pancreas and lymphoid organs as a permissive genetic mechanism for autoimmunity against the IA-2 type 1 diabetes autoantigen. Diabetes 50(4):895–900

Dobersen MJ, Scharff JE, Ginsberg-Fellner F, Notkins AL (1980) Cytotoxic autoantibodies to β cells in the serum of patients with insulin-dependent diabetes mellitus. N Engl J Med 303(26):1493–1498

Dotta F, Falorni A, Tiberti C et al (1997) Autoantibodies to the GM2-1 islet ganglioside and to GAD-65 at type 1 diabetes onset. J Autoimmun 10(6):585–588

Dotta F, Censini S, van Halteren AG et al (2007) Coxsackie B4 virus infection of β cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. Proc Natl Acad Sci USA 104(12):5115–5120

Dudda JC, Martin SF (2004) Tissue targeting of T cells by DCs and microenvironments. Trends Immunol 25(8):417–421

Eisenbarth GS (1986) Type I, diabetes mellitus. A chronic autoimmune disease. N Engl J Med 314(21):1360–1368

Eisenbarth GS, Jeffrey J (2008) The natural history of type 1A diabetes. Arq Bras Endocrinol Metabol 52(2):146–155

Elding Larsson H, Vehik K, Gesualdo P et al (2014) Children followed in the TEDDY study are diagnosed with type 1 diabetes at an early stage of disease. Pediatr Diabetes 15:118-26

Endl J, Otto H, Jung G et al (1997) Identification of naturally processed T cell epitopes from glutamic acid decarboxylase presented in the context of HLA-DR alleles by T lymphocytes of recent onset IDDM patients. J Clin Invest 99(10):2405–2415

Esposti MD, Ngo A, Myers MA (1996) Inhibition of mitochondrial complex I may account for IDDM induced by intoxication with the rodenticide Vacor. Diabetes 45(11):1531–1534

EURODIAB ACE Study Group (2000) Variation and trends in incidence of childhood diabetes in Europe. Lancet 355(9207):873–876

Faresjo MK, Vaarala O, Thuswaldner S, Ilonen J, Hinkkanen A, Ludvigsson J (2006) Diminished IFN-gamma response to diabetes-associated autoantigens in children at diagnosis and during follow up of type 1 diabetes. Diabetes Metab Res Rev 22(6):462–470

Fenalti G, Buckle AM (2010) Structural biology of the GAD autoantigen. Autoimmun Rev 9(3):148–152

Fenalti G, Hampe CS, Arafat Y et al (2008) COOH-terminal clustering of autoantibody and T-cell determinants on the structure of GAD65 provide insights into the molecular basis of autoreactivity. Diabetes 57(5):1293–1301

Foulis AK, McGill M, Farquharson MA (1991) Insulitis in type 1 (insulin-dependent) diabetes mellitus in man–macrophages, lymphocytes, and interferon-gamma containing cells. J Pathol 165(2):97–103

Fourlanos S, Varney MD, Tait BD et al (2008) The rising incidence of type 1 diabetes is accounted for by cases with lower-risk human leukocyte antigen genotypes. Diabetes Care 31(8): 1546–1549

Gambelunghe G, Ghaderi M, Cosentino A, Falorni A, Brunetti P, Sanjeevi CB (2000) Association of MHC Class I chain-related A (MIC-A) gene polymorphism with Type I diabetes. Diabetologia 43(4):507–514

Gambineri E, Torgerson TR, Ochs HD (2003) Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. Curr Opin Rheumatol 15(4):430–435

Garcia CA, Prabakar KR, Diez J et al (2005) Dendritic cells in human thymus and periphery display a proinsulin epitope in a transcription-dependent, capture-independent fashion. J Immunol 175(4):2111–2122

Geenen V, Mottet M, Dardenne O et al (2010) Thymic self-antigens for the design of a negative/tolerogenic self-vaccination against type 1 diabetes. Curr Opin Pharmacol 10(4):461–472

Geluk A, van Meijgaarden KE, Schloot NC, Drijfhout JW, Ottenhoff TH, Roep BO (1998) HLA-DR binding analysis of peptides from islet antigens in IDDM. Diabetes 47(10):1594–1601

Gepts W (1965) Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes 14(10):619–633

Gepts W, De Mey J (1978) Islet cell survival determined by morphology. An immunocytochemical study of the islets of Langerhans in juvenile diabetes mellitus. Diabetes 27(Suppl 1):251–261

Gepts W, Lecompte PM (1981) The pancreatic islets in diabetes. Am J Med 70(1):105–115

Graham J, Hagopian WA, Kockum I et al (2002) Genetic effects on age-dependent onset and islet cell autoantibody markers in type 1 diabetes. Diabetes 51(5):1346–1355

Greening JE, Tree TI, Kotowicz KT et al (2003) Processing and presentation of the islet autoantigen GAD by vascular endothelial cells promotes transmigration of autoreactive T-cells. Diabetes 52(3):717–725

Gregersen PK (2005) Gaining insight into PTPN22 and autoimmunity. Nat Genet 37(12): 1300–1302

Hagopian WA, Sanjeevi CB, Kockum I et al (1995) Glutamate decarboxylase-, insulin-, and islet cell-antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. J Clin Invest 95(4):1505–1511

Hagopian WA, Erlich H, Lernmark A et al (2011) The Environmental Determinants of Diabetes in the Young (TEDDY): genetic criteria and international diabetes risk screening of 421 000 infants. Pediatr Diabetes 12(8):733–743

Hampe CS, Hammerle LP, Bekris L et al (2000) Recognition of glutamic acid decarboxylase (GAD) by autoantibodies from different GAD antibody-positive phenotypes. J Clin Endocrinol Metab 85(12):4671–4679

Hanninen A, Jalkanen S, Salmi M, Toikkanen S, Nikolakaros G, Simell O (1992) Macrophages, T cell receptor usage, and endothelial cell activation in the pancreas at the onset of insulin-dependent diabetes mellitus. J Clin Invest 90(5):1901–1910

Hanninen A, Soilu-Hanninen M, Hampe CS et al (2010) Characterization of $CD4^+$ T cells specific for glutamic acid decarboxylase (GAD65) and proinsulin in a patient with stiff-person syndrome but without type 1 diabetes. Diabetes Metab Res Rev 26(4):271–279

Harfouch-Hammoud E, Walk T, Otto H et al (1999) Identification of peptides from autoantigens GAD65 and IA-2 that bind to HLA class II molecules predisposing to or protecting from type 1 diabetes. Diabetes 48(10):1937–1947

Hatfield EC, Hawkes CJ, Payton MA, Christie MR (1997) Cross reactivity between IA-2 and phogrin/IA-2β in binding of autoantibodies in IDDM. Diabetologia 40(11):1327–1333

Helmke K, Otten A, Willems WR et al (1986) Islet cell antibodies and the development of diabetes mellitus in relation to mumps infection and mumps vaccination. Diabetologia 29(1):30–33

Heninger AK, Monti P, Wilhelm C et al (2013) Activation of islet autoreactive naive T cells in infants is influenced by homeostatic mechanisms and antigen-presenting capacity. Diabetes 62(6):2059–2066

Hermann R, Lipponen K, Kiviniemi M et al (2006) Lymphoid tyrosine phosphatase (LYP/PTPN22) Arg620Trp variant regulates insulin autoimmunity and progression to type 1 diabetes. Diabetologia 49(6):1198–1208

Hernandez J, Aung S, Redmond WL, Sherman LA (2001) Phenotypic and functional analysis of $CD8^+$ T cells undergoing peripheral deletion in response to cross-presentation of self-antigen. J Exp Med 194(6):707–717

Herold KC, Brooks-Worrell B, Palmer J et al (2009) Validity and reproducibility of measurement of islet autoreactivity by T-cell assays in subjects with early type 1 diabetes. Diabetes 58(11):2588–2595

Hirai H, Miura J, Hu Y et al (2008) Selective screening of secretory vesicle-associated proteins for autoantigens in type 1 diabetes: VAMP2 and NPY are new minor autoantigens. Clin Immunol 127(3):366–374

Hjorth M, Axelsson S, Ryden A, Faresjo M, Ludvigsson J, Casas R (2011) GAD-alum treatment induces GAD65-specific $CD4^+$ $CD25highFOXP3^+$ cells in type 1 diabetic patients. Clin Immunol 138(1):117–126

Honeyman MC, Stone NL, Harrison LC (1998) T-cell epitopes in type 1 diabetes autoantigen tyrosine phosphatase IA-2: potential for mimicry with rotavirus and other environmental agents. Mol Med 4(4):231–239

Hultcrantz M, Huhn MH, Wolf M et al (2007) Interferons induce an antiviral state in human pancreatic islet cells. Virology 367(1):92–101

Imagawa A, Hanafusa T, Tamura S et al (2001) Pancreatic biopsy as a procedure for detecting in situ autoimmune phenomena in type 1 diabetes: close correlation between serological markers and histological evidence of cellular autoimmunity. Diabetes 50(6):1269–1273

In't Veld P, Lievens D, De Grijse J et al (2007) Screening for insulitis in adult autoantibody-positive organ donors. Diabetes 56(9):2400–2404

Itoh M (1989) Immunological aspects of diabetes mellitus: prospects for pharmacological modification. Pharmacol Ther 44(3):351–406

Jarchum I, Nichol L, Trucco M, Santamaria P, DiLorenzo TP (2008) Identification of novel IGRP epitopes targeted in type 1 diabetes patients. Clin Immunol 127(3):359–365

Jaume JC, Parry SL, Madec AM, Sonderstrup G, Baekkeskov S (2002) Suppressive effect of glutamic acid decarboxylase 65-specific autoimmune B lymphocytes on processing of T cell determinants located within the antibody epitope. J Immunol 169(2):665–672

Jun HS, Yoon JW (1994) Initiation of autoimmune type 1 diabetes and molecular cloning of a gene encoding for islet cell-specific 37kd autoantigen. Adv Exp Med Biol 347:207–220

Kanatsuna N, Taneera J, Vaziri-Sani F et al (2013) Autoimmunity against INS-IGF2 expressed in human pancreatic islets. J Biol Chem 288:29013–29023

Karam JH, Lewitt PA, Young CW et al (1980) Insulinopenic diabetes after rodenticide (Vacor) ingestion: a unique model of acquired diabetes in man. Diabetes 29(12):971–978

Karlsen AE, Hagopian WA, Grubin CE et al (1991) Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10. Proc Natl Acad Sci USA 88(19):8337–8341

Karlsen AE, Hagopian WA, Petersen JS et al (1992) Recombinant glutamic acid decarboxylase (representing the single isoform expressed in human islets) detects IDDM-associated 64,000-M(r) autoantibodies. Diabetes 41(10):1355–1359

Karvonen M, Viik-Kajander M, Moltchanova E, Libman I, LaPorte R, Tuomilehto J (2000) Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group. Diabetes Care 23(10):1516–1526

Kawasaki E, Eisenbarth GS, Wasmeier C, Hutton JC (1996) Autoantibodies to protein tyrosine phosphatase-like proteins in type I diabetes. Overlapping specificities to phogrin and ICA512/IA-2. Diabetes 45(10):1344–1349

Kelemen K, Gottlieb PA, Putnam AL, Davidson HW, Wegmann DR, Hutton JC (2004) HLA-DQ8-associated T cell responses to the diabetes autoantigen phogrin (IA-2 β) in human prediabetes. J Immunol 172(6):3955–3962

Kerokoski P, Ilonen J, Gaedigk R et al (1999) Production of the islet cell antigen ICA69 (p69) with baculovirus expression system: analysis with a solid-phase time-resolved fluorescence method of sera from patients with IDDM and rheumatoid arthritis. Autoimmunity 29(4):281–289

Kobayashi T, Tanaka S, Okubo M, Nakanishi K, Murase T, Lernmark A (2003) Unique epitopes of glutamic acid decarboxylase autoantibodies in slowly progressive type 1 diabetes. J Clin Endocrinol Metab 88(10):4768–4775

Kronenberg M (2005) Toward an understanding of NKT cell biology: progress and paradoxes. Annu Rev Immunol 23:877–900

Kubosaki A, Nakamura S, Notkins AL (2005) Dense core vesicle proteins IA-2 and IA-2β: metabolic alterations in double knockout mice. Diabetes 54(Suppl 2):S46–S51

Kukko M, Kimpimaki T, Korhonen S et al (2005) Dynamics of diabetes-associated autoantibodies in young children with human leukocyte antigen-conferred risk of type 1 diabetes recruited from the general population. J Clin Endocrinol Metab 90(5):2712–2717

Lamb MM, Yin X, Barriga K et al (2008) Dietary glycemic index, development of islet autoimmunity, and subsequent progression to type 1 diabetes in young children. J Clin Endocrinol Metab 93(10):3936–3942

Lampeter ER, Kishimoto TK, Rothlein R et al (1992) Elevated levels of circulating adhesion molecules in IDDM patients and in subjects at risk for IDDM. Diabetes 41(12):1668–1671

Lan MS, Lu J, Goto Y, Notkins AL (1994) Molecular cloning and identification of a receptor-type protein tyrosine phosphatase, IA-2, from human insulinoma. DNA Cell Biol 13(5):505–514

Lan MS, Wasserfall C, Maclaren NK, Notkins AL (1996) IA-2, a transmembrane protein of the protein tyrosine phosphatase family, is a major autoantigen in insulin-dependent diabetes mellitus. Proc Natl Acad Sci USA 93(13):6367–6370

Landin-Olsson M, Hillman M, Erlanson-Albertsson C (2013) Is type 1 diabetes a food-induced disease? Med Hypotheses 81(2):338–342

Lee HS, Briese T, Winkler C et al (2013) Next-generation sequencing for viruses in children with rapid-onset type 1 diabetes. Diabetologia 56(8):1705–1711

Lemaire K, Ravier MA, Schraenen A et al (2009) Insulin crystallization depends on zinc transporter ZnT8 expression, but is not required for normal glucose homeostasis in mice. Proc Natl Acad Sci USA 106(35):14872–14877
Lernmark A, Larsson HE (2013) Immune therapy in type 1 diabetes mellitus. Nat Rev Endocrinol 9(2):92–103
Lernmark A, Freedman ZR, Hofmann C et al (1978) Islet-cell-surface antibodies in juvenile diabetes mellitus. N Engl J Med 299(8):375–380
Lernmark A, Kloppel G, Stenger D et al (1995) Heterogeneity of islet pathology in two infants with recent onset diabetes mellitus. Virchows Arch 425(6):631–640
Lindehammer SR, Hansson I, Midberg B et al (2011) Seroconversion to islet autoantibodies between early pregnancy and delivery in non-diabetic mothers. J Reprod Immunol 88(1):72–79
Lindley S, Dayan CM, Bishop A, Roep BO, Peakman M, Tree TI (2005) Defective suppressor function in $CD4^+CD25^+$ T-cells from patients with type 1 diabetes. Diabetes 54(1):92–99
Lio D, Candore G, Romano GC et al (1997) Modification of cytokine patterns in subjects bearing the HLA-B8, DR3 phenotype: implications for autoimmunity. Cytokines Cell Mol Ther 3(4):217–224
Liu YJ (2005) IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol 23:275–306
Mallone R, Scotto M, Janicki CN et al (2011a) Immunology of Diabetes Society T-cell workshop: HLA class I tetramer-directed epitope validation initiative T-cell workshop report – HLA class I tetramer validation initiative. Diabetes Metab Res Rev 27(8):720–726
Mallone R, Brezar V, Boitard C (2011b) T cell recognition of autoantigens in human type 1 diabetes: clinical perspectives. Clin Dev Immunol 2011:513210
Mannering SI, Pang SH, Williamson NA et al (2009) The A-chain of insulin is a hot-spot for $CD4^+$ T cell epitopes in human type 1 diabetes. Clin Exp Immunol 156(2):226–231
Marshall MO, Hoyer PE, Petersen JS et al (1994) Contribution of glutamate decarboxylase antibodies to the reactivity of islet cell cytoplasmic antibodies. J Autoimmun 7(4):497–508
Maziarz M, Janer M, Roach JC et al (2010) The association between the PTPN22 1858C > T variant and type 1 diabetes depends on HLA risk and GAD65 autoantibodies. Genes Immun 11(5):406–415
Mollah ZU, Pai S, Moore C et al (2008) Abnormal NF-kappa B function characterizes human type 1 diabetes dendritic cells and monocytes. J Immunol 180(5):3166–3175
Monti P, Scirpoli M, Rigamonti A et al (2007) Evidence for in vivo primed and expanded autoreactive T cells as a specific feature of patients with type 1 diabetes. J Immunol 179(9):5785–5792
Monti P, Heninger AK, Bonifacio E (2009) Differentiation, expansion, and homeostasis of autoreactive T cells in type 1 diabetes mellitus. Curr Diab Rep 9(2):113–118
Myers MA, Hettiarachchi KD, Ludeman JP, Wilson AJ, Wilson CR, Zimmet PZ (2003) Dietary microbial toxins and type 1 diabetes. Ann N Y Acad Sci 1005:418–422
Nagata M, Kotani R, Moriyama H, Yokono K, Roep BO, Peakman M (2004) Detection of autoreactive T cells in type 1 diabetes using coded autoantigens and an immunoglobulin-free cytokine ELISPOT assay: report from the fourth immunology of diabetes society T cell workshop. Ann N Y Acad Sci 1037:10–15
Nepom GT (2012) MHC class II tetramers. J Immunol 188(6):2477–2482
Nepom GT, Buckner JH (2012) A functional framework for interpretation of genetic associations in T1D. Curr Opin Immunol 24(5):516–521
Nerup J, Binder C (1973) Thyroid, gastric and adrenal auto-immunity in diabetes mellitus. Acta Endocrinol (Copenh) 72(2):279–286
Nerup J, Andersen OO, Bendixen G et al (1973) Anti-pancreatic, cellular hypersensitivity in diabetes mellitus. Experimental induction of anti-pancreatic, cellular hypersensitivity and associated morphological β-cell changes in the rat. Acta Allergol 28(4):231–249
Nerup J, Platz P, Andersen OO et al (1974) HL-A antigens and diabetes mellitus. Lancet 2(7885):864–866

Nerup J, Christy M, Kromann H et al (1979) HLA and insulin-dependent diabetes mellitus. Postgrad Med J 55(Suppl 2):8–13

Nicoletti F, Conget I, Di Mauro M et al (2002) Serum concentrations of the interferon-gamma-inducible chemokine IP-10/CXCL10 are augmented in both newly diagnosed Type I diabetes mellitus patients and subjects at risk of developing the disease. Diabetologia 45(8):1107–1110

Nielsen LB, Mortensen HB, Chiarelli F et al (2006) Impact of IDDM2 on disease pathogenesis and progression in children with newly diagnosed type 1 diabetes: reduced insulin antibody titres and preserved β cell function. Diabetologia 49(1):71–74

Oak S, Gilliam LK, Landin-Olsson M et al (2008) The lack of anti-idiotypic antibodies, not the presence of the corresponding autoantibodies to glutamate decarboxylase, defines type 1 diabetes. Proc Natl Acad Sci USA 105(14):5471–5476

Oikarinen M, Tauriainen S, Oikarinen S et al (2012) Type 1 diabetes is associated with enterovirus infection in gut mucosa. Diabetes 61(3):687–691

Oling V, Reijonen H, Simell O, Knip M, Ilonen J (2012) Autoantigen-specific memory $CD4^+$ T cells are prevalent early in progression to Type 1 diabetes. Cell Immunol 273(2):133–139

Onkamo P, Vaananen S, Karvonen M, Tuomilehto J (1999) Worldwide increase in incidence of type 1 diabetes – the analysis of the data on published incidence trends. Diabetologia 42(12):1395–1403

Ortqvist E, Brooks-Worrell B, Lynch K et al (2010) Changes in GAD65Ab-specific antiidiotypic antibody levels correlate with changes in C-peptide levels and progression to islet cell autoimmunity. J Clin Endocrinol Metab 95(11):E310–E318

Osugi Y, Vuckovic S, Hart DN (2002) Myeloid blood $CD11c^+$ dendritic cells and monocyte-derived dendritic cells differ in their ability to stimulate T lymphocytes. Blood 100(8):2858–2866

Owerbach D, Lernmark A, Platz P et al (1983) HLA-D region β-chain DNA endonuclease fragments differ between HLA-DR identical healthy and insulin-dependent diabetic individuals. Nature 303(5920):815–817

Ozawa Y, Kasuga A, Nomaguchi H et al (1996) Detection of autoantibodies to the pancreatic islet heat shock protein 60 in insulin-dependent diabetes mellitus. J Autoimmun 9(4):517–524

Padoa CJ, Banga JP, Madec AM et al (2003) Recombinant Fabs of human monoclonal antibodies specific to the middle epitope of GAD65 inhibit type 1 diabetes-specific GAD65Abs. Diabetes 52(11):2689–2695

Palmer JP, Asplin CM, Clemons P et al (1983) Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science 222(4630):1337–1339

Parikka V, Nanto-Salonen K, Saarinen M et al (2012) Early seroconversion and rapidly increasing autoantibody concentrations predict prepubertal manifestation of type 1 diabetes in children at genetic risk. Diabetologia 55(7):1926–1936

Parker KC, Bednarek MA, Coligan JE (1994) Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. J Immunol 152(1):163–175

Patterson CC, Dahlquist GG, Gyurus E, Green A, Soltesz G (2009) Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. Lancet 373(9680):2027–2033

Peakman M, Stevens EJ, Lohmann T et al (1999) Naturally processed and presented epitopes of the islet cell autoantigen IA-2 eluted from HLA-DR4. J Clin Invest 104(10):1449–1457

Peakman M, Tree TI, Endl J, van Endert P, Atkinson MA, Roep BO (2001) Characterization of preparations of GAD65, proinsulin, and the islet tyrosine phosphatase IA-2 for use in detection of autoreactive T-cells in type 1 diabetes: report of phase II of the Second International Immunology of Diabetes Society Workshop for Standardization of T-cell assays in type 1 diabetes. Diabetes 50(8):1749–1754

Pietropaolo M, Castano L, Babu S et al (1993) Islet cell autoantigen 69 kD (ICA69). Molecular cloning and characterization of a novel diabetes-associated autoantigen. J Clin Invest 92(1):359–371

Pihoker C, Gilliam LK, Hampe CS, Lernmark A (2005) Autoantibodies in diabetes. Diabetes 54 (Suppl 2):S52–S61

Pociot F, Akolkar B, Concannon P et al (2010) Genetics of type 1 diabetes: what's next? Diabetes 59(7):1561–1571

Pugliese A, Zeller M, Fernandez A Jr et al (1997) The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. Nat Genet 15(3):293–297

Pugliese A, Brown D, Garza D et al (2001) Self-antigen-presenting cells expressing diabetes-associated autoantigens exist in both thymus and peripheral lymphoid organs. J Clin Invest 107(5):555–564

Qin HY, Mahon JL, Atkinson MA, Chaturvedi P, Lee-Chan E, Singh B (2003) Type 1 diabetes alters anti-hsp90 autoantibody isotype. J Autoimmun 20(3):237–245

Rammensee H, Bachmann J, Emmerich NP, Bachor OA, Stevanovic S (1999) SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics 50(3–4):213–219

Reijonen H, Mallone R, Heninger AK et al (2004) GAD65-specific $CD4^+$ T-cells with high antigen avidity are prevalent in peripheral blood of patients with type 1 diabetes. Diabetes 53(8):1987–1994

Resic-Lindehammer S, Larsson K, Ortqvist E et al (2008) Temporal trends of HLA genotype frequencies of type 1 diabetes patients in Sweden from 1986 to 2005 suggest altered risk. Acta Diabetol 45(4):231–235

Rich SS, Akolkar B, Concannon P et al (2009) Overview of the type I diabetes genetics consortium. Genes Immun 10(Suppl 1):S1–S4

Richter W, Shi Y, Baekkeskov S (1993) Autoreactive epitopes defined by diabetes-associated human monoclonal antibodies are localized in the middle and C-terminal domains of the smaller form of glutamate decarboxylase. Proc Natl Acad Sci USA 90(7):2832–2836

Rodacki M, Milech A, de Oliveira JE (2006) NK cells and type 1 diabetes. Clin Dev Immunol 13(2–4):101–107

Rodacki M, Svoren B, Butty V et al (2007) Altered natural killer cells in type 1 diabetic patients. Diabetes 56(1):177–185

Roep BO, Peakman M (2011) Diabetogenic T lymphocytes in human Type 1 diabetes. Curr Opin Immunol 23(6):746–753

Roep BO, Peakman M (2012) Antigen targets of type 1 diabetes autoimmunity. Cold Spring Harb Perspect Med 2(4):a007781

Rolandsson O, Hagg E, Hampe C et al (1999) Glutamate decarboxylase (GAD65) and tyrosine phosphatase-like protein (IA-2) autoantibodies index in a regional population is related to glucose intolerance and body mass index. Diabetologia 42(5):555–559

Rudy G, Stone N, Harrison LC et al (1995) Similar peptides from two β cell autoantigens, proinsulin and glutamic acid decarboxylase, stimulate T cells of individuals at risk for insulin-dependent diabetes. Mol Med 1(6):625–633

Sakaguchi S, Yamaguchi T, Nomura T, Ono M (2008) Regulatory T cells and immune tolerance. Cell 133(5):775–787

Sanda S, Roep BO, von Herrath M (2008) Islet antigen specific IL-10^+ immune responses but not $CD4^+$ $CD25^+$ $FoxP3^+$ cells at diagnosis predict glycemic control in type 1 diabetes. Clin Immunol 127(2):138–143

Sanjeevi CB, Lybrand TP, DeWeese C et al (1995) Polymorphic amino acid variations in HLA-DQ are associated with systematic physical property changes and occurrence of IDDM. Members of the Swedish Childhood Diabetes Study. Diabetes 44(1):125–131

Savinov AY, Wong FS, Stonebraker AC, Chervonsky AV (2003) Presentation of antigen by endothelial cells and chemoattraction are required for homing of insulin-specific $CD8^+$ T cells. J Exp Med 197(5):643–656

Schloot NC, Roep BO, Wegmann DR, Yu L, Wang TB, Eisenbarth GS (1997) T-cell reactivity to GAD65 peptide sequences shared with coxsackie virus protein in recent-onset IDDM, post-onset IDDM patients and control subjects. Diabetologia 40(3):332–338

Seyfert-Margolis V, Gisler TD, Asare AL et al (2006) Analysis of T-cell assays to measure autoimmune responses in subjects with type 1 diabetes: results of a blinded controlled study. Diabetes 55(9):2588–2594

Shlomchik MJ (2008) Sites and stages of autoreactive B cell activation and regulation. Immunity 28(1):18–28

Sibley RK, Sutherland DE, Goetz F, Michael AF (1985) Recurrent diabetes mellitus in the pancreas iso- and allograft. A light and electron microscopic and immunohistochemical analysis of four cases. Lab Invest 53(2):132–144

Skarstrand H, Dahlin LB, Lernmark A, Vaziri-Sani F (2013a) Neuropeptide Y autoantibodies in patients with long-term type 1 and type 2 diabetes and neuropathy. J Diabetes Complications 27:609–617

Skarstrand H, Lernmark A, Vaziri-Sani F (2013b) Antigenicity and epitope specificity of ZnT8 autoantibodies in type 1 diabetes. Scand J Immunol 77(1):21–29

Skowera A, Ellis RJ, Varela-Calvino R et al (2008) CTLs are targeted to kill β cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope. J Clin Invest 118(10):3390–3402

Somoza N, Vargas F, Roura-Mir C et al (1994) Pancreas in recent onset insulin-dependent diabetes mellitus. Changes in HLA, adhesion molecules and autoantigens, restricted T cell receptor V β usage, and cytokine profile. J Immunol 153(3):1360–1377

Sosenko JM, Skyler JS, Palmer JP et al (2011a) A longitudinal study of GAD65 and ICA512 autoantibodies during the progression to type 1 diabetes in Diabetes Prevention Trial-Type 1 (DPT-1) participants. Diabetes Care 34(11):2435–2437

Sosenko JM, Skyler JS, Mahon J et al (2011b) Validation of the Diabetes Prevention Trial-Type 1 Risk Score in the TrialNet Natural History Study. Diabetes Care 34(8):1785–1787

Sosenko JM, Skyler JS, Palmer JP et al (2013) The prediction of type 1 diabetes by multiple autoantibody levels and their incorporation into an autoantibody risk score in relatives of type 1 diabetic patients. Diabetes Care 36:2615–2620

Standifer NE, Ouyang Q, Panagiotopoulos C et al (2006) Identification of Novel HLA-A*0201-restricted epitopes in recent-onset type 1 diabetic subjects and antibody-positive relatives. Diabetes 55(11):3061–3067

Steed J, Gilliam LK, Harris RA, Lernmark A, Hampe CS (2008) Antigen presentation of detergent-free glutamate decarboxylase (GAD65) is affected by human serum albumin as carrier protein. J Immunol Methods 334(1–2):114–121

Stene LC, Oikarinen S, Hyoty H et al (2010) Enterovirus infection and progression from islet autoimmunity to type 1 diabetes: the Diabetes and Autoimmunity Study in the Young (DAISY). Diabetes 59(12):3174–3180

Tang Q, Bluestone JA (2008) The Foxp3^{+} regulatory T cell: a jack of all trades, master of regulation. Nat Immunol 9(3):239–244

TEDDY Study Group (2008) The Environmental Determinants of Diabetes in the Young (TEDDY) study. Ann N Y Acad Sci 1150:1–13

Thorsby E, Lie BA (2005) HLA associated genetic predisposition to autoimmune diseases: genes involved and possible mechanisms. Transpl Immunol 14(3–4):175–182

Todd JA (2010) Etiology of type 1 diabetes. Immunity 32(4):457–467

Todd JA, Farrall M (1996) Panning for gold: genome-wide scanning for linkage in type 1 diabetes. Hum Mol Genet 5:1443–1448

Todd JA, Bell JI, McDevitt HO (1987) HLA-DQ β gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature 329(6140):599–604

Todd JA, Bell JI, McDevitt HO (1988) A molecular basis for genetic susceptibility to insulin-dependent diabetes mellitus. Trends Genet 4(5):129–134

Torn C, Shtauvere-Brameus A, Sanjeevi CB, Landin-Olsson M (2002) Increased autoantibodies to SOX13 in Swedish patients with type 1 diabetes. Ann N Y Acad Sci 958:218–223
Torn C, Mueller PW, Schlosser M, Bonifacio E, Bingley PJ (2008) Diabetes antibody standardization program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. Diabetologia 51(5):846–852
Tree TI, O'Byrne D, Tremble JM et al (2000) Evidence for recognition of novel islet T cell antigens by granule-specific T cell lines from new onset type 1 diabetic patients. Clin Exp Immunol 121(1):100–105
Tree TI, Roep BO, Peakman M (2006) A mini meta-analysis of studies on $CD4^+$ $CD25^+$ T cells in human type 1 diabetes: report of the Immunology of Diabetes Society T Cell Workshop. Ann N Y Acad Sci 1079:9–18
Uibo R, Lernmark A (2008) GAD65 autoimmunity-clinical studies. Adv Immunol 100:39–78
Ungar B, Stocks AE, Martin FI, Whittingham S, Mackay IR (1968) Intrinsic-factor antibody, parietal-cell antibody, and latent pernicious anaemia in diabetes mellitus. Lancet 2(7565):415–417
Unger WW, Pinkse GG, Van der Kracht SM et al (2007) Human clonal CD8 autoreactivity to an IGRP islet epitope shared between mice and men. Ann N Y Acad Sci 1103:192–195
Uno S, Imagawa A, Okita K et al (2007) Macrophages and dendritic cells infiltrating islets with or without β cells produce tumour necrosis factor-α in patients with recent-onset type 1 diabetes. Diabetologia 50(3):596–601
van Endert P, Hassainya Y, Lindo V et al (2006) HLA class I epitope discovery in type 1 diabetes. Ann N Y Acad Sci 1079:190–197
Vang T, Miletic AV, Bottini N, Mustelin T (2007) Protein tyrosine phosphatase PTPN22 in human autoimmunity. Autoimmunity 40(6):453–461
Vaziri-Sani F, Oak S, Radtke J et al (2010) ZnT8 autoantibody titers in type 1 diabetes patients decline rapidly after clinical onset. Autoimmunity 43(8):598–606
Vehik K, Beam CA, Mahon JL et al (2011) Development of autoantibodies in the TrialNet Natural History Study. Diabetes Care 34(9):1897–1901
Velthuis JH, Unger WW, Abreu JR et al (2010) Simultaneous detection of circulating autoreactive $CD8^+$ T-cells specific for different islet cell-associated epitopes using combinatorial MHC multimers. Diabetes 59(7):1721–1730
Vidard L, Rock KL, Benacerraf B (1992) Heterogeneity in antigen processing by different types of antigen-presenting cells. Effect of cell culture on antigen processing ability. J Immunol 149 (6):1905–1911
Viglietta V, Kent SC, Orban T, Hafler DA (2002) GAD65-reactive T cells are activated in patients with autoimmune type 1a diabetes. J Clin Invest 109(7):895–903
Virtanen SM, Nevalainen J, Kronberg-Kippila C et al (2012) Food consumption and advanced β cell autoimmunity in young children with HLA-conferred susceptibility to type 1 diabetes: a nested case-control design. Am J Clin Nutr 95(2):471–478
Von Meyenburg H (1940) Über "insulitis" bei diabetes. Schweitz Med Wochenschr 21:554–561
Walter M, Albert E, Conrad M et al (2003) IDDM2/insulin VNTR modifies risk conferred by IDDM1/HLA for development of type 1 diabetes and associated autoimmunity. Diabetologia 46(5):712–720
Wang X, Zhang A, Liu Y et al (2012) Anti-idiotypic antibody specific to GAD65 autoantibody prevents type 1 diabetes in the NOD mouse. PLoS One 7(2):e32515
Wardemann H, Nussenzweig MC (2007) β-cell self-tolerance in humans. Adv Immunol 95:83–110
Wasmeier C, Hutton JC (1996) Molecular cloning of phogrin, a protein-tyrosine phosphatase homologue localized to insulin secretory granule membranes. J Biol Chem 271(30):18161–18170
Weenink SM, Lo J, Stephenson CR et al (2009) Autoantibodies and associated T-cell responses to determinants within the 831–860 region of the autoantigen IA-2 in Type 1 diabetes. J Autoimmun 33(2):147–154
Weichselbaum A (1910) Über die veranderungen des pankreas bei diabetes mellitus. Sitzungsber Akad Wiss Wien Math Naturw Klasse 119:73–281

Wenzlau JM, Juhl K, Yu L et al (2007) The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci USA 104(43):17040–17045

Wenzlau JM, Liu Y, Yu L et al (2008) A common nonsynonymous single nucleotide polymorphism in the SLC30A8 gene determines ZnT8 autoantibody specificity in type 1 diabetes. Diabetes 57(10):2693–2697

Wicker LS, Chen SL, Nepom GT et al (1996) Naturally processed T cell epitopes from human glutamic acid decarboxylase identified using mice transgenic for the type 1 diabetes-associated human MHC class II allele, DRB1*0401. J Clin Invest 98(11):2597–2603

Yang J, Danke N, Roti M et al (2008) $CD4^+$ T cells from type 1 diabetic and healthy subjects exhibit different thresholds of activation to a naturally processed proinsulin epitope. J Autoimmun 31(1):30–41

Yoon JW, Ihm SH, Kim KW (1989) Viruses as a triggering factor of type 1 diabetes and genetic markers related to the susceptibility to the virus-associated diabetes. Diabetes Res Clin Pract 7 (Suppl 1):S47–S58

Yu L, Miao D, Scrimgeour L, Johnson K, Rewers M, Eisenbarth GS (2012) Distinguishing persistent insulin autoantibodies with differential risk: nonradioactive bivalent proinsulin/insulin autoantibody assay. Diabetes 61(1):179–186

Zhang B, Lan MS, Notkins AL (1997) Autoantibodies to IA-2 in IDDM: location of major antigenic determinants. Diabetes 46(1):40–43

Zhou X, Bailey-Bucktrout S, Jeker LT, Bluestone JA (2009) Plasticity of $CD4^+$ $FoxP3^+$ T cells. Curr Opin Immunol 21(3):281–285

Ziegler AG, Nepom GT (2010) Prediction and pathogenesis in type 1 diabetes. Immunity 32(4):468–478

Ziegler AG, Rewers M, Simell O et al (2013) Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA 309(23):2473–2479

Current Approaches and Future Prospects for the Prevention of β-Cell Destruction in Autoimmune Diabetes

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_19,

Abstract

Type 1 diabetes (T1D) is an autoimmune disease resulting from the destruction of pancreatic β-cells. The main aim of treatment for T1D should be to prevent β-cell destruction and preserve existing β-cells in individuals with progressive autoimmunity. This can be achieved in several ways, and in this chapter, the authors have reviewed recent approaches that are currently being tested in animal models and human T1D patients under the following categories: (i) - antigen-based therapy, (ii) antibody-based therapy, (iii) immunomodulating therapy, and (iv) other form of therapies.

Keywords

Type 1 diabetes • β-cell • Antigen-based therapy • Biologics • Immunomodulation

Introduction

Type 1 diabetes mellitus (T1D) results from autoimmune destruction of pancreatic β-cells. Autoimmunity is thought to occur in genetically predisposed individuals after exposure to one or more environmental triggers such as dietary factors, viral infections, etc. Infiltrating T cells, B cells, and NK cells in pancreatic islets initiate the autoimmune response and progressively destroy the insulin-producing β-cells. The entire process of β-cell destruction can take anywhere from a few months to a few years, finally resulting in hyperglycemia. HLA-DQ8 and HLA-DQ2 have been associated with high risk to T1D, and 89 % of newly diagnosed children from Sweden are positive for these HLA alleles (Sanjeevi et al. 1994, 1995a, b, 1996). Association of these HLA alleles with T1D has been shown to be inversely proportional to age (Graham et al. 1999).

Latent autoimmune diabetes in adults (LADA) is a slowly progressive form of autoimmune diabetes. Patients initially diagnosed as classical type 2 diabetics are identified as LADA according to the following criteria of the Immunology of Diabetes Society: (i) adult age (>30 years) at onset of diabetes, (ii) the presence

Table 1 List of therapeutics used in prevention of β-cell death in autoimmune diabetes

Therapy	Reference
1. Antigen-based therapy	
Insulin (subcutaneous)	DPT-1 Study Group 2002
Insulin (oral)	Skyler et al. 2005; Barker et al. 2007
Insulin (intranasal)	Achenbach et al. 2008; Nanto-Salonen et al. 2008
Insulin (B chain with adjuvant)	Orban et al. 2010
alum-formulated GAD65	Agardh et al. 2005; Ludvigsson et al. 2008, 2011; Wherrett et al. 2011
DiaPep277	Raz et al. 2001; Elias et al. 2006; Lazar et al. 2007; Ziegler et al. 2010; Buzzetti et al. 2011
2. Monoclonal antibody-based therapy	
Anti-CD3 (otelixizumab)	Keymeulen et al. 2005, 2010
Anti-CD3 (teplizumab)	Herold et al. 2002, 2005, 2009
Anti-CD20 (rituximab)	Pescovitz et al. 2009
CTLA4 Ig (abatacept)	Orban et al. 2011
Anti-IL-1 (IL-1R antagonist, anakinra and anti-IL-1β antibody, canakinumab)	Moran et al. 2013
Anti-TNF-α (etanercept)	Mastrandrea et al. 2009
3. Immunomodulating therapy	
Autologous hematopoietic stem cell transplantation	Voltarelli et al. 2007; Couri et al. 2009
Umbilical cord blood transfusion	Haller et al. 2009
PBMCs educated by cord blood stem cell	Zhao et al. 2012
IFN-α (ingestion)	Brod and Burns 1994; Brod et al. 1997; Rother et al. 2009
Anti-T-cell globulin	Eisenbarth et al. 1985; Saudek et al. 2004
4. Miscellaneous	
Classic anti-inflammatory agents	Hundal et al. 2002; Tan et al. 2002; van de Ree et al. 2003
Vitamin D	Bizzarri et al. 2010; Walter et al. 2010; Gabbay et al. 2012
Atorvastatin	Strom et al. 2012
Diazoxide	Radtke et al. 2010
5. Past trials	
Immunosuppression drugs	Elliott et al. 1981; Harrison et al. 1985; Behme et al. 1988; Bougneres et al. 1988; Silverstein et al. 1988; Cook et al. 1989
Nicotinamide	Yamada et al. 1982; Gale et al. 2004
BCG vaccination	Allen et al. 1999; Huppmann et al. 2005

of circulating islet autoantibodies, and (iii) lack of a requirement for insulin for at least 6 months after diagnosis.

Considering this sequence of events, preventing β-cell destruction is vital to preserving the residual β-cells in individuals with progressive β-cell loss and those

at risk of developing T1D and LADA (referred to as autoimmune diabetes in adults). Antigen-specific and antigen-nonspecific immune therapies that aim to reduce islet cell autoimmunity are in different stages of clinical development. Recent insights into the autoimmune process are elucidating the etiology of autoimmune diabetes, conceivably identifying therapeutic targets. Stand-alone and/or combinational therapies that reduce autoimmunity in islets, regenerate β-cells, and restore insulin secretion are ongoing and appear to be the future of autoimmune diabetes intervention. Aggressive autoimmunity appears significantly earlier than overt disease, and therefore, pursuing therapeutic strategies before disease presentation should be beneficial for susceptible patients. Early intervention before the autoimmunity is initiated is the best. Second best is intervention after autoimmunity is initiated but before the disease becomes insulin dependent. The detailed discussion of immunological β-cell destruction can be found here as follows: "▶ Immunology of β-cell Destruction" and "▶ Inflammatory Pathways Linked to β Cell Demise in Diabetes".

Preservation of β-cells is advantageous in autoimmune diabetes as it may significantly reduce both short- and long-term complications (hypoglycemia, retinopathies, etc.) while at the same time stabilize blood glucose levels and improve quality of life. To this end, pharmaceuticals are being developed using the available knowledge to generate target antigen-specific immune response. Ideally, tolerance induction would be a short time course, leading to a long-lasting tolerant stage, without debilitating the capability of the immune system to mount effective immune response against invading pathogens. In the following sections, the authors have discussed recent strategies employed to prevent β-cell destruction and preserve residual β-cells in autoimmune diabetic patients in the following categories: (i) antigen-based therapy, (ii) antibody-based therapy, (iii) other forms of therapy, and (iv) failed therapies in the past (summarized in Table 1).

Antigen-Based Therapy

Insulin

Autoimmunity against insulin in T1D has long been observed since the 1980s that T1D patient had circulation insulin autoantibodies (IAA) before the insulin treatment (Palmer et al. 1983). As one of the major autoantigens in T1D, insulin is also among the earliest used antigens to induce immune tolerance in T1D patients (to preserve β-cells) as well as T1D relatives (to prevent the disease). The diabetes prevention trial 1 (DPT-1) was performed to access the capability of insulin administered as injections to prevent T1D among T1D relatives. The study, however, failed to demonstrate any beneficial preventive outcome (2002). The insignificant outcome led to subsequent change in insulin administration in similar trials.

Oral tolerance is a term used to describe the immune tolerance, which can be induced by the exogenous administration of antigen to the peripheral immune system via the gut. The active suppression of low doses of administered antigen

appears to be mediated by the oral antigen-generating regulatory T cells that migrate to lymphoid organs and to organs expressing the antigen, thus conferring suppression via the secretion of downregulatory cytokines including IL-4, IL-10, and TGF-β. Since Oral administration is one of the easiest ways to induce immune tolerance. Another prevention approach in the DPT-1 was to administer insulin orally in first-degree relatives of T1D patients; however, the treatment failed to delay or prevent T1D (Skyler et al. 2005). At the same time, it was also found that in DPT-1 trial, oral administration did not alter IAA levels over time in those already positive for IAA at the start of treatment (Barker et al. 2007).

Similar to oral tolerance, immune tolerance could also be induced by administration of antigen to the respiratory tract (mucosal tolerance). At first, insulin administration through the respiratory tract was developed as an alternative to subcutaneous insulin injection. However, the inhaled insulin is associated with increased risk of lung cancer (Gatto et al. 2012). The use of inhaled insulin to prevent β-cell destruction requires further safety studies. While at the same time, the safety of nasal insulin administration is well documented. However, nasal insulin administration in children carrying high-risk HLA (for T1D) soon after detection of autoantibodies failed to prevent or delay the disease (Nanto-Salonen et al. 2008).

Exposure of the nasal mucous membranes to insulin may also cause act like a vaccine effect whereby protective immune cells are stimulated and then counteract the autoreactive immune cells which damage the β cells. There is an ongoing trial that aims to determine if intranasal insulin can protect β cells and stop progression to diabetes in individuals who are at risk (NCT00336674). The Pre-POINT (Primary Oral/intranasal Insulin Trial) is a dose-finding safety and immune efficacy pilot study aiming primary prevention in children genetically at risk to T1D, using oral or intranasal insulin (Achenbach et al. 2008). The results from Pre-POINT, in the future can give us more information on the effectiveness of insulin tolerance induction in T1D.

While most of the above trials end with insignificant outcomes, trials using insulin with modifications was then carried out to treat or prevent T1D. A recent published phase I trial used intramuscular human insulin B chain in incomplete Freund's adjuvant for T1D treatment (Orban et al. 2010). After 2-year follow-up, although there was no statistical difference in stimulated C-peptide responses between treated and untreated patients, a robust insulin-specific humoral and regulatory T-cell (Treg) response was developed in treated patients (Orban et al. 2010). Results from long-term follow-up or trials using upgraded insulin modification can probably give us more data on the treatment.

Insulin and Cholera Toxin

A mechanism of tolerance induction that is currently showing promise is oral insulin conjugated to β-subunit of the cholera toxin (CTB) (Bergerot et al. 1997). It has been shown recently that oral administration of microgram amounts of

antigen coupled to the CTB subunit can effectively suppress systemic T-cell reactivity in animal models. Bergerot et al. report that feeding small amounts (2–20 μg) of human insulin conjugated to CTB can effectively suppress β-cell destruction and clinical diabetes in adult nonobese diabetic (NOD) mice (Bergerot et al. 1997). The protective effect could be transferred by T cells from CTB-insulin-treated animals and was associated with reduced lesions of insulitis. Furthermore, adoptive cotransfer experiments show concomitant reduction in islet cell infiltration. These results suggest that protection against autoimmune diabetes can be achieved by feeding minute amounts of a pancreas islet cell autoantigen linked to CTB and appears to involve the selective migration and retention of protective T cells into lymphoid tissues draining the site of organ injury.

CTB subunit carries the insulin to the intestine and helps in the transfer of the insulin molecule across the intestinal barrier. The CTB conjugation also helps in the reduction of the dosage of insulin that can be administered orally without causing hypoglycemia. Further, this approach has also been tried successfully by intranasal administration. Both approaches have prevented the development of diabetes in the NOD mouse model of the autoimmune disease. CTB-insulin β-chain fusion protein produced in silk worms has been shown to suppress insulitis in NOD mice (Gong et al. 2007).

GAD65

Glutamic acid decarboxylase isoform 65 (GAD65) is a major autoantigen in T1D. Studies in NOD mouse have shown that destruction of islet β-cells was associated with T cells recognizing GAD65. Kaufman et al. showed that in NOD mice, intravenously injection of GAD65 before diabetes onset effectively prevents autoimmune β-cell destruction and reduce the development of spontaneous diabetes (Kaufman et al. 1993).

Diamyd AB evaluated this by using alum-formulated human recombinant GAD65 in LADA patients. They selected diabetic patients of both sexes aged 30–70 years, diagnosed with type 2 diabetes (T2D) and positive for GAD65 antibodies in their phase I/II trial. These patients were treated with either diet or oral tablets. A total of 34 patients and 13 controls were tested with 4, 20, 100, and 500 μg dose. This was injected subcutaneously twice but 4 weeks apart. No serious adverse effects were reported. In the follow-up, the C-peptide level (both fasting and stimulated) was significantly elevated in the group receiving 20 μg dose compared to placebo. Likewise, the HbA1c and mean glucose levels were significantly lowered in the 20 μg dose compared to placebo. The $CD4^{+}CD25^{+}$ T cells which reflect the increase in regulatory T cells associated with nondestructive response to β-cell were elevated in the 20 μg dose but not in other doses. All these findings were relevant even after a follow-up period of 24 months and 5 years (www.diamyd.com; Agardh et al. 2005). It is thought that the prevention of β-cell destruction and β-cell recovery is due to shifting of immune response from destructive to nondestructive which is mediated by the Diamyd GAD65 vaccine.

Subsequent phase IIb trials in Swedish T1D patients with alum-formulated GAD65 showed significant preservation of β-cell function 30 months after the first 20 μg dose administrations. It also induced antigen-specific T-cell population, cytokines involved in regulation of the immune system, and a long-lasting B cell memory, suggesting that modulation of general immune responses to GAD65 can be helpful in preserving residual β-cells (Ludvigsson et al. 2008). In the extended evaluation of the Swedish phase IIb trials, it showed that the alum-formulated GAD65 was able to delay the progressive β-cell destruction (Ludvigsson et al. 2011). However, the phase IIb trial in Canada did not significantly differ C-peptide levels between alum-formulated GAD65 treated and control groups at 1 year (Wherrett et al. 2011). Whether it is due to the shorter follow-up duration or the immunological difference between populations is not clear. A phase III trial recently concluded to verify the previous observed effect of alum-formulated GAD65 did not meet the end point when the results were analyzed from the European sites. If Sweden and Finland were excluded in the analysis, the results in the rest of the European sites showed significant end point. The reason why Sweden and Finland sites showed nonsignificant end point was because all the children in the trial had taken state-recommended H1N1 vaccine, which were not a part of the inclusion criteria. Even in Sweden, if the results were analyzed in those children who completed the study before the H1N1 vaccine was recommended by the state, significant result was obtained. It is not clear what H1N1 does to the protective effect of the alum-formulated GAD65 vaccine. Meanwhile, few studies are in progress like the phase II trial which is ongoing in Norway to see whether the difference in the effect of alum-formulated GAD65 is in association with enterovirus infections (NCT01129232). In supplementation with other anti-inflammatory drugs, another phase II trial is ongoing in Sweden to see the effect of using alum-formulated GAD65, vitamin D, and ibuprofen (NCT01785108).

Alum-formulated GAD65 is the only antigen-based vaccine candidate which has been shown to be effective in LADA. LADA is often misdiagnosed as type 2 diabetes and treated accordingly. This may lead to additional stress on an already declining β-cell mass (due to autoimmune destruction). Hence, diagnosis and treatment of LADA are vital.

DiaPeP277

Heat shock protein 60 (hsp60) is a 60 kDa protein which is one of the self-antigens in T1D. DiaPeP277 is a 24-amino-acid peptide which comprises 24 residues (437–460) analog to hsp60 (www.develogen.com). Administration of DiaPep277 in NOD mice arrested diabetes (Elias et al. 1990). A randomized double-blind phase II trial using DiaPeP277 in human subjects with newly onset disease (<6 months) resulted in preservation of the endogenous insulin production compared to the placebo group (Raz et al. 2001). In a follow-up study (Elias et al. 2006), the findings were reiterated. In both studies, immunomodulation was observed and associated with downregulation of Th1 cells and upregulation of IL-10 producing

T cells. The immune responses were antigen specific as T-cell responses to bacterial antigens remained unaffected. However, studies performed in children did not show any improvement in the preservation of β-cell function or metabolic control (Lazar et al. 2007).

The preliminary results from phase III trials of DiaPep277 showed that T1D patients treated with DiaPep277 maintained C-peptide level better than in placebo group. Meanwhile, patients in the treated group had lower HbA1c level (Ziegler et al. 2010). Subsequent analysis found that T1D adults with low- and moderate-risk HLA genotypes would benefit the most from the intervention with DiaPep277 (Buzzetti et al. 2011). Additional phase III trial is ongoing for further evaluation on the efficacy/safety (NCT01103284, NCT01898086) and treatment effect (NCT01460251) of DiaPep277.

Monoclonal Antibody-Based Therapy

Anti-CD3 Antibodies

Experiments in the early 1990s in NOD mice demonstrated that hamster-derived anti-CD3 monoclonal antibodies reversed diabetes in hyperglycemic mice (Chatenoud et al. 1994, 1997). In order to increase safety in future clinical application, Fc-mutated (Fc-nonbinding) monoclonal anti-CD3 antibodies were engineered and were found to be less mitogenic, but were equally tolerogenic compared to functional Fc anti-CD3 antibodies (Chatenoud et al. 1994, 1997). These series of experiments demonstrated several unique features of the antibody therapy. First, continuous immunosuppression was not required, and second, the ability of the antibody to reverse disease after hyperglycemia has occurred was demonstrated. Treated NOD mice were resistant to transfer of diabetes by diabetogenic spleen cells, implying the involvement of active immune regulation preventing diabetes (Chatenoud et al. 1997).

Two of the earliest monoclonal antibody specific for the CD3 T-cell epitope that were tested in clinical trials are the ChAglyCD3 antibody (known after as otelixizumab), having a single mutation (Asn→Ala) at residue 297 in the Fc region that prevents glycosylation, derived from rat YTH 12.5 antibody (Routledge et al. 1995), and the hOKT3Ala-Ala antibody (known after as teplizumab), having two mutations at residues 234 (Lue→Ala) and 235 (Lue→Ala) in the Fc region. This antibody is derived from OKT3 (Bolt et al. 1993). A 6-day otelixizumab treatment in newly diagnosed T1D patients was found to be associated with lower requirement of insulin, however, susceptibility to infection at 1-year follow-up time (Keymeulen et al. 2005). When these T1D patients were followed longer (4 years), the treatment with otelixizumab after their T1D diagnosis was still able to suppress the rise in insulin requirements (Keymeulen et al. 2010). At the same time, teplizumab was also showed to change the ratio of $CD4^+$ T cells to $CD8^+$ T cells within 3 months and subsequently preserved insulin production up to 5 years (Herold et al. 2002, 2009) with better clinical parameters found in teplizumab-treated

T1D patients (Herold et al. 2005). A current ongoing trial is undergoing to evaluate the effect of teplizumab to prevent or delay the onset of T1D in relatives determined to be at very high risk for developing T1D (NCT01030816).

The FcR-nonbinding anti-CD3 antibody (anti-CD3 antibody with a mutated Fc portion) therapy was effective only if the immune response was primed and ongoing. Locally, they target autoreactive T cells, and the strength of the T-cell receptor (TCR)/CD3 is important in determining the efficacy. Thus, it can be hypothesized that though CD3 is expressed on all T cells, anti-CD3 antibodies mediate signaling depending on the functional stage of the target T cell whether it is naive or effector or memory. Administration of anti-CD3 antibodies induces depletion of effector T cells in the target tissue and lymphoid organs. In the pancreas-draining lymph nodes, apoptosis is induced in effector T cells compared to regulatory T cells and resting T cells (Hirsch et al. 1988; Wong and Colvin 1991; Carpenter et al. 2000). Apoptotic effector T cells are engulfed and digested by phagocytes (macrophages and immature dendritic cells [DC]). These phagocytes secrete large amounts of transforming growth factor (TGF-β) which creates a noninflammatory environment and also plays a major role in the maturation of DCs. TGF-β production has been suggested and experimental data demonstrate that TGF-β is central to the tolerance induced by FcR-nonbinding anti-CD3 antibodies. TGF-β-neutralizing antibodies are shown to completely neutralize the tolerogenicity induced by anti-CD3 therapy (Belghith et al. 2003). Local production of TGF-β has been shown to have the capability to convert a proinflammatory environment to a noninflammatory and tolerogenic environment (Li et al. 2006). A high concentration of TGF-β also promotes upregulation of inhibitory receptor ligands (programmed cell death ligand 1, ICOS ligand) and downregulation of MHC and costimulatory molecules on antigen-presenting cells (Li et al. 2006; Rutella et al. 2006). This in turn induces the induction or expansion of $CD4^+CD25^+FOXP3^+$ T-regulatory cells (Treg) (Rutella et al. 2006). From the available experimental data, it has been proposed that the FcR-nonbinding anti-CD3 antibody treatment triggers a massive local production of TGF-β, by phagocytes engulfing activated effector T cells (You et al. 2008).

Improving the Existing anti-CD3 Antibody Therapy

Administration of drugs which promote β-cell survival and growth (such as exendin-4) may increase the β-cell growth and replication in the "tolerant" environment. In NOD mice, combination of exendin-4 and anti-CD3 monoclonal antibodies led to effective reversal or the disease with increased insulin content of the β-cell as compared with individual exendin-4 or anti-CD3 monoclonal antibody treatment (Sherry et al. 2007). Frequent side effects because of interferences with the T-cell population in proximity with treatment periods and recurrent autoimmunity might be a problem in anti-CD3 antibody-treated individuals. Repetitive treatment can be a possible way out in such a situation but formation of anti-idiotypic antibodies should be taken into consideration.

Anti-CD20 Antibodies

B cells constitute about 60–70 % of the immune cells infiltrating the pancreatic islets (Green and Flavell 1999). Until recently B cells were thought to play an important role in priming T cells (Wong and Wen 2005). However, a recent study showed for the first time that B cells promote the survival of $CD8^+$ T cells in the islets and thereby promote the disease (Brodie et al. 2008). CD20 is a cell surface marker expressed on all mature B cells. Rituximab (Roche/Genentech), a humanized anti-CD20 monoclonal antibody (CD20 mAb), has been shown to successfully deplete human B cells from peripheral circulation via mechanisms involving Fc- and complement-mediated cytotoxicity and probably via proapoptotic signals (Rastetter et al. 2004; Martin and Chan 2006). Given the important role of B cells in the pathogenesis of T1D, depleting B cells is a very interesting therapeutic option. Transgenic NOD mice engineered to express human CD20 on B cells, when treated with a single dose of CD20 mAb, gave interesting results (Hu et al. 2007; Xiu et al. 2008). First, treatment of mice in the early stage of the disease (insulitis) prevented or delayed the progression to disease; second, clinical hyperglycemia could be reversed in over one-third of the experimental animals; third, B cell levels were restored to pre-depletion levels within 3 months of treatment, but the progression to T1D was delayed almost indefinitely. Recent published data from TrailNet anti-CD20 study group showed that four infusions of rituximab in the first month after diagnosis of T1D can preserve β-cell function at 1-year follow-up (Pescovitz et al. 2009). A recent clinical trial (NCT01280682) is ongoing to investigate further the immunomodulating role of rituximab in the treatment of T1D.

CTLA4 Immunoglobulin (CTLA4 Ig)

Cytotoxic T-lymphocyte antigen 4 (CTLA4) is expressed on the surface of T-helper (Th) cells. CTLA4 binds CD80 and CD86 on the antigen-presenting cells and blockades the most important second signal (costimulation signal) for full activation of T cells transduced by the binding between CD80/CD86 and CD28 on the T cells. Thus, CTLA4 functions as a negative regulator of T-cell activation, and the costimulation blockade has been proposed as a therapeutic modality for autoimmunity and transplantation (Bluestone et al. 2006; Teft et al. 2006). The CTLA4 Ig (or lately named abatacept) is a fusion protein of human CTLA4 and the Ig–Fc region designed to bind CD80/CD86 and block the T-cell costimulatory signal (Lenschow et al. 1992). A study in NOD mice showed that costimulatory blockade with CTLA4 Ig fusion protein prevented diabetes when administered before overt diabetes (Lenschow et al. 1995). A recent phase II trial showed that abatacept infusion regularly after T1D diagnosis can preserve β-cell function throughout the 2 years during the investigation (Orban et al. 2011). Further trial is ongoing (NCT01773707) to determine whether the infusion of abatacept can delay or prevent the development of T1D among T1D relatives at risk.

IL-1 antagonist and anti-IL-1β Antibody

Interleukin-1β (IL-1β) is a proinflammatory factor secreted by several cell types in response to tissue insult. It has been shown that IL-1β bound to pancreatic β-cell interleukin-1 (IL-1) type 1 receptors (IL-1R), IL-1 induces β-cell dysfunction and apoptosis through mitogen-activated protein kinase pathways (Mandrup-Poulsen et al. 2010). In addition, IL-1β enhances expansion and survival of T cells, promotes differentiation of T cells toward pathological phenotypes, and enables effector T cells to proliferate despite the presence of Tregs (Dinarello et al. 2012). These evidence makes blockade of IL-1β is an attractive therapeutic target. A recent report combined results from two independent studies blocking IL-1 (anakinra, IL-1 receptor antagonist, and canakinumab, anti-IL-1β mAb) showed that both treatment did not preserve C-peptide level at 9-month or 12-month time (Moran et al. 2013). Although future results of long follow-up duration might give us more information, the investigator questioned that the IL-1 blockade treatment should be given as prevention for T1D before T1D onset. However, up to date, there is no registered trial investigating the preventive effect of IL-1 blockade treatment in T1D. There are currently trials investigating the use of other IL-1 blockade in the treatment of T1D (rilonacept, IL-1 Trap, NCT00962026, and gevokizumab, anti-IL-1β antibody, NCT01788033 and NCT00998699).

Anti-TNF-α Antibody

It was found in NOD mouse that TNF-α mRNA is produced by $CD4^+$ T cells within inflamed islets during the development of diabetes (Held et al. 1990). In vitro models show that TNF-α potentiates the destruction of β-cells by other cytokines (Mandrup-Poulsen et al. 1987). Transgenic mice with increased β-cell expression of TNF-α have significant lymphocytic insulitis, which is abrogated in TNF receptor knockout mice (Herrera et al. 2000). It was also indicated clinically that anti-TNF therapy may induce IL-10-secreting regulatory cells with a consequent resolution of the inflammation in the pancreatic islets (Arif et al. 2010). Etanercept is a recombinant fusion protein of TNF receptor to the constant end of the IgG1 antibody that binds to TNF-α, thereby clearing the TNF-α from circulation. The usage of etanercept twice a week subcutaneously resulted in lower A1C and increased endogenous insulin production at 6-month follow-up (Mastrandrea et al. 2009). However, long-term effect of etanercept in T1D is currently unknown and awaiting future investigations.

Others (Anti-CD52 Antibody and Anti-CD2 Antibody)

Mycophenolate mofetil (MMF) is rapidly absorbed after oral administration and hydrolyzed to MPA, an inhibitor of inosine monophosphate dehydrogenase that inhibits guanosine nucleotide synthesis and thus inhibits T and B cell proliferation

with no obvious effect on other cell types. It was found that MMF was effective in diabetic animal models (Hao et al. 1992, 1993). Recent study in DRBB rat model demonstrated a synergistic effect of MMF with daclizumab in the treatment of diabetes (Ugrasbul et al. 2008). Daclizumab is a humanized monoclonal antibody that binds to CD25, the α subunit of the interleukin-2 (IL-2) receptor on the surface of activated lymphocytes, functioning to arrest the proliferation of activated lymphocytes. However, clinical trial in human using MMF and daclizumab or alone did not have an effect on the loss of C-peptide in subjects with new-onset T1D (Gottlieb et al. 2010). In addition, the increased risk of virus infection after coadministration of MMF and daclizumab might impede further investigations (Loechelt et al. 2013). An ongoing trial using daclizumab alone (NCT00198146) is under investigation.

Alefacept is a soluble LFA3/IgG1 fusion protein that binds CD2 on T cells. Alefacept was found recently to be efficacious in treatment of chronic psoriasis (Ellis and Krueger 2001). Alefacept was found to downregulate circulating memory ($CD45RO^+$) T cells (da Silva et al. 2002). The trial using alefacept in the treatment of T1D (NCT00965458) is ongoing.

Immunomodulating Therapy

Autologous Hematopoietic Stem Cell Transplantation

It was shown as early as in the late 1980s that diabetes in NOD mice may be prevented by allogeneic transplantation of hematopoietic stem cells (HSC) from a non-disease-prone strain (LaFace and Peck 1989). In the 1990s, it was demonstrated that environmentally induced animal autoimmune diseases such as experimental autoimmune encephalomyelitis and adjuvant arthritis could be cured by syngeneic, autologous, or pseudo-autologous (using syngeneic animals in the same stage of disease as the recipient) HSC transplants (Burt et al. 1995; Kroger et al. 1998; Karussis et al. 1999). These studies in animal models indicated that HSC transplantation can probably reintroduce tolerance to autoantigens. Indeed, in a recent published uncontrolled clinical trial, HSC transplantation procedure in human showed that it is associated with more insulin independence and lower insulin usage and increased C-peptide level (Couri et al. 2009). Aside from classic HSC transplantation, autologous non-myeloablative hematopoietic stem cell transplantation also showed effectiveness in slowing C-peptide decrease, decreasing GAD65 antibody, and better diabetic metabolism measurements (Voltarelli et al. 2007). However, since earlier trials had shown that low-dose immunosuppression can induce a slow decline or even improvement in C-peptide level (see below in section "Immunosuppression Drugs"), it was argued whether the effect is due to repopulation of the immune cells or the high-dose immunosuppression itself before the transplantation. There are two ongoing trials in China (NCT01341899 and NCT00807651) that might provide more knowledge on the HSC transplantation in T1D.

While the transplantation of HSC was showed to halt the autoimmune destruction of β-cells, research and trials on the transplantation of islets heightened further the importance of transplantation in the treatment of T1D (discussed elsewhere: "▶ Human Islet Autotransplantation," "▶ Islet Encapsulation," "▶ Islet Xenotrans plantation: Recent Advances and Future Prospect," and "▶ Successes and Disap pointments with Clinical Islet Transplantation").

Stem Cell Therapies

Mesenchymal stem cells (MSCs) were found to be able to protect NOD mice from diabetes by induction of Tregs (Fiorina et al. 2009; Madec et al. 2009). On one side, it was shown that MSC and PBMC mixture is capable of switching T-cell response from Th1 to Th2 when it encounters autoantigen GAD65 (Zanone et al. 2010), and on the other hand, MSCs were found to be able to home to injured tissue and stimulate endogenous islet regeneration (Yagi et al. 2010; Bell et al. 2012). Being capable of shooting two birds with one stone, MSC attracts researchers' interest and there are several ongoing trials investigating different types of MSCs (NCT00690066, NCT01322789, NCT01374854, NCT01068951, NCT01219465, NCT01496339).

In addition to MSC, immature unprimed functional regulatory T cells (Tregs) were found abundant in the umbilical cord blood (Godfrey et al. 2005). These highly functional Tregs might limit inflammatory cytokine responses and anergize effector T cells in autoimmune processes (Fruchtman 2003). Thus, the umbilical cord blood has become a focus of researchers to design cell-based therapies for T1D patients. However, umbilical cord blood transfusion did not demonstrate efficacy in preserving C-peptide in the newly diagnosed after 1 year of transfusion (Haller et al. 2009). Larger randomized studies as well as 2-year post-infusion follow-up of this cohort are needed to determine whether autologous cord blood-based approaches can be used to slow the decline of endogenous insulin production in children with T1D is an idea source to be transplanted to patients with T1D. An ongoing trial is investigating the effectiveness of autologous cord blood transfusion in the treatment of T1D (NCT00989547). At the same time, the combination of MSC transplantation and umbilical cord blood transfusion is also under investigation (NCT01143168).

It has been reported in NOD mouse model that the autologous $CD4^+CD62L^+$ Tregs co-cultured with the human cord blood stem cells (CB-SC) can eliminate hyperglycemia and promote β-cell regeneration (Zhao et al. 2009). Although the underlying mechanism is not clear, it was proposed that CB-SC could suppress the proliferation of β-cell-specific autoreactive T cells (Zhao et al. 2007). The phase I trial was published recently using CB-SC-treated peripheral lymphocytes (stem cell educator) reinfusion to patients with existing T1D (Zhao et al. 2012). The stem cell educator showed its safety and capability of persistently improving metabolic control after a single treatment (Zhao et al. 2012). A phase II trial (NCT01350219) is currently ongoing to investigate the effectiveness of this method in the treatment of T1D.

The other usage of stem cell therapies prospered in the production of islets in vitro as well as in vivo. The in vitro islets generated from human embryonic stem cells and its potential clinical implementation is further discussed in "► Making Islets From Human Embryonic Stem Cells". Potential usage of in vivo stem cells is discussed in "► Stem Cells in Pancreatic Islets".

Other Cell Therapies

Dendritic cell (DC) is one of the most important antigen-presenting cells that also showed immunoregulatory characteristics (Hackstein et al. 2001). Studies in mouse models showed that treating NOD mice dendritic cells ex vivo with antisense oligonucleotides targeting the primary transcripts of CD40, CD80, and CD86 (costimulatory molecules) can downregulate those cell surface molecules, thus increasing $CD4^+CD25^+$ T cells (Tregs) in NOD recipients (Machen et al. 2004). This regulation effect in mice was then found to be mediated by IL-7 produced by the treated DCs (Harnaha et al. 2006). The trial using this method (NCT00445913) is ongoing and awaiting results.

Tregs (T-regulatory cells) can act through DC to prevent autoreactive T-cell differentiation, thus preventing or slowing down the progression of autoimmune diseases (Tang and Bluestone 2006). The shortage of Tregs can lead to the development and accumulation of Treg-resistant pathogenic T cells in patients with autoimmune diseases (Tang and Bluestone 2006). Thus, restoration of self-tolerance using Tregs in these patients will likely be able to control ongoing tissue injury. A recent report showed the feasibility of expanding Tregs isolated from patient with recent-onset T1D (Putnam et al. 2009). The ongoing trial using autologous expanded Tregs transfusion in T1D patients (NCT01210664) will investigate the effectiveness of this hypothesis.

IFN-α and IL-2

Early trial showed that parenteral IFN-α (interferon-α) provided no benefits in patients with newly diagnosed T1D patients (Koivisto et al. 1984). However, it was found later that ingested IFN-α has immunomodulatory effect in experimental autoimmune animal model and in multiple sclerosis in humans (Brod and Burns 1994; Brod et al. 1997). In a recent phase I trial using ingested human recombinant IFN-α in T1D, it was found that 5,000 units of IFN-α administered once daily in newly diagnosed T1D patients for 1 year could maintain more β-cell function (Rother et al. 2009). Future study is required to verify the results and pave the way for clinical use of IFN-α.

Interleukin 2 (IL-2) is a dependant cytokine for Tregs to maintain viability and function. IL-2 down signaling events activate Akt/Erk pathway and targets CTLA4 gene. At the same time, IL-2 also signals through STAT5 pathway and activates FOXP3 and CD25 genes (Hulme et al. 2012). Reduction in IL-2 in T1D may

contribute to Treg decline. Aldesleukin (Proleukin) is a commercialized IL-2 and currently under the investigation of several trials (NCT01353833, NCT01827735, NCT01862120).

Anti-T-Cell Globulin

Early clinical experience on immunomodulating therapy using anti-T-cell globulin (ATG) in T1D showed effectiveness in delaying and lowering insulin requirement (Eisenbarth et al. 1985) A recent clinical trial using polyclonal ATG confirmed that short-term ATG therapy in newly diagnosed T1D contributes to the preservation of residual C-peptide production and to lower insulin requirements at 1 year after diagnosis (Saudek et al. 2004). A current ongoing trial (NCT00515099) is further investigating the therapeutic effect of ATG in T1D.

Other Forms of Therapy

DNA Vaccination

DNA vaccination involves administration of a gene that encodes the target antigen, instead of the antigen as in classical vaccination. Variety of vectors can be used to transfer the target gene as DNA or RNA, along with genes encoding immunomodulatory molecules. Several studies have been performed using administration of plasmids encoding antigens such as insulin B chain, GAD65, and immunoglobulin G–Fc fusion constructs in animal models. However, plasmids carry unmethylated CpG motifs (ISS, immunostimulatory sequences) which activate the innate immune system. Therefore, DNA vaccination against T1D should block or overcome the effect of such stimulatory elements. DNA vaccine hold good promise in treatment of autoimmune diseases as they have been used, in experimental models, to direct the immune response toward a Th1 or a Th2 response (Prud'homme 2003).

DNA Vaccination with GAD65

Intramuscular injections of plasmid containing GAD65 fused with IgG–Fc and IL-4 were reported to generate a GAD65-specific Th2 response, protecting NOD mice from developing T1D (Tisch et al. 2001). A study performed to evaluate two different modes of delivery of a plasmid coding for GAD65 reported the elicitation of IL-4 secreting T-cell response. Two methods of plasmid delivery, intramuscular and a novel gene gun method, were tested in this study. Intramuscular injections fail to stop the ongoing β-cell autoimmunity, whereas the gene gun method was successful in eliciting immunomodulation, significantly delaying the disease onset in NOD mice (Goudy et al. 2008).

Microsphere-Based Vaccine

Microparticulate carriers have the capability to shape the functional phenotype of dendritic cells (DC) (Waeckerle-Men et al. 2006; Yoshida and Babensee 2006). A nucleic acid-based vaccine using antisense oligonucleotides coated on microspheres, directed against CD40, CD80, and CD86 (costimulatory molecules important in DC maturation), has been shown to prevent T1D in NOD mice as well as reverse new-onset disease (Phillips et al. 2008). Microspheres administered are taken up by DCs by phagocytosis. Inflammation in the pancreatic islets associated with β-cell apoptosis is suggested to drive the antisense oligonucleotide-loaded DCs to acquire the β-cell antigen(s). This is followed by the accumulation of these DCs in the pancreatic lymph nodes, where they are hypothesized to interact with regulatory T cells inducing a β-cell-specific immune hyporesponsiveness or functional tolerance to β-cell antigens (Tarbell et al. 2006). The detailed mode of action of the microsphere-based vaccine is yet to be established and clinical trials in human subjects will decide the efficacy of this approach in the prevention of T1D.

Anti-Inflammatory Agents

Use of anti-inflammatory drugs such as aspirin (Hundal et al. 2002), statins (Tan et al. 2002), and glitazone (van de Ree et al. 2003) has been shown to be beneficial in type 2 diabetes. These drugs have been shown to have anti-inflammatory effect by affecting either the signaling pathways (such as NFkB signaling) or cytokines involved in inflammation. Such drugs can be vital in bringing down the overall islet inflammation and thereby creating a better islet environment which can respond to other forms of treatment.

Newly developed anti-inflammatory drugs have joined the tide against T1D recently. α-1 antitrypsin or α1-antitrypsin (AAT) is a naturally occurring anti-inflammatory glycoprotein; AAT is a protease inhibitor belonging to the serpin superfamily. AAT has been shown to facilitate Treg expansion in the NOD mice mode, protecting the mice from diabetes (Koulmanda et al. 2008). AAT alters CCR7 expression on DC surface; thus, promoting semimature DC migration to the lymph nodes subsequently activates Tregs (Ozeri et al. 2012). There are two trials now investigating AAT in the treatment of T1D (NCT01319331, NCT01661192).

Vitamin D

Vitamin D has been shown to suppress proinflammatory responses by suppressing enhanced activity of immune cells taking part in autoimmune processes. In NOD mice, vitamin D has been shown to prevent autoimmune diabetes (Mathieu et al. 1994). Supplementation of vitamin D has been shown to be protective in children against T1D. High dosage and the timing of the dose have also been shown to play a role. A randomized open-label, pilot trial is currently under way

(NCT00141986), where increased dose of vitamin D (2,000 IU/day instead of the current practice of 400 IU/day) is administered to children genetically at risk of developing T1D. However, trials with vitamin D in new-onset T1D have shown mixed results, with one showing benefit (Gabbay et al. 2012) while the other two did not (Bizzarri et al. 2010; Walter et al. 2010). The dosing and timing of the treatment using vitamin D require future studies.

N-3 Polyunsaturated Fatty Acids and Other Dietary Supplements

It has been shown early that synthesis of IL-1β, IL-1α, and tumor necrosis factor can be suppressed by dietary supplementation with long-chain n-3 fatty acids (Endres et al. 1989). An ongoing trial (NCT00333554) investigating the effect of long-chain n-3 fatty acids in the prevention of T1D might provide us more useful information.

In addition, study using streptozotocin-treated diabetic rats showed that chromium supplementation lowered blood levels of proinflammatory cytokines. Although there is no benefit in plasma glucose level from chromium supplement found in the animal study (Vinson 2007), the investigators believed T1D patients could benefit from chromium supplement. The effect of chromium supplement human T1D is currently under investigation (NCT01709123).

Other Drugs

Lansoprazole and other proton-pump inhibitors consistently elevated serum gastrin levels (Ligumsky et al. 2001). It has already been found as early as the mid-1950s that gastrin has the potential to increase new β-cell formation (Zollinger and Ellison 1955). Thus, it was hypothesized that lansoprazole could probably induce β-cell regeneration by increasing serum gastrin level. There are two current trials investigating the safety of coadministration of cyclosporin and lansoprazole among patients with existing T1D (NCT01762657) and newly diagnosed T1D (NCT01762644).

The lipid-lowering drug atorvastatin was also found to have immunomodulating effect in rheumatoid arthritis intervention trials (McCarey et al. 2004). Atorvastatin treatment was found to be able to preserve β-cell function in T1D patients with median inflammation mediator levels (Strom et al. 2012). There is an ongoing trial (NCT00529191) which might give further information on the effect of atorvastatin treatment in T1D. Similarly, another lipid-lowering drug simvastatin is also under investigation in a current trial (NCT 00441844).

Imatinib is originally designed as a specific inhibitor of Abl protein tyrosine kinases and used in the treatment of chronic myeloid leukemia (Druker et al. 1996). Recent studies showed that imatinib had a strong anti-inflammatory effect by inhibiting TNF-α production in macrophages (Wolf et al. 2005). A recent study showed that both imatinib and sunitinib treatments led to durable remission in NOD mice (Louvet et al. 2008). The underlying mechanism is probably due to the

multikinase inhibiting characteristics of imatinib and sunitinib which inhibited platelet-derived growth factor receptor (PDGFR) (Louvet et al. 2008). The phase I trial using imatinib for the treatment of T1D (NCT01781975) is planned and awaiting participant recruitment.

Table 2 Ongoing trials

Therapy	NCT number
1. Antigen-based therapy	
Insulin (intranasal, prevention)	NCT00336674
alum-formulated GAD65	NCT01122446, NCT01129232, NCT01785108
DiaPep277	NCT01103284, NCT01898086, NCT01460251
2. Monoclonal antibody-based therapy	
Anti-CD3 (teplizumab, prevention)	NCT01030816
Anti-CD20 (rituximab)	NCT01280682
CTLA4 Ig (abatacept)	NCT01773707
Anti-IL-1 (IL-1 Trap, rilonacept)	NCT00962026
Anti-IL-1 (IL-1β antibody, gevokizumab)	NCT01788033, NCT00998699
Anti-CD25 (daclizumab)	NCT00198146
Anti-CD2 (alefacept)	NCT00965458
3. Immunomodulating therapy	
Autologous hematopoietic stem cell transplantation	NCT01341899, NCT00807651
Mesenchymal stem cells transfusion	NCT00690066, NCT01322789, NCT01374854, NCT01068951, NCT01219465, NCT01496339
Umbilical cord blood transfusion	NCT00989547, NCT01143168
PBMCs educated by cord blood stem cell	NCT01350219
Dendritic cell treated ex vivo	NCT00445913
Tregs expanded ex vivo	NCT01210664
IL-2	NCT01353833, NCT01827735, NCT01862120
Anti-T-cell globulin	NCT00515099
4. Miscellaneous	
Novel anti-inflammatory agents (AAT)	NCT01319331, NCT01661192
Vitamin D (prevention)	NCT00141986
N-3 polyunsaturated fatty acid (dietary supplement)	NCT00333554
Chromium (dietary supplement)	NCT01709123
Lansoprazole	NCT01762657, NCT01762644
Atorvastatin and simvastatin	NCT00529191 and NCT 00441844
Imatinib	NCT01781975

Diazoxide is a potassium channel activator that is frequently used in the treatment for hypertension. It was found that diazoxide can provide β-cell rest by reversibly suppressing glucose-induced insulin secretion through opening ATP-sensitive K^+ channels in the β-cell (Trube et al. 1986). Early trial using diazoxide in T1D showed that those treated T1D displayed higher residual insulin secretion than the placebo group (Bjork et al. 1996; Ortqvist et al. 2004). However, a recent trial did not observe the preservation effect from diazoxide, although better metabolic control was found among diazoxide-treated T1D patients (Radtke et al. 2010). More studies in the future with regard to diazoxide function may unveil more on the effect of diazoxide as well as T1D pathogenesis. Ongoing clinical trials for new therapy in managing type 1 diabetes is listed in Table 2.

Past Trials

Immunosuppression Drugs

Cyclosporin was one of the first immunosuppressive drugs used in treatment of T1D, which could delay the onset of the disease (Bougneres et al. 1988). However, cyclosporin achieved immunosuppression by targeting intracellular processes, which is nonspecific and unrelated to autoantigens involved in the disease. Withdrawal of the treatment resulted in invariable recurrence of the pathogenic immune response. Considering the nephrotoxic potential of the drug, it was not a choice of long-term treatment, and therefore, it was not considered for therapy (Behme et al. 1988).

Other early used drugs are prednisone (Elliott et al. 1981), azathioprine (Harrison et al. 1985; Cook et al. 1989), and coadministration of prednisone plus azathioprine (Silverstein et al. 1988). Although these drugs showed slow decline or even improvement in C-peptide level, they were not used in clinic afterward due to considerable side effects.

Nicotinamide

The European Nicotinamide Diabetes Intervention Trial (ENDIT) tested the efficacy of nicotinamide in preventing diabetes in human subjects. Previous studies in animal models demonstrated that the administration of nicotinamide can prevent T1D (Yamada et al. 1982). Nicotinamide is speculated to confer protection by inhibiting DNA repair enzyme poly-ADP-ribose polymerase and prevent the depletion of β-cell NAD. However, in the ENDIT, nicotinamide treatment did not result in successful prevention of T1D (Gale et al. 2004).

BCG

Bacille Calmette–Guerin (BCG) vaccination has been proposed as an adjuvant therapy to prevent T1D. A study reported that administration of BCG

vaccination soon after T1D onset preserves β-cell function (Allen et al. 1999). However, this was not the case in the trials that followed. BCG vaccination could not prevent the development of T1D in children genetically at risk (Huppmann et al. 2005).

Ongoing Prediction Studies

Several international collaborative efforts are under way. These studies will identify potential population/risk groups who would benefit from various therapies for prevention of β-cell death.

Potential therapies aiming at prevention of β-cell death would directly benefit patients suffering from autoimmune diabetes (T1D/LADA). Successful therapies can also benefit prediabetics, first-degree relatives of T1D patients, and individuals at risk of developing autoimmune diabetes.

TEDDY: The Environmental Determinants of Diabetes in the Young (TEDDY) study are an effort to screen more than 360,000 children around the world to the environmental factors that might play a role in T1D pathogenesis (2008). Several genome-wide association scans have been completed and are under way, with an aim to identify the T1D risk loci across the human genome. Identification of environmental and genetic factors involved in the etiology of T1D can broaden the scope of therapeutic interventions.

TrailNet: It is an international consortium of clinical research centers working toward achieving prevention of T1D (Skyler et al. 2008).

TRIGR: Trial to reduce T1M in the genetically at-risk (TRIGR) study is another collaborative effort, which aims at testing the hypothesis that weaning to an extensively hydrolyzed infant formula will decrease the incidence of T1D in children who carry high-risk HLA and in those who have a first-degree relative with T1D (2007). Initial findings from TRIGR suggest that introduction of cow's milk at an early age in children with dysfunctional gut immune system might result in aberrant immune response, leading to T1D (Luopajarvi et al. 2008).

DAISY: The DAISY study (the Diabetes Autoimmunity Study in the Young) aims at elucidating the interaction between genes and the environment that can trigger T1D. Children who are genetically at risk or those who have a first-degree T1D relative are being studied and followed up.

BABY-DIAB: BABY-DIAB is (Roll et al. 1996) a prospective study conducted from birth among children of mothers with T1D or gestational diabetes or fathers with T1D to investigate the temporal sequence of antibody responses to islet cells (ICA), insulin (IAA), GAD65 (GADA), and the protein tyrosine phosphatase IA-2/ICA512 (IA-2A). A total of 78.6 % of children (17,055 out of 21,700) born in the southeast of Sweden were entered in the *ABIS* (All Babies in Southeast Sweden) study with an aim to study environmental factors affecting the development of immune-mediated diseases in children, with special focus on T1D (Ludvigsson et al. 2002).

DIPP: The DIabetes Prediction and Prevention Project was launched in 1994 in Finland. In the study, general population newborns are screened for increased genetic risk for T1D in the University Hospitals of Turku, Tampere, and Oulu.

BABYDIET: BABYDIET study (Schmid et al. 2004) is a primary prevention trial in Germany initiated to investigate whether delay of the introduction of dietary gluten can prevent the development of islet autoimmunity in newborns with a first-degree relative with T1D, who are at genetically high risk of T1D. However, the result showed that delaying gluten exposure until the age of 12 months is safe but does not substantially reduce the risk for islet autoimmunity in genetically at-risk children (Hummel et al. 2011).

TIRGR: The Trial to Reduce IDDM in the Genetically at Risk (TRIGR) study is an international randomized double-blind controlled intervention trial that was designed to establish whether weaning to a highly hydrolyzed formula in infancy reduces the risk of T1D later in childhood (Group 2007). A recent report from the study showed that dietary intervention using casein hydrolysate formula had a long-lasting effect on markers of β-cell autoimmunity (Knip et al. 2010). And the difference in fecal microbiota composition between children with β-cell autoimmunity and those without has been found (de Goffau et al. 2013). Further trial (NCT01735123) is ongoing to investigate whether extensively hydrolyzed casein formula is able to protect children at risk for type 1 diabetes.

Future Directions

Intervention/prevention of β-cell destruction in T1D is the final goal resulting in good metabolic control of blood glucose. Balancing the risks and benefits in intervention/prevention of T1D is very complicated. Individual response to a particular therapy might differ. Biomarkers which can identify individuals who would or would not respond to a particular therapy are the need of the hour. T1D is associated with end-organ complications. The number of adverse events in an individual undergoing a particular therapy might differ from another, depending upon the time and intensity of progress to end-organ complications. Therefore, identification of those at risk becomes important while considering therapy.

Disease diagnosis is another important factor. T1D is usually diagnosed when the existing β-cells fail to meet the insulin needs of the body and thereby insufficient metabolic control. Earlier identification of existing autoimmunity is very crucial.

Research in the past few decades has highlighted many ways in which this can be achieved. Several promising candidates (such as alum-formulated GAD65 and anti-CD3 antibodies) have also reached different stages of clinical trials. Alum-formulated GAD65 was tested in several phase III clinical trials, including a trial in nine countries in Europe apart from Norway and a 4-year follow-up in Sweden (www.diamyd.com). It is interesting to note that although alum-formulated GAD65 and anti-CD3 seem to show similar efficacy the alum-formulated GAD65 product has not been associated with any relevant side effects and moreover it is easy to administer. Considering the complex etiology of the disease, involving several

susceptibility factors and immune cells, it is possible that multi-therapy, involving more than one therapeutic agent, may be of advantage. With the increasing insights into the etiology of the disease, more and more targets are being identified for prevention/intervention.

Strategies on Islet Expansion

Nutrient ingestion stimulates the gastrointestinal tract to secrete incretin hormones to enhance glucose-dependent insulin secretion, thereby maintaining glucose homeostasis. The success of several therapies in reversing islet cell autoimmunity has led to the search of agents that enhance β-cell preservation or restoration. The safety of the combined usage of the above strategies was under investigation (NCT00873925, NCT00064714) which might give exciting results in the near future.

Glucagon-like peptide-1 (GLP-1) is a gut hormone secreted from the intestinal L cells. GLP-1 has a very short circulating half-life due to rapid inactivation by the enzyme dipeptidyl peptidase IV (DPP-4). Since GLP-1 has been well identified as an insulin stimulator and glucagon inhibitor, both GLP-1 and DPP-4 inhibitor were widely tested in the treatment for T2D (Mari et al. 2005; Mu et al. 2006; Duttaroy et al. 2011). It was recently found that GLP-1 peptide could not only induce β-cell proliferation and neogenesis but also suppress β-cell apoptosis and delay the onset of T1D in mouse model (Hadjiyanni and Drucker 2007; Zhang et al. 2007; Hadjiyanni et al. 2008; Xue et al. 2010). Meanwhile, clinical trials showed that GLP-1 improve glucose control in T1D patients (Behme et al. 2003). Sitagliptin (a DPP-4 inhibitor) improved glucose control in T1D patients (Ellis et al. 2011). There are several ongoing trials investigating GLP-1 agonist (NCT01722227, NCT01722240 and NCT01879917), DPP-4 inhibitor (NCT00813228, NCT01159847, NCT01099618 and NCT01559025), and the co-application of both GLP-1 agonist and DPP-4 inhibitor (NCT01782261). The effect of GLP-1 in T1D might soon be revealed in the near future.

Islet Regeneration

When the treatment aimed to suppress autoimmunity is developing rapidly, treatment aimed to induce islet/β-cell regeneration is also under way. Currently, there are several stem cell therapies under different stages of investigation. An ongoing trial (NCT00465478) is using autologous bone marrow stem cell transplantation to stimulate islet stem cell regeneration. Similarly, another trial (NCT00703599) using autologous adipose-derived stem cells to stimulate islet stem cell regeneration is also under investigation. The combination of stem cell therapies is also interesting, a current trial (NCT01143168) combining bone marrow mononuclear cells and umbilical cord MSC for treating T1D patients is also ongoing.

Probiotic Approach

Identification of the role of environmental agents (viruses and more recently bacteria) and their potential use as therapeutics throws open a vast range of possibilities. Use of food supplements or even a probiotic yoghurt containing "friendly bacteria" in prevention of autoimmune diabetes has been suggested. The idea seems farfetched but considering the influx of information on the disease etiology, it is not completely impractical; however, such concepts should be approached with extreme caution. In conclusion, therapies aiming at preserving/preventing β-cell function should aim at providing safe, long-term, and clinically relevant improvements over standard insulin therapy.

Promising Therapies

- *Alum-formulated GAD65:* Specific modulation of long-lasting immune response to β-cells GAD65
- *Anti-CD3 antibodies:* Prevention of β-cell destruction by depletion of T cells
- *Anti-CD20 antibodies:* Prevention of β-cell destruction by depletion of B cells
- *DiaPep277:* Immunomodulation and shift from Th1 response to a Th2 response
- *Cell/stem cell therapies:* Reintroduce immune tolerance and induce islet regeneration

Key Points

- Therapeutic interventions can be beneficial to individuals identified at risk and to individuals with existing autoimmunity to prevent the damage to residual β-cells.
- Modern therapies aimed at reducing β-cell autoimmunity should ideally be short-term treatment which can induce long-lasting "tolerance," but does not debilitate the capacity of the immune system to fight pathogens.
- Successful intervention using autoantigen-specific therapies like alum-formulated GAD65 is the need of the hour.
- Combinatorial therapies can be very helpful in β-cell regeneration and arresting aggressive β-cell autoimmunity.
- The correct timing of immunomodulating therapies could be very important.
- New approaches such as DNA vaccines can be beneficial, but should be approached with caution.

Cross-References

► Generating Pancreatic Endocrine Cells from Pluripotent Stem Cells
► Human Islet Autotransplantation

▶ Immunology of β-Cell Destruction
▶ Inflammatory Pathways Linked to β Cell Demise in Diabetes
▶ Islet Encapsulation
▶ Islet Xenotransplantation: Recent Advances and Future Prospects
▶ Stem Cells in Pancreatic Islets
▶ Successes and Disappointments with Clinical Islet Transplantation

References

Achenbach P, Barker J, Bonifacio E (2008) Modulating the natural history of type 1 diabetes in children at high genetic risk by mucosal insulin immunization. Curr Diab Rep 8(2):87–93

Agardh CD, Cilio CM, Lethagen A, Lynch K, Leslie RD, Palmer M, Harris RA, Robertson JA, Lernmark A (2005) Clinical evidence for the safety of GAD65 immunomodulation in adult-onset autoimmune diabetes. J Diabetes Complications 19(4):238–246

Allen HF, Klingensmith GJ, Jensen P, Simoes E, Hayward A, Chase HP (1999) Effect of Bacillus Calmette-Guerin vaccination on new-onset type 1 diabetes. A randomized clinical study. Diabetes Care 22(10):1703–1707

Arif S, Cox P, Afzali B, Lombardi G, Lechler RI, Peakman M, Mirenda V (2010) Anti-TNFα therapy–killing two birds with one stone? Lancet 375(9733):2278

Barker JM, McFann KK, Orban T (2007) Effect of oral insulin on insulin autoantibody levels in the Diabetes Prevention Trial Type 1 oral insulin study. Diabetologia 50(8):1603–1606

Behme MT, Dupre J, Stiller CR (1988) Effect of cyclosporine on insulin binding to erythrocytes in type 1 diabetes mellitus of recent onset. Clin Invest Med 11(2):113–122

Behme MT, Dupre J, McDonald TJ (2003) Glucagon-like peptide 1 improved glycemic control in type 1 diabetes. BMC Endocr Disord 3(1):3

Belghith M, Bluestone JA, Barriot S, Megret J, Bach JF, Chatenoud L (2003) TGF-β-dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes. Nat Med 9(9):1202–1208

Bell GI, Broughton HC, Levac KD, Allan DA, Xenocostas A, Hess DA (2012) Transplanted human bone marrow progenitor subtypes stimulate endogenous islet regeneration and revascularization. Stem Cells Dev 21(1):97–109

Bergerot I, Ploix C, Petersen J, Moulin V, Rask C, Fabien N, Lindblad M, Mayer A, Czerkinsky C, Holmgren J, Thivolet C (1997) A cholera toxoid-insulin conjugate as an oral vaccine against spontaneous autoimmune diabetes. Proc Natl Acad Sci USA 94(9):4610–4614

Bizzarri C, Pitocco D, Napoli N, Di Stasio E, Maggi D, Manfrini S, Suraci C, Cavallo MG, Cappa M, Ghirlanda G, Pozzilli P, Group I (2010) No protective effect of calcitriol on β-cell function in recent-onset type 1 diabetes: the IMDIAB XIII trial. Diabetes Care 33(9):1962–1963

Bjork E, Berne C, Kampe O, Wibell L, Oskarsson P, Karlsson FA (1996) Diazoxide treatment at onset preserves residual insulin secretion in adults with autoimmune diabetes. Diabetes 45 (10):1427–1430

Bluestone JA, St Clair EW, Turka LA (2006) CTLA4Ig: bridging the basic immunology with clinical application. Immunity 24(3):233–238

Bolt S, Routledge E, Lloyd I, Chatenoud L, Pope H, Gorman SD, Clark M, Waldmann H (1993) The generation of a humanized, non-mitogenic CD3 monoclonal antibody which retains in vitro immunosuppressive properties. Eur J Immunol 23(2):403–411

Bougneres PF, Carel JC, Castano L, Boitard C, Gardin JP, Landais P, Hors J, Mihatsch MJ, Paillard M, Chaussain JL et al (1988) Factors associated with early remission of type I diabetes in children treated with cyclosporine. N Engl J Med 318(11):663–670

Brod SA, Burns DK (1994) Suppression of relapsing experimental autoimmune encephalomyelitis in the SJL/J mouse by oral administration of type I interferons. Neurology 44(6):1144–1148

Brod SA, Kerman RH, Nelson LD, Marshall GD Jr, Henninger EM, Khan M, Jin R, Wolinsky JS (1997) Ingested IFN-α has biological effects in humans with relapsing-remitting multiple sclerosis. Mult Scler 3(1):1–7

Brodie GM, Wallberg M, Santamaria P, Wong FS, Green EA (2008) β-cells promote intra-islet $CD8^+$ cytotoxic T-cell survival to enhance type 1 diabetes. Diabetes 57(4):909–917

Burt RK, Burns W, Ruvolo P, Fischer A, Shiao C, Guimaraes A, Barrett J, Hess A (1995) Syngeneic bone marrow transplantation eliminates V β 8.2 T lymphocytes from the spinal cord of Lewis rats with experimental allergic encephalomyelitis. J Neurosci Res 41(4):526–531

Buzzetti R, Cernea S, Petrone A, Capizzi M, Spoletini M, Zampetti S, Guglielmi C, Venditti C, Pozzilli P, Grp DT (2011) C-peptide response and HLA genotypes in subjects with recent-onset type 1 diabetes after immunotherapy with DiaPep277 an exploratory study. Diabetes 60 (11):3067–3072

Carpenter PA, Pavlovic S, Tso JY, Press OW, Gooley T, Yu XZ, Anasetti C (2000) Non-Fc receptor-binding humanized anti-CD3 antibodies induce apoptosis of activated human T cells. J Immunol 165(11):6205–6213

Chatenoud L, Thervet E, Primo J, Bach JF (1994) Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. Proc Natl Acad Sci USA 91(1):123–127

Chatenoud L, Primo J, Bach JF (1997) CD3 antibody-induced dominant self tolerance in overtly diabetic NOD mice. J Immunol 158(6):2947–2954

Cook JJ, Hudson I, Harrison LC, Dean B, Colman PG, Werther GA, Warne GL, Court JM (1989) Double-blind controlled trial of azathioprine in children with newly diagnosed type I diabetes. Diabetes 38(6):779–783

Couri CE, Oliveira MC, Stracieri AB, Moraes DA, Pieroni F, Barros GM, Madeira MI, Malmegrim KC, Foss-Freitas MC, Simoes BP, Martinez EZ, Foss MC, Burt RK, Voltarelli JC (2009) C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA 301(15):1573–1579

da Silva AJ, Brickelmaier M, Majeau GR, Li Z, Su L, Hsu YM, Hochman PS (2002) Alefacept, an immunomodulatory recombinant LFA-3/IgG1 fusion protein, induces CD16 signaling and CD2/CD16-dependent apoptosis of $CD2^+$ cells. J Immunol 168(9):4462–4471

de Goffau MC, Luopajarvi K, Knip M, Ilonen J, Ruohtula T, Harkonen T, Orivuori L, Hakala S, Welling GW, Harmsen HJ, Vaarala O (2013) Fecal microbiota composition differs between children with β-cell autoimmunity and those without. Diabetes 62(4):1238–1244

Diabetes Prevention Trial–Type 1 Diabetes Study Group (2002) Effects of insulin in relatives of patients with type 1 diabetes mellitus. N Engl J Med 346(22):1685–1691

Dinarello CA, Simon A, van der Meer JW (2012) Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nat Rev Drug Discov 11(8):633–652

Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J, Lydon NB (1996) Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 2(5):561–566

Duttaroy A, Voelker F, Merriam K, Zhang X, Ren X, Subramanian K, Hughes TE, Burkey BF (2011) The DPP-4 inhibitor vildagliptin increases pancreatic β cell mass in neonatal rats. Eur J Pharmacol 650(2–3):703–707

Eisenbarth GS, Srikanta S, Jackson R, Rabinowe S, Dolinar R, Aoki T, Morris MA (1985) Anti-thymocyte globulin and prednisone immunotherapy of recent onset type 1 diabetes mellitus. Diabetes Res 2(6):271–276

Elias D, Markovits D, Reshef T, van der Zee R, Cohen IR (1990) Induction and therapy of autoimmune diabetes in the non-obese diabetic (NOD/Lt) mouse by a 65-kDa heat shock protein. Proc Natl Acad Sci USA 87(4):1576–1580

Elias D, Avron A, Tamir M, Raz I (2006) DiaPep277 preserves endogenous insulin production by immunomodulation in type 1 diabetes. Ann N Y Acad Sci 1079:340–344

Elliott RB, Crossley JR, Berryman CC, James AG (1981) Partial preservation of pancreatic β-cell function in children with diabetes. Lancet 2(8247):631–632

Ellis CN, Krueger GG (2001) Treatment of chronic plaque psoriasis by selective targeting of memory effector T lymphocytes. N Engl J Med 345(4):248–255

Ellis SL, Moser EG, Snell-Bergeon JK, Rodionova AS, Hazenfield RM, Garg SK (2011) Effect of sitagliptin on glucose control in adult patients with type 1 diabetes: a pilot, double-blind, randomized, crossover trial. Diabet Med 28(10):1176–1181

Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JW, Cannon JG, Rogers TS, Klempner MS, Weber PC et al (1989) The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Engl J Med 320(5):265–271

Fiorina P, Jurewicz M, Augello A, Vergani A, Dada S, La Rosa S, Selig M, Godwin J, Law K, Placidi C, Smith RN, Capella C, Rodig S, Adra CN, Atkinson M, Sayegh MH, Abdi R (2009) Immunomodulatory function of bone marrow-derived mesenchymal stem cells in experimental autoimmune type 1 diabetes. J Immunol 183(2):993–1004

Fruchtman S (2003) Stem cell transplantation. Mt Sinai J Med 70(3):166–170

Gabbay MA, Sato MN, Finazzo C, Duarte AJ, Dib SA (2012) Effect of cholecalciferol as adjunctive therapy with insulin on protective immunologic profile and decline of residual β-cell function in new-onset type 1 diabetes mellitus. Arch Pediatr Adolesc Med 166(7): 601–607

Gale EA, Bingley PJ, Emmett CL, Collier T (2004) European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes. Lancet 363(9413):925–931

Gatto NM, Koralek DO, Bracken MB, Duggan WT, Lem J, Klioze SS, Jackson NC (2012) Comparative lung cancer mortality with inhaled insulin or comparator: an observational follow-up study of patients previously enrolled in exubera controlled clinical trials (FUSE). Pharmacoepidemiol Drug Saf 21:26

Godfrey WR, Spoden DJ, Ge YG, Baker SR, Liu B, Levine BL, June CH, Blazar BR, Porter SB (2005) Cord blood $CD4^{+}CD25^{+}$-derived T regulatory cell lines express FoxP3 protein and manifest potent suppressor function. Blood 105(2):750–758

Gong Z, Jin Y, Zhang Y (2007) Suppression of diabetes in Non-Obese Diabetic (NOD) mice by oral administration of a cholera toxin B subunit-insulin B chain fusion protein vaccine produced in silkworm. Vaccine 25(8):1444–1451

Gottlieb PA, Quinlan S, Krause-Steinrauf H, Greenbaum CJ, Wilson DM, Rodriguez H, Schatz DA, Moran AM, Lachin JM, Skyler JS (2010) Failure to preserve β-cell function with mycophenolate mofetil and daclizumab combined therapy in patients with new- onset type 1 diabetes. Diabetes Care 33(4):826–832

Goudy KS, Wang B, Tisch R (2008) Gene gun-mediated DNA vaccination enhances antigen-specific immunotherapy at a late preclinical stage of type 1 diabetes in nonobese diabetic mice. Clin Immunol 129(1):49–57

Graham J, Kockum I, Sanjeevi CB, Landin-Olsson M, Nystrom L, Sundkvist G, Arnqvist H, Blohme G, Lithner F, Littorin B, Schersten B, Wibell L, Ostman J, Lernmark A, Breslow N, Dahlquist G (1999) Negative association between type 1 diabetes and HLA DQB1*0602-DQA1*0102 is attenuated with age at onset. Swedish Childhood Diabetes Study Group. Eur J Immunogenet 26(2–3):117–127

Green EA, Flavell RA (1999) Tumor necrosis factor-α and the progression of diabetes in non-obese diabetic mice. Immunol Rev 169:11–22

Group TS (2007) Study design of the Trial to Reduce IDDM in the Genetically at Risk (TRIGR). Pediatr Diabetes 8(3):117–137

Hackstein H, Morelli AE, Thomson AW (2001) Designer dendritic cells for tolerance induction: guided not misguided missiles. Trends Immunol 22(8):437–442

Hadjiyanni I, Drucker DJ (2007) Glucagon-like peptide 1 and type 1 diabetes: NOD ready for prime time? Endocrinology 148(11):5133–5135

Hadjiyanni I, Baggio LL, Poussier P, Drucker DJ (2008) Exendin-4 modulates diabetes onset in nonobese diabetic mice. Endocrinology 149(3):1338–1349

Haller MJ, Wasserfall CH, McGrail KM, Cintron M, Brusko TM, Wingard JR, Kelly SS, Shuster JJ, Atkinson MA, Schatz DA (2009) Autologous umbilical cord blood transfusion in very young children with type 1 diabetes. Diabetes Care 32(11):2041–2046

Hao L, Calcinaro F, Gill RG, Eugui EM, Allison AC, Lafferty KJ (1992) Facilitation of specific tolerance induction in adult mice by RS-61443. Transplantation 53(3):590–595

Hao L, Chan SM, Lafferty KJ (1993) Mycophenolate mofetil can prevent the development of diabetes in BB rats. Ann N Y Acad Sci 696:328–332

Harnaha J, Machen J, Wright M, Lakomy R, Styche A, Trucco M, Makaroun S, Giannoukakis N (2006) Interleukin-7 is a survival factor for $CD4^+$ $CD25^+$ T-cells and is expressed by diabetes-suppressive dendritic cells. Diabetes 55(1):158–170

Harrison LC, Colman PG, Dean B, Baxter R, Martin FI (1985) Increase in remission rate in newly diagnosed type I diabetic subjects treated with azathioprine. Diabetes 34(12):1306–1308

Held W, MacDonald HR, Weissman IL, Hess MW, Mueller C (1990) Genes encoding tumor necrosis factor α and granzyme A are expressed during development of autoimmune diabetes. Proc Natl Acad Sci USA 87(6):2239–2243

Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, Gitelman SE, Harlan DM, Xu D, Zivin RA, Bluestone JA (2002) Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. N Engl J Med 346(22):1692–1698

Herold KC, Gitelman SE, Masharani U, Hagopian W, Bisikirska B, Donaldson D, Rother K, Diamond B, Harlan DM, Bluestone JA (2005) A single course of anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. Diabetes 54(6):1763–1769

Herold KC, Gitelman S, Greenbaum C, Puck J, Hagopian W, Gottlieb P, Sayre P, Bianchine P, Wong E, Seyfert-Margolis V, Bourcier K, Bluestone JA, I. T. N. A. I. S. G. Immune Tolerance Network (2009) Treatment of patients with new onset Type 1 diabetes with a single course of anti-CD3 mAb Teplizumab preserves insulin production for up to 5 years. Clin Immunol 132 (2):166–173

Herrera PL, Harlan DM, Vassalli P (2000) A mouse CD8 T cell-mediated acute autoimmune diabetes independent of the perforin and Fas cytotoxic pathways: possible role of membrane TNF. Proc Natl Acad Sci USA 97(1):279–284

Hirsch R, Eckhaus M, Auchincloss H Jr, Sachs DH, Bluestone JA (1988) Effects of in vivo administration of anti-T3 monoclonal antibody on T cell function in mice. I. Immunosuppression of transplantation responses. J Immunol 140(11):3766–3772

Hu CY, Rodriguez-Pinto D, Du W, Ahuja A, Henegariu O, Wong FS, Shlomchik MJ, Wen L (2007) Treatment with CD20-specific antibody prevents and reverses autoimmune diabetes in mice. J Clin Invest 117(12):3857–3867

Hulme MA, Wasserfall CH, Atkinson MA, Brusko TM (2012) Central role for interleukin-2 in type 1 diabetes. Diabetes 61(1):14–22

Hummel S, Pfluger M, Hummel M, Bonifacio E, Ziegler AG (2011) Primary dietary intervention study to reduce the risk of islet autoimmunity in children at increased risk for type 1 diabetes: the BABYDIET study. Diabetes Care 34(6):1301–1305

Hundal RS, Petersen KF, Mayerson AB, Randhawa PS, Inzucchi S, Shoelson SE, Shulman GI (2002) Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. J Clin Invest 109(10):1321–1326

Huppmann M, Baumgarten A, Ziegler AG, Bonifacio E (2005) Neonatal Bacille Calmette-Guerin vaccination and type 1 diabetes. Diabetes Care 28(5):1204–1206

Karussis D, Vourka-Karussis U, Mizrachi-Koll R, Abramsky O (1999) Acute/relapsing experimental autoimmune encephalomyelitis: induction of long lasting, antigen-specific tolerance by syngeneic bone marrow transplantation. Mult Scler 5(1):17–21

Kaufman DL, Clare-Salzler M, Tian J, Forsthuber T, Ting GS, Robinson P, Atkinson MA, Sercarz EE, Tobin AJ, Lehmann PV (1993) Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. Nature 366(6450):69–72

Keymeulen B, Vandemeulebroucke E, Ziegler AG, Mathieu C, Kaufman L, Hale G, Gorus F, Goldman M, Walter M, Candon S, Schandene L, Crenier L, De Block C, Seigneurin JM, De Pauw P, Pierard D, Weets I, Rebello P, Bird P, Berrie E, Frewin M, Waldmann H, Bach JF, Pipeleers D, Chatenoud L (2005) Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. N Engl J Med 352(25):2598–2608

Keymeulen B, Walter M, Mathieu C, Kaufman L, Gorus F, Hilbrands R, Vandemeulebroucke E, Van de Velde U, Crenier L, De Block C, Candon S, Waldmann H, Ziegler AG, Chatenoud L, Pipeleers D (2010) Four-year metabolic outcome of a randomised controlled CD3-antibody trial in recent-onset type 1 diabetic patients depends on their age and baseline residual β cell mass. Diabetologia 53(4):614–623

Knip M, Virtanen SM, Seppa K, Ilonen J, Savilahti E, Vaarala O, Reunanen A, Teramo K, Hamalainen AM, Paronen J, Dosch HM, Hakulinen T, Akerblom HK, Finnish TSG (2010) Dietary intervention in infancy and later signs of β-cell autoimmunity. N Engl J Med 363 (20):1900–1908

Koivisto VA, Aro A, Cantell K, Haataja M, Huttunen J, Karonen SL, Mustajoki P, Pelkonen R, Seppala P (1984) Remissions in newly diagnosed type 1 (insulin-dependent) diabetes: influence of interferon as an adjunct to insulin therapy. Diabetologia 27(2):193–197

Koulmanda M, Bhasin M, Hoffman L, Fan Z, Qipo A, Shi H, Bonner-Weir S, Putheti P, Degauque N, Libermann TA, Auchincloss H Jr, Flier JS, Strom TB (2008) Curative and β cell regenerative effects of α1-antitrypsin treatment in autoimmune diabetic NOD mice. Proc Natl Acad Sci USA 105(42):16242–16247

Kroger N, Kruger W, Wacker-Backhaus G, Hegewisch-Becker S, Stockschlader M, Fuchs N, Russmann B, Renges H, Durken M, Bielack S, de Wit M, Schuch G, Bartels H, Braumann D, Kuse R, Kabisch H, Erttmann R, Zander AR (1998) Intensified conditioning regimen in bone marrow transplantation for Philadelphia chromosome-positive acute lymphoblastic leukemia. Bone Marrow Transplant 22(11):1029–1033

LaFace DM, Peck AB (1989) Reciprocal allogeneic bone marrow transplantation between NOD mice and diabetes-nonsusceptible mice associated with transfer and prevention of autoimmune diabetes. Diabetes 38(7):894–901

Lazar L, Ofan R, Weintrob N, Avron A, Tamir M, Elias D, Phillip M, Josefsberg Z (2007) Heat-shock protein peptide DiaPep277 treatment in children with newly diagnosed type 1 diabetes: a randomised, double-blind phase II study. Diabetes Metab Res Rev 23(4):286–291

Lenschow DJ, Zeng Y, Thistlethwaite JR, Montag A, Brady W, Gibson MG, Linsley PS, Bluestone JA (1992) Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4lg. Science 257(5071):789–792

Lenschow DJ, Ho SC, Sattar H, Rhee L, Gray G, Nabavi N, Herold KC, Bluestone JA (1995) Differential effects of anti-B7-1 and anti-B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse. J Exp Med 181(3):1145–1155

Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA (2006) Transforming growth factor-β regulation of immune responses. Annu Rev Immunol 24:99–146

Ligumsky M, Lysy J, Siguencia G, Friedlander Y (2001) Effect of long-term, continuous versus alternate-day omeprazole therapy on serum gastrin in patients treated for reflux esophagitis. J Clin Gastroenterol 33(1):32–35

Loechelt BJ, Boulware D, Green M, Baden LR, Gottlieb P, Krause-Steinrauf H, Weinberg A, G. Type 1 Diabetes TrialNet Daclizumab/Mycophenolic Acid Study (2013) Epstein-Barr and other herpesvirus infections in patients with early onset type 1 diabetes treated with daclizumab and mycophenolate mofetil. Clin Infect Dis 56(2):248–254

Louvet C, Szot GL, Lang J, Lee MR, Martinier N, Bollag G, Zhu S, Weiss A, Bluestone JA (2008) Tyrosine kinase inhibitors reverse type 1 diabetes in nonobese diabetic mice. Proc Natl Acad Sci USA 105(48):18895–18900

Ludvigsson J, Gustafsson-Stolt U, Liss PE, Svensson T (2002) Mothers of children in ABIS, a population-based screening for prediabetes, experience few ethical conflicts and have a positive attitude. Ann N Y Acad Sci 958:376–381

Ludvigsson J, Faresjo M, Hjorth M, Axelsson S, Cheramy M, Pihl M, Vaarala O, Forsander G, Ivarsson S, Johansson C, Lindh A, Nilsson NO, Aman J, Ortqvist E, Zerhouni P, Casas R (2008) GAD treatment and insulin secretion in recent-onset type 1 diabetes. N Engl J Med 359 (18):1909–1920

Ludvigsson J, Hjorth M, Cheramy M, Axelsson S, Pihl M, Forsander G, Nilsson NO, Samuelsson BO, Wood T, Aman J, Ortqvist E, Casas R (2011) Extended evaluation of the safety and efficacy of GAD treatment of children and adolescents with recent-onset type 1 diabetes: a randomised controlled trial. Diabetologia 54(3):634–640

Luopajarvi K, Savilahti E, Virtanen SM, Ilonen J, Knip M, Akerblom HK, Vaarala O (2008) Enhanced levels of cow's milk antibodies in infancy in children who develop type 1 diabetes later in childhood. Pediatr Diabetes 9(5):434–441

Machen J, Harnaha J, Lakomy R, Styche A, Trucco M, Giannoukakis N (2004) Antisense oligonucleotides down-regulating costimulation confer diabetes-preventive properties to nonobese diabetic mouse dendritic cells. J Immunol 173(7):4331–4341

Madec AM, Mallone R, Afonso G, Abou Mrad E, Mesnier A, Eljaafari A, Thivolet C (2009) Mesenchymal stem cells protect NOD mice from diabetes by inducing regulatory T cells. Diabetologia 52(7):1391–1399

Mandrup-Poulsen T, Bendtzen K, Dinarello CA, Nerup J (1987) Human tumor necrosis factor potentiates human interleukin 1-mediated rat pancreatic β-cell cytotoxicity. J Immunol 139 (12):4077–4082

Mandrup-Poulsen T, Pickersgill L, Donath MY (2010) Blockade of interleukin 1 in type 1 diabetes mellitus. Nat Rev Endocrinol 6(3):158–166

Mari A, Sallas WM, He YL, Watson C, Ligueros-Saylan M, Dunning BE, Deacon CF, Holst JJ, Foley JE (2005) Vildagliptin, a dipeptidyl peptidase-IV inhibitor, improves model-assessed β-cell function in patients with type 2 diabetes. J Clin Endocrinol Metab 90(8):4888–4894

Martin F, Chan AC (2006) B cell immunobiology in disease: evolving concepts from the clinic. Annu Rev Immunol 24:467–496

Mastrandrea L, Yu J, Behrens T, Buchlis J, Albini C, Fourtner S, Quattrin T (2009) Etanercept treatment in children with new-onset type 1 diabetes: pilot randomized, placebo-controlled, double-blind study. Diabetes Care 32(7):1244–1249

Mathieu C, Waer M, Laureys J, Rutgeerts O, Bouillon R (1994) Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D3. Diabetologia 37(6):552–558

McCarey DW, McInnes IB, Madhok R, Hampson R, Scherbakov O, Ford I, Capell HA, Sattar N (2004) Trial of Atorvastatin in Rheumatoid Arthritis (TARA): double-blind, randomised placebo-controlled trial. Lancet 363(9426):2015–2021

Moran A, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Greenbaum CJ, Herold KC, Marks JB, Raskin P, Sanda S, Schatz D, Wherrett DK, Wilson DM, Krischer JP, Skyler JS, G. Type 1 Diabetes TrialNet Canakinumab Study, Pickersgill L, de Koning E, Ziegler AG, Boehm B, Badenhoop K, Schloot N, Bak JF, Pozzilli P, Mauricio D, Donath MY, Castano L, Wagner A, Lervang HH, Perrild H, Mandrup-Poulsen T, Group AS, Pociot F, Dinarello CA (2013) Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. Lancet 381(9881):1905–1915

Mu J, Woods J, Zhou YP, Roy RS, Li Z, Zycband E, Feng Y, Zhu L, Li C, Howard AD, Moller DE, Thornberry NA, Zhang BB (2006) Chronic inhibition of dipeptidyl peptidase-4 with a sitagliptin analog preserves pancreatic β-cell mass and function in a rodent model of type 2 diabetes. Diabetes 55(6):1695–1704

Nanto-Salonen K, Kupila A, Simell S, Siljander H, Salonsaari T, Hekkala A, Korhonen S, Erkkola R, Sipila JI, Haavisto L, Siltala M, Tuominen J, Hakalax J, Hyoty H, Ilonen J, Veijola R, Simell T, Knip M, Simell O (2008) Nasal insulin to prevent type 1 diabetes in children with HLA genotypes and autoantibodies conferring increased risk of disease: a double-blind, randomised controlled trial. Lancet 372(9651):1746–1755

Orban T, Farkas K, Jalahej H, Kis J, Treszl A, Falk B, Reijonen H, Wolfsdorf J, Ricker A, Matthews JB, Tchao N, Sayre P, Bianchine P (2010) Autoantigen-specific regulatory T cells

induced in patients with type 1 diabetes mellitus by insulin B-chain immunotherapy. J Autoimmun 34(4):408–415

Orban T, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Gottlieb PA, Greenbaum CJ, Marks JB, Monzavi R, Moran A, Raskin P, Rodriguez H, Russell WE, Schatz D, Wherrett D, Wilson DM, Krischer JP, Skyler JS, G. Type 1 Diabetes TrialNet Abatacept Study (2011) Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. Lancet 378(9789):412–419

Ortqvist E, Bjork E, Wallensteen M, Ludvigsson J, Aman J, Johansson C, Forsander G, Lindgren F, Berglund L, Bengtsson M, Berne C, Persson B, Karlsson FA (2004) Temporary preservation of β-cell function by diazoxide treatment in childhood type 1 diabetes. Diabetes Care 27(9):2191–2197

Ozeri E, Mizrahi M, Shahaf G, Lewis EC (2012) α-1 antitrypsin promotes semimature, IL-10-producing and readily migrating tolerogenic dendritic cells. J Immunol 189(1):146–153

Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, Paquette TL (1983) Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science 222 (4630):1337–1339

Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, Becker DJ, Gitelman SE, Goland R, Gottlieb PA, Marks JB, McGee PF, Moran AM, Raskin P, Rodriguez H, Schatz DA, Wherrett D, Wilson DM, Lachin JM, Skyler JS, C. D. S. G. Type 1 Diabetes TrialNet Anti (2009) Rituximab, B-lymphocyte depletion, and preservation of β-cell function. N Engl J Med 361(22):2143–2152

Phillips B, Nylander K, Harnaha J, Machen J, Lakomy R, Styche A, Gillis K, Brown L, Lafreniere D, Gallo M, Knox J, Hogeland K, Trucco M, Giannoukakis N (2008) A microsphere-based vaccine prevents and reverses new-onset autoimmune diabetes. Diabetes 57(6):1544–1555

Prud'homme GJ (2003) Prevention of autoimmune diabetes by DNA vaccination. Expert Rev Vaccines 2(4):533–540

Putnam AL, Brusko TM, Lee MR, Liu W, Szot GL, Ghosh T, Atkinson MA, Bluestone JA (2009) Expansion of human regulatory T-cells from patients with type 1 diabetes. Diabetes 58(3):652–662

Radtke MA, Nermoen I, Kollind M, Skeie S, Sorheim JI, Svartberg J, Hals I, Moen T, Dorflinger GH, Grill V (2010) Six months of diazoxide treatment at bedtime in newly diagnosed subjects with type 1 diabetes does not influence parameters of β-cell function and autoimmunity but improves glycemic control. Diabetes Care 33(3):589–594

Rastetter W, Molina A, White CA (2004) Rituximab: expanding role in therapy for lymphomas and autoimmune diseases. Annu Rev Med 55:477–503

Raz I, Elias D, Avron A, Tamir M, Metzger M, Cohen IR (2001) β-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. Lancet 358(9295):1749–1753

Roll U, Christie MR, Fuchtenbusch M, Payton MA, Hawkes CJ, Ziegler AG (1996) Perinatal autoimmunity in offspring of diabetic parents. The German multicenter BABY-DIAB study: detection of humoral immune responses to islet antigens in early childhood. Diabetes 45 (7):967–973

Rother KI, Brown RJ, Morales MM, Wright E, Duan Z, Campbell C, Hardin DS, Popovic J, McEvoy RC, Harlan DM, Orlander PR, Brod SA (2009) Effect of ingested interferon-α on β-cell function in children with new-onset type 1 diabetes. Diabetes Care 32(7):1250–1255

Routledge EG, Falconer ME, Pope H, Lloyd IS, Waldmann H (1995) The effect of aglycosylation on the immunogenicity of a humanized therapeutic CD3 monoclonal antibody. Transplantation 60(8):847–853

Rutella S, Danese S, Leone G (2006) Tolerogenic dendritic cells: cytokine modulation comes of age. Blood 108(5):1435–1440

Sanjeevi CB, Lybrand TP, Landin-Olsson M, Kockum I, Dahlquist G, Hagopian WA, Palmer JP, Lernmark A (1994) Analysis of antibody markers, DRB1, DRB5, DQA1 and DQB1 genes and modeling of DR2 molecules in DR2-positive patients with insulin-dependent diabetes mellitus. Tissue Antigens 44(2):110–119

Sanjeevi CB, Landin-Olsson M, Kockum I, Dahlquist G, Lernmark A (1995a) Effects of the second HLA-DQ haplotype on the association with childhood insulin-dependent diabetes mellitus. Tissue Antigens 45(2):148–152

Sanjeevi CB, Lybrand TP, DeWeese C, Landin-Olsson M, Kockum I, Dahlquist G, Sundkvist G, Stenger D, Lernmark A (1995b) Polymorphic amino acid variations in HLA-DQ are associated with systematic physical property changes and occurrence of IDDM. Members of the Swedish Childhood Diabetes Study. Diabetes 44(1):125–131

Sanjeevi CB, Hook P, Landin-Olsson M, Kockum I, Dahlquist G, Lybrand TP, Lernmark A (1996) DR4 subtypes and their molecular properties in a population-based study of Swedish childhood diabetes. Tissue Antigens 47(4):275–283

Saudek F, Havrdova T, Boucek P, Karasova L, Novota P, Skibova J (2004) Polyclonal anti-T-cell therapy for type 1 diabetes mellitus of recent onset. Rev Diabet Stud 1(2):80–88

Schmid S, Buuck D, Knopff A, Bonifacio E, Ziegler AG (2004) BABY DIET, a feasibility study to prevent the appearance of islet autoantibodies in relatives of patients with Type 1 diabetes by delaying exposure to gluten. Diabetologia 47(6):1130–1131

Sherry NA, Chen W, Kushner JA, Glandt M, Tang Q, Tsai S, Santamaria P, Bluestone JA, Brillantes AM, Herold KC (2007) Exendin-4 improves reversal of diabetes in NOD mice treated with anti-CD3 monoclonal antibody by enhancing recovery of β-cells. Endocrinology 148(11):5136–5144

Silverstein J, Maclaren N, Riley W, Spillar R, Radjenovic D, Johnson S (1988) Immunosuppression with azathioprine and prednisone in recent-onset insulin-dependent diabetes mellitus. N Engl J Med 319(10):599–604

Skyler JS, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, Greenbaum C, Cuthbertson D, Rafkin-Mervis LE, Chase HP, Leschek E (2005) Effects of oral insulin in relatives of patients with type 1 diabetes: the Diabetes Prevention Trial–Type 1. Diabetes Care 28(5):1068–1076

Skyler JS, Greenbaum CJ, Lachin JM, Leschek E, Rafkin-Mervis L, Savage P, Spain L (2008) Type 1 Diabetes TrialNet–an international collaborative clinical trials network. Ann N Y Acad Sci 1150:14–24

Strom A, Kolb H, Martin S, Herder C, Simon MC, Koenig W, Heise T, Heinemann L, Roden M, Schloot NC, Group DS (2012) Improved preservation of residual β cell function by atorvastatin in patients with recent onset type 1 diabetes and high CRP levels (DIATOR trial). PLoS One 7 (3):e33108

Tan KC, Chow WS, Tam SC, Ai VH, Lam CH, Lam KS (2002) Atorvastatin lowers C-reactive protein and improves endothelium-dependent vasodilation in type 2 diabetes mellitus. J Clin Endocrinol Metab 87(2):563–568

Tang Q, Bluestone JA (2006) Regulatory T-cell physiology and application to treat autoimmunity. Immunol Rev 212:217–237

Tarbell KV, Yamazaki S, Steinman RM (2006) The interactions of dendritic cells with antigen-specific, regulatory T cells that suppress autoimmunity. Semin Immunol 18(2):93–102

TEDDY Study Group (2008) The Environmental Determinants of Diabetes in the Young (TEDDY) study. Ann N Y Acad Sci 1150:1–13

Teft WA, Kirchhof MG, Madrenas J (2006) A molecular perspective of CTLA-4 function. Annu Rev Immunol 24:65–97

Tisch R, Wang B, Weaver DJ, Liu B, Bui T, Arthos J, Serreze DV (2001) Antigen-specific mediated suppression of β cell autoimmunity by plasmid DNA vaccination. J Immunol 166 (3):2122–2132

TRIGR Study Group (2007) Study design of the Trial to Reduce IDDM in the Genetically at Risk (TRIGR). Pediatr Diabetes 8(3):117–137

Trube G, Rorsman P, Ohno-Shosaku T (1986) Opposite effects of tolbutamide and diazoxide on the ATP-dependent K^+ channel in mouse pancreatic β-cells. Pflugers Arch 407(5):493–499

Ugrasbul F, Moore WV, Tong PY, Kover KL (2008) Prevention of diabetes: effect of mycophenolate mofetil and anti-CD25 on onset of diabetes in the DRBB rat. Pediatr Diabetes 9(6):596–601

van de Ree MA, Huisman MV, Princen HM, Meinders AE, Kluft C (2003) Strong decrease of high sensitivity C-reactive protein with high-dose atorvastatin in patients with type 2 diabetes mellitus. Atherosclerosis 166(1):129–135

Vinson JA (2007) So many choices, so what's a consumer to do?: a commentary on effect of chromium niacinate and chromium picolinate supplementation on lipid peroxidation, TNF-α, IL-6, CRP, glycated hemoglobin, triglycerides, and cholesterol levels in blood of streptozotocin-treated diabetic rats. Free Radic Biol Med 43(8):1121–1123

Voltarelli JC, Couri CE, Stracieri AB, Oliveira MC, Moraes DA, Pieroni F, Coutinho M, Malmegrim KC, Foss-Freitas MC, Simoes BP, Foss MC, Squiers E, Burt RK (2007) Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA 297(14):1568–1576

Waeckerle-Men Y, Allmen EU, Gander B, Scandella E, Schlosser E, Schmidtke G, Merkle HP, Groettrup M (2006) Encapsulation of proteins and peptides into biodegradable poly(D, L-lactide-co-glycolide) microspheres prolongs and enhances antigen presentation by human dendritic cells. Vaccine 24(11):1847–1857

Walter M, Kaupper T, Adler K, Foersch J, Bonifacio E, Ziegler AG (2010) No effect of the 1α,25-dihydroxyvitamin D3 on β-cell residual function and insulin requirement in adults with new-onset type 1 diabetes. Diabetes Care 33(7):1443–1448

Wherrett DK, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Gottlieb PA, Greenbaum CJ, Herold KC, Marks JB, Monzavi R, Moran A, Orban T, Palmer JP, Raskin P, Rodriguez H, Schatz D, Wilson DM, Krischer JP, Skyler JS, T. D. T. G. S. G (2011) Antigen-based therapy with Glutamic Acid Decarboxylase (GAD) vaccine in patients with recent-onset type 1 diabetes: a randomised double-blind trial. Lancet 378(9788):319–327

Wolf AM, Wolf D, Rumpold H, Ludwiczek S, Enrich B, Gastl G, Weiss G, Tilg H (2005) The kinase inhibitor imatinib mesylate inhibits TNF-α production in vitro and prevents TNF-dependent acute hepatic inflammation. Proc Natl Acad Sci USA 102(38):13622–13627

Wong JT, Colvin RB (1991) Selective reduction and proliferation of the $CD4^+$ and $CD8^+$ T cell subsets with bispecific monoclonal antibodies: evidence for inter-T cell-mediated cytolysis. Clin Immunol Immunopathol 58(2):236–250

Wong FS, Wen L (2005) B cells in autoimmune diabetes. Rev Diabet Stud 2(3):121–135

Xiu Y, Wong CP, Bouaziz JD, Hamaguchi Y, Wang Y, Pop SM, Tisch RM, Tedder TF (2008) B lymphocyte depletion by CD20 monoclonal antibody prevents diabetes in nonobese diabetic mice despite isotype-specific differences in Fc gamma R effector functions. J Immunol 180 (5):2863–2875

Xue S, Wasserfall C, Parker M, McGrail S, McGrail K, Campbell-Thompson M, Schatz DA, Atkinson MA, Haller MJ (2010) Exendin-4 treatment of nonobese diabetic mice increases β-cell proliferation and fractional insulin reactive area. J Diabetes Complications 24(3):163–167

Yagi H, Soto-Gutierrez A, Parekkadan B, Kitagawa Y, Tompkins RG, Kobayashi N, Yarmush ML (2010) Mesenchymal stem cells: mechanisms of immunomodulation and homing. Cell Transplant 19(6):667–679

Yamada K, Nonaka K, Hanafusa T, Miyazaki A, Toyoshima H, Tarui S (1982) Preventive and therapeutic effects of large-dose nicotinamide injections on diabetes associated with insulitis. An observation in nonobese diabetic (NOD) mice. Diabetes 31(9):749–753

Yoshida M, Babensee JE (2006) Molecular aspects of microparticle phagocytosis by dendritic cells. J Biomater Sci Polym Ed 17(8):893–907

You S, Candon S, Kuhn C, Bach JF, Chatenoud L (2008) CD3 antibodies as unique tools to restore self-tolerance in established autoimmunity their mode of action and clinical application in type 1 diabetes. Adv Immunol 100:13–37

Zanone MM, Favaro E, Miceli I, Grassi G, Camussi E, Caorsi C, Amoroso A, Giovarelli M, Perin PC, Camussi G (2010) Human mesenchymal stem cells modulate cellular immune response to islet antigen glutamic acid decarboxylase in type 1 diabetes. J Clin Endocrinol Metab 95 (8):3788–3797

Zhang J, Tokui Y, Yamagata K, Kozawa J, Sayama K, Iwahashi H, Okita K, Miuchi M, Konya H, Hamaguchi T, Namba M, Shimomura I, Miyagawa JI (2007) Continuous stimulation of human glucagon-like peptide-1 (7–36) amide in a mouse model (NOD) delays onset of autoimmune type 1 diabetes. Diabetologia 50(9):1900–1909

Zhao Y, Huang Z, Qi M, Lazzarini P, Mazzone T (2007) Immune regulation of T lymphocyte by a newly characterized human umbilical cord blood stem cell. Immunol Lett 108(1):78–87

Zhao Y, Lin B, Darflinger R, Zhang Y, Holterman MJ, Skidgel RA (2009) Human cord blood stem cell-modulated regulatory T lymphocytes reverse the autoimmune-caused type 1 diabetes in nonobese diabetic (NOD) mice. PLoS One 4(1):e4226

Zhao Y, Jiang Z, Zhao T, Ye M, Hu C, Yin Z, Li H, Zhang Y, Diao Y, Li Y, Chen Y, Sun X, Fisk MB, Skidgel R, Holterman M, Prabhakar B, Mazzone T (2012) Reversal of type 1 diabetes via islet β cell regeneration following immune modulation by cord blood-derived multipotent stem cells. BMC Med 10:3

Ziegler AG, Elias D, Dagan S, Raz I, Grp D-AS (2010) DIA-AID 1-An international phase III clinical study to evaluate the biological effect of DiaPep277 (R) in preservation of β cell function in newly diagnosed T1D patients. Diabetologia 53:S190

Zollinger RM, Ellison EH (1955) Primary peptic ulcerations of the jejunum associated with islet cell tumors of the pancreas. Ann Surg 142(4):709–723, discussion, 724–8

In Vivo Biomarkers for Detection of β Cell Death

39

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Contents

Abstract

There is an immediate need for a noninvasive approach to detect β cell destruction during the initial stages of type I diabetes and late stages of type II diabetes in order to permit early preventative and interventional treatment strategies. The appearance of circulating biomarkers has been highly useful in diagnosing specific organ damage and early detection of some types of cancer. Hence, for nearly two decades, there have been efforts to identify analogous prognosticating factors in diabetes. To date, studies have identified several β cell-selective proteins which have recently been validated as biomarkers for detection of acute islet insulin cell death in vivo: glutamic acid decarboxylase 65 (GAD65), doublecortin (DCX), and protein phosphatase inhibitor 1 (PPP1R1A). More recently, the PCR detection of circulating genomic DNA originating from the β cell by its methylation fingerprint, as well as circulating β cell-specific microRNA, has offered more sensitive means to discern in vivo islet damage.

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_35,

This chapter surveys the current state of β cell biomarkers for real-time detection of cell death in vivo. While preclinical data suggests that we still have a long way to go, the successful translation of the biomarker approach to humans will revolutionize the way in which diabetes is treated.

Keywords
β cell mass • Streptozotocin • Islet transplantation • Necrosis • Apoptosis

Introduction

Both type 1 and type 2 diabetes mellitus are diseases of inadequate β cell mass. In type 1 diabetes (T1D), autoimmune T-cell-mediated destruction of islet β cells results in a progressive loss of β cell mass which presents itself clinically as hyperglycemia after >70 % of the insulin-secreting cells have been destroyed (Cnop et al. 2005; Eisenbarth 1986). In contrast, type 2 diabetic (T2D) patients suffer from insulin resistance that induces an initial compensatory expansion of β cell mass and hyperinsulinemia; subsequently as the disease progresses, hyperglycemia can be restrained in T2D by lifestyle modification (diet and exercise), insulin secretagogues (e.g., sulfonylureas, incretin mimetics) or insulin-sensitizing agents (e.g., biguanides, glitazones). Later in T2D, β cell apoptosis is also observed, and once patients become refractory to current pharmaceutical agents, they too become dependent upon injection of exogenous insulin to adequately control their blood glucose levels (Butler et al. 2003; Pan et al. 1997; Prentki and Nolan 2006; Tuomilehto et al. 2001).

The preclinical progression of both type 1 and type 2 diabetes may go undetected until hyperglycemia is diagnosed, and thus, a tool to monitor β cell mass in susceptible patient cohorts would permit interventional strategies at earlier time points to prevent ongoing β cell stress and injury (reviewed in the accompanying chapters: "► Clinical Approaches to Preserve β-Cell Function in Diabetes; "► Prevention of β-Cell Destruction in Autoimmune Diabetes: Current Approaches and Future Prospects"). Currently, our capabilities for selecting candidates for prevention or intervention trials are based upon genetic susceptibility (including diabetes in first degree relatives) and the presence of one or more islet autoantibodies (Hinke 2011). While the presence of islet-reactive antibodies in the circulation is highly predictive of diabetes development (Wenzlau et al. 2007; Winter and Schatz 2011), the degree of β cell damage at the point of autoantibody detection is unclear. Other classical biomarkers of β cell function are primarily metabolic parameters: glycated hemoglobin (HbA1C), fasting plasma glucose, oral glucose tolerance testing, circulating proinsulin or proinsulin:insulin ratio, and C-peptide (Lebastchi and Herold 2012; Neutzsky-Wulff et al. 2012). However, these are end-stage markers, giving little or no therapeutic window for prevention or reversal of disease progression.

Biomarkers released upon cell death or injury have been used to rapidly detect or assess tissue damage, for example, troponin I in myocardial ischemia (Babuin and

Jaffe 2005), prostate-specific antigen for prostate cancer (Makarov et al. 2009), and alanine aminotransferase in liver injury (Schomaker et al. 2013). Extensive effort has been made to elucidate similar candidate biomarkers in β cells to permit real-time detection of β cell destruction, and recently several novel candidates have been identified. In addition to the real-time detection of progressive β cell destruction in vivo during diabetes, such biomarkers will allow greater optimization of islet transplantation, where there are still many inefficiencies in the procedure leading to significant β cell death during cadaveric isolation and culture, as much as 50 % β cell death during transplantation, and then varying degrees of engraftment.

Qualities of Good Biomarkers

One must consider a number of criteria when evaluating the utility of potential biomarkers for detection of β cell destruction. Furthermore, the qualities of several good examples of clinically useful biomarkers can be compared to putative novel biomarkers as a yardstick. Perhaps the most desirable traits of good biomarkers are tissue selectivity and a highly sensitive detection method. The former quality ensures that measurement of the circulating molecule is unequivocally detectable due to selective tissue damage and is not confounded by other pathological conditions. If this condition is not met, or if the biomarker is upregulated during tumorigenesis, it will require additional diagnostic confirmation. Importantly, it is undesirable to observe expression in tissues contributing the majority of plasma proteins, liver and muscle, as they could be the source of false positives. The expression level of a protein can also directly influence the ease of measurement, whether by mass spectrometry, a biochemical enzymatic spectrophotometric method, or antibody-based approaches such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), or immunoprecipitation and immunoblotting. The method of detection, combined with the abundance of the biomarker, will determine the lower limit of detection, which in turn dictates the actual worth of the analyte in research or clinical diagnosis. A more recent advance in the biomarker field has been the finding that tissue-specific microRNA species and methylated genomic DNA can be released into the circulation and subsequently detected by PCR-based approaches in the days or weeks following tissue injury. While this methodology, by virtue of its signal amplification during detection, may result in a higher frequency of erroneous positive results, the ease and sensitivity of the detection method greatly favor this approach over protein-based biomarkers.

For β cell-specific biomarkers, proteins and miRNA species should likely not be metabolically active agents, be actively secreted by metabolic stimuli, nor their expression be metabolically regulated in order to properly interpret results. Generally, avoidance of transmembrane proteins would also be advisable. For both protein and nucleic acid biomarkers, a detailed pharmacokinetic profile should be characterized to help identify the detection window duration. Diabetes is a progressive disease, and thus, there is unlikely to be an acute insult causing a spike of biomarker appearance in the circulation – rather, a low level of biomarker release

over a period of years (in contrast, during islet transplantation, acute necrosis during engraftment may show a rapid peak in released biomarker). The following sections examine the current successes and challenges reported in early stage biomarker validation studies in rodent models and human diabetes.

GAD65

Glutamic acid decarboxylase (GAD) 65 was one of the first candidate biomarkers for detection of β cell death in vitro and in vivo. GAD65 along with GAD67 are the two predominant forms of the enzyme responsible for generation of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Hinke 2007). GAD expression is restricted to neuroendocrine cell types, with the human isoform of GAD65 being abundantly expressed in brain and α, β, and δ cells of the endocrine pancreas (Mally et al. 1996); rats express both GAD65 and GAD67 in islet cells, whereas mice appear to synthesize exclusively GAD67 (Chessler and Lernmark 2000). Studies on rat islet tissue indicate GAD65 to be localized to membrane structures via palmitoylation, while GAD67 is cytosolic (Dirkx et al. 1995). GAD65 has many of the characteristics of a decent biomarker, with respect to tissue selectivity and sufficient molar abundance, however, because of the prevalence of anti-GAD65 autoantibodies in the same population of patients in which use of GAD65 as a biomarker is sought, the potential use of this enzyme is limited by antibody interference.

The first in vitro evidence that GAD65 might serve as a biomarker for β cell death came from Smismans et al. (1996), showing enzyme release into the culture media following streptozotocin (STZ) toxin treatment of flow-sorted purified rat β cells. Biochemical assays detected a significant reduction in cellular GAD activity and GABA content in STZ-treated cells, with a concomitant appearance of GAD activity in concentrated media extracts. In a preclinical study examining auto- and allotransplantation of islets into dogs under temporary immunosuppression, an enzymatic assay for GAD achieved limited success in tracking islet graft rejection but left authors optimistic to the possibility of detection of islet-specific proteins prior clinical manifestation of hyperglycemia in diabetes (Shapiro et al. 2001). Development of more sensitive detection methods, sandwich ELISA, RIA, and time-resolved fluorescent immunoassay technologies (Hao et al. 1999; Rui et al. 2007; Schlosser et al. 1997), have shown promise for in vivo applications but were still limited by the relative abundance of GAD65 in islets, the scarce but vital organ, and the dilution of the enzyme in the circulation below the lower limits of detection of these assays. This has prompted subsequent investigators of similar biomarkers to use GAD65 abundance as an internal benchmark criterion, hypothesizing proteins with lower expression than GAD will prove to be too difficult to detect in the circulation.

In 2006, a new magnetic bead immunoassay to GAD65 using a modified dot blot chemiluminescent detection strategy was described by Waldrop et al. (2006). The authors were able to achieve a quantitation limit of 31 pg/mL, and more

importantly, as the method employed a denaturation step prior to detection, it was not susceptible to autoantibody interference. The subsequent application of this assay to a proof-of-concept in vivo study in rats was the first to measure acute release of GAD65 into the circulation in response to β cell toxin administration (Waldrop et al. 2007). Remarkably, it was possible to temporally separate β cell death as being well prior to the onset of hyperglycemia. Two β cell-specific toxic compounds, STZ and alloxan, were used at high doses to severely damage β cells in Wistar rats; markers of β cell function were assessed over time. Either toxin treatment induced acute GAD65 appearance in the peripheral circulation or eventually elevated blood sugar – in the same animals, β cell death was evident by significantly reduced circulating insulin and C-peptide levels. Increasing doses of STZ administered to rats caused a concentration-dependent GAD65 shedding into the circulation within 6 h, while blood glucose remained close to basal values. By 24 h postinjection, overt hyperglycemia (>16 mM) and significantly elevated circulating GAD65 were observed at all STZ doses above 40 mg/kg. At a lower dose of 20 mg/kg STZ, there was no induction of β cell TUNEL positivity in rat pancreatic sections at the 24-h time point, nor was there hyperglycemia, yet GAD65 was elevated more than fourfold in the circulation (Waldrop et al. 2007). The latter result implies that it may be possible to detect mild insults to insulin-secreting cells by tracking GAD65 as a circulating biomarker.

These results show promise for use of this type of strategy to study the early stages of diabetes initiation in animal models and human subjects, possibly permitting elucidation of the triggering events during the etiology of diabetes prior to the onset of hyperglycemia. The major hurdle in this regard is the window of detection: Waldrop and colleagues estimated a circulating half-life of recombinant GAD65 of 2.9 h, and following acute toxin, the biomarker was detectable in the circulation for 24 h, but returned to baseline within 2 days (Waldrop et al. 2007). During human T1D, there is a gradual decline in β cell mass over a period of years and a persistence of a small number of β cells in later stages of diagnosis (Pipeleers et al. 2001). Mild acute STZ injury of β cells in rats did cause a progressive appearance of circulating GAD65 (Waldrop et al. 2007), but it is unclear if the prolonged shedding of GAD65 during the development of human autoimmune diabetes will be detectable. To date, no follow-up reports have been published to describe preclinical experiments in rodent models of spontaneous progressive β cell death, such as the BioBreeding rat model of autoimmune islet destruction or late-stage glucolipotoxic β cell death in the ZDF Fatty Zucker rat (Hinke 2007).

Doublecortin

Using the GAD65 proof-of-concept study as the established gold standard of in vivo β cell death biomarkers, Jiang and co-workers sought to improve on the diagnostic sensitivity by focusing on more abundant proteins in insulin-secreting cells. The Belgium-based group used a bottom-up LC-MS/MS unlabeled proteomic strategy, which is inherently biased towards proteins with higher expression, to identify

candidate alternate biomarkers in flow-sorted primary rat β and α cell populations (Jiang et al. 2013b) (also refer to the chapter "► Proteomics and Islet Research" for greater detail on the identification of β cell-specific proteins). This approach provided a list of 521 proteins above the limit of detection that could be identified with confidence, of which 164 were specific to insulin-secreting cells and 54 to glucagon-enriched cells. This data set was cross-referenced to tissue comparative gene transcript array data from the same group on the same islet populations, as well as brain, pituitary, liver, muscle, and white adipose tissues (Martens et al. 2011), thus permitting candidate selection based upon tissue selectivity. As β cells share a similar neuroendocrine gene expression program to α cells, brain, and pituitary, the authors did not set exclusion criteria based on expression of transcripts in these tissues; however, it was possible to examine the relative levels of mRNAs corresponding to the mass spectrometry identified β cell-expressed proteins and narrow the putative candidate biomarkers to a field of 36 proteins with at least 1.5-fold greater mRNA expression than liver, muscle, and adipose. These candidates were then further restricted by excluding plasmalemmal and intravesicular proteins (Jiang et al. 2013b).

The cytoplasmic protein doublecortin (DCX) was chosen for further in vitro validation, as it showed similar β cell selectivity as islet amyloid polypeptide (IAPP) and prohormone convertase 1 (PC1) and was not detected in the glucagon-positive-enriched cell population (Jiang et al. 2013b). Doublecortin expression in insulin-positive cells had not been previously described, and this protein was primarily used as a marker of neurogenesis during migration of newly formed neurons into the neocortex (Couillard-Despres et al. 2005; Gleeson et al. 1999). Using quantitative immunoblotting, Jiang et al. estimated that the DCX content of human and rat β cells was 5 and 40 times more abundant that GAD65, respectively (Jiang et al. 2013b). The sensitivity of the LC-MS/MS proteomic approach was unable to detect GAD65 in tryptic digests of rat β cell proteins. Quantitative PCR and Western blotting confirmed DCX expression was restricted to islet and brain, with about 20-fold enrichment in β cells as compared to whole brain or pituitary lysates. Fluorescence microscopy localized DCX immunoreactivity to the cytosol of all insulin-positive cells and rare weak staining in some glucagon-positive cells (Jiang et al. 2013b).

In vitro validation studies to evaluate DCX as a biomarker of β cell necrosis were performed using STZ or H_2O_2 treatments and performed in parallel with GAD65 measurements. Using a magnetic bead-based quantitative immunoprecipitation method, a detection limit of 24 nM DCX was obtained, which was sufficient to measure the proportional appearance of DCX in the islet culture media and disappearance from cell extracts following toxin treatment (Jiang et al. 2013b). While this offered proof-of-principle, the rudimentary method to assay DCX prevented further validation in vivo due to lack of sensitivity. Regardless, it was possible to show that DCX was stable in plasma and pharmacokinetic data measuring the removal of recombinant DCX from the circulation indicated an in vivo half-life of around 3 h (remarkably similar to the $t_{1/2}$ calculated for GAD65, perhaps reflecting exclusion from renal filtration) (Jiang et al. 2013b; Waldrop

et al. 2007). Additionally, no disease-related DCX autoreactivity was observed in human T1D samples, suggesting that antibody interference is unlikely to cause complications in biomarker detection (Jiang et al. 2013b). Hence, DCX appears to be a promising candidate biomarker for tracking β cell death, with several apparent advantages over GAD65 for this purpose. However, full in vivo validation is precluded pending development of more sensitive DCX detection assays.

Protein Phosphatase Inhibitor 1

A second candidate biomarker came out of the combined efforts of the Martens' group examining β cell-selective transcripts by gene array and abundant protein by label-free mass spectrometry. Protein phosphatase inhibitor 1 (PPP1R1A) fits the criteria for tracking β cell death in vivo based upon its high protein abundance in rat β cells, with relatively good tissue-specific expression (Jiang et al. 2013a). PPP1R1A is an endogenous regulatory inhibitor of protein phosphatase 1 (PP1); when PPP1R1A is phosphorylated by protein kinase A (PKA) on Thr^{35}, it potently inhibits PP1 activity with a K_i of 1.6 nM (Oliver and Shenolikar 1998). It has been studied primarily in regard to its role in PP1 regulation of skeletal muscle glycogen metabolism and contraction, synaptic plasticity, and cell growth. The presence of PPP1R1A in muscle tissue causes some concern for its application as a circulating biomarker, particularly considering its expression is elevated in some cancers (Wai et al. 2002). Nevertheless, Jiang et al. demonstrated expression in purified rat primary β cells and a transformed rat insulin-secreting cell line (INS-1) by Western blot, and protein expression of PPP1R1A was at least one order of magnitude greater in the islet-derived tissue than in rodent skeletal muscle or whole brain, consistent with other studies (Lilja et al. 2005; Martens et al. 2010, 2011; Nicolaou et al. 2009; Vander Mierde et al. 2007). Fluorescent immunostaining human pancreatic sections indicated cytoplasmic PPP1R1A co-staining only in insulin-positive cells (Jiang et al. 2013a).

Using a similar study design to in vitro validation of DCX as a putative biomarker (Jiang et al. 2013b), flow-sorted rat β cells were treated with STZ or cultured cryopreserved human islets were exposed to H_2O_2. PPP1R1A release into the culture media and remaining content in the damaged tissue was assessed by a combination of magnetic Dynabead immunoprecipitation and Western blot detection, followed by densitometry (Jiang et al. 2013a). STZ treatment caused 60–70 % β cell death by 24 h, and similarly, hydrogen peroxide exposure stimulated islet disintegration and 40–50 % cell necrosis at the same time point. In the case of rodent β cells, a 20-fold increase in immunoprecipitated PPP1R1A was observed in media following STZ administration relative to controls, with a reciprocal loss of cellular PPP1R1A protein content. Concordant results from human islet cultures were observed following H_2O_2 treatment (Jiang et al. 2013a).

For in vivo validation, rats were injected with a diabetogenic dose of STZ (60 mg/kg), and in addition to tracking blood glucose and plasma insulin, samples were obtained at 2-h intervals for immunohistochemical analysis, total pancreatic

PPP1R1A protein content, and immunoprecipitated circulating PPP1R1A. Pancreatic PPP1R1A protein expression began to drop within the first 2 h and fell to barely detectable levels after 4 h; likewise, fluorescent staining of PPP1R1A could no longer be visualized by 4 h (Jiang et al. 2013a). At the same time, Jiang and colleagues were able to show a rise in the protein phosphatase 1 inhibitor in the circulation within 2 h and a significant peak at 4 h post-STZ injection. Subsequently, the circulating PPP1R1A rapidly returned to baseline values and was undetectable beyond the 8-h time point (Jiang et al. 2013a). This latter finding contrasts to the in vivo validation results with GAD65, where the protein was undetectable in the circulation in the first 3 h, peaking at 6 h post-STZ and remaining detectable in the circulation for at least 24 h (Waldrop et al. 2007). While these disparate results are likely partially due to the sensitivity of the assays used to detect the circulating proteins, the circulating half-life of PPP1R1A was found to be only 15 min (Jiang et al. 2013a). Injection of recombinant PPP1R1A was cleared quite rapidly from the blood stream, most likely because protein is only approximately 19 kDa and thus freely filtered by the glomerulus. This highlights the need to fully characterize the pharmacokinetic properties of putative biomarkers as part of the validation process.

While this window for detection is quite narrow, it was possible to show in vivo substantiation of PPP1R1A as a biomarker using an alternative model system. During islet transplantation, as many as 50 % of portally injected islets fail to functionally engraft due to ischemic, inflammatory, and mechanical stress during the procedure (Emamaullee and Shapiro 2006; Piemonti et al. 2010) (also refer to chapter "► Successes and Disappointments with Clinical Islet Transplantation"). As such, similar to the preclinical canine islet transplantation study examining serum GAD activity (Shapiro et al. 2001), it was hoped that PPP1R1A could be measured in plasma samples of human islet recipients and give some index of the engraftment efficiency. Four islet transplant patients were studied, and 75 % had detectable circulating PPP1R1A biomarker that appeared to correlate with the number of islets transplanted (graft size) (Jiang et al. 2013a). In these graft recipients, the biomarker peaked within the first 15 min and then rapidly returned to baseline following similar kinetics to the pharmacokinetic experiment suggesting complete renal filtration. Control subject serum (kidney transplant, stroke, pancreatitis, type 2 diabetes) did not show any detectable PPP1R1A, nor were any autoantibodies to PPP1R1A measured in GAD autoantibody-positive sera from type 1 diabetics (Jiang et al. 2013a).

Methylated Insulin Gene

Epigenetic regulation of genes can control tissue-specific expression. DNA methylation typically favors a transcriptionally repressed state, whereas demethylation is transcriptionally permissive (Miranda and Jones 2007). Differential methylation of specific oncogenes has been demonstrated in specific cancers, and their detection in serum has been applied as a diagnostic biomarker in these patients

(Grady et al. 2001; Muller et al. 2003; Wallner et al. 2006). Two groups have searched for diabetic biomarkers using the same strategy. Initial efforts by Kuroda et al. (2009) examined the methylation state of the mouse (*Ins2*) and human (*INS*) insulin promoters to characterize the tissue-specific expression of insulin in β cells and during differentiation of stem cells to insulin-positive tissue. Three methylation sites were found in the mouse *Ins2* promoter and 9 in the human *INS* promoter, with the striking observation of all sites being methylated in non-islet tissues. Insulin promoter methylation was shown to directly repress gene transcription by inhibiting transcription factor binding to the upstream CRE site (Kuroda et al. 2009). In a follow-up study from the same group, researchers describe the development and thorough characterization of a quantitative, methylation-specific PCR assay for circulating β cell *Ins2* exon 2 DNA and apply it to a mouse model of T1D (Husseiny et al. 2012). It was estimated that the assay sensitivity achieved could detect the β cell-specific DNA from as few as one thousand cells in the circulation of a mouse. NOD/scid mice were injected with 50 mg/kg STZ on three consecutive days, and glycemia- and methylation-specific PCR were performed on bisulfite-converted gDNA from blood for 5 weeks post-STZ. Hyperglycemia was evident within 5–6 days, but a significant increase in demethylated β cell-specific *Ins2* DNA could be measured as early as 2 days following STZ (Husseiny et al. 2012). The sensitivity of this method and the ability to detect islet injury prior to hyperglycemia hopefully point towards the future ability to intervene in the progression of human diabetes to prevent further loss of β cell mass.

In a similar approach, the Herold lab examined the methylation state of the coding region of the mouse *Ins1* gene and identified seven methylation sites that were modified in a tissue-specific manner (Akirav et al. 2011). Optimization of a nested two-step methylation-specific PCR reaction was used to show more than 12-fold greater demethylated *Ins1* DNA in crude islet preparations compared to a panel of non-islet tissues. Using a single high dose of STZ (200 mg/kg) and analyzing circulating *Ins1* DNA, these researchers measured a significant 2.6-fold increase in the demethylation index at 8 h after STZ, which increased to 3.8-fold by 24 h. Notably, at the early time point, immunohistochemical indicators of β cell injury were present in the absence of elevated blood glucose (Akirav et al. 2011). Application of these methods to the NOD mouse pre-diabetic state showed a significant, but variable elevation of demethylated *Ins1* DNA in the circulation, which was inversely correlated with pancreatic insulin content. In extending this study to humans, Akirav et al. were able to show demethylation of *INS* DNA in sorted human β cells at eight putative sites. The most promising result shown was the detection of demethylated insulin DNA in the sera from five newly diagnosed type 1 diabetic patients (<1.5 years since diagnosis), and the proportion of demethylated *INS* DNA was significantly greater than in control subjects (Akirav et al. 2011).

Broader application of the two-step nested methylation-sensitive PCR method to human diabetes was reported by Lebastchi et al. (2013). Serum samples were obtained during a clinical trial (DELAY) at enrollment and 1 year later from 13 age-matched nondiabetic control patients, 43 patients

recently diagnosed with type 1 diabetes, and a third group of 37 diabetic patients treated with anti-CD3 monoclonal antibody. At entry, newly diagnosed T1D patients were found to have significantly more circulating unmethylated insulin DNA compared to control subjects. Patients receiving the anti-CD3 intervention treatment had a reduced decline in C-peptide levels and reduced insulin requirement after 1 year, relative to placebo-treated diabetics; at the same time, a significant decline in abundance of unmethylated *INS* DNA was measured in the sera from the treatment group, suggesting that treatment was associated with less β cell loss than the placebo group (Lebastchi et al. 2013). Hence, proof-of-concept has been verified for use of demethylated insulin DNA as a circulating biomarker for β cell death and independently replicated by two laboratories. Possibly the most challenging aspect for the future of this technology in diabetes research will be the adaptation of the technique to facilitate larger clinical screening efforts.

miR-375

MicroRNAs (miRNAs) are single-stranded RNA molecules (approx. 21–23 nt in length) that have emerged as epigenetic regulators of gene expression and cellular function (Bartel 2009). Several β cell-specific miRNAs have been identified, of which the most abundant and best characterized is miR-375 (Fernandez-Valverde et al. 2011; Poy et al. 2004). These microRNAs have been shown to play a role in endocrine pancreas development, α and β cell mass, insulin production, and glucose-stimulated insulin release (El Ouaamari et al. 2008; Fernandez-Valverde et al. 2011; Kredo-Russo et al. 2012; Melkman-Zehavi et al. 2011; Poy et al. 2009). Kieffer's group examined the potential of miR-375 as marker of β cell death, as it was the first islet-specific miRNA identified and the most abundant (Landgraf et al. 2007; Poy et al. 2004). Furthermore, studies have recently shown some extracellular miRNAs to have remarkable stability due to complex formation (Koberle et al. 2013); the use of quantitative PCR to detect circulating miRNAs similarly avoids the limitations in sensitivity-hampering application of protein biomarkers.

Initially, results were confirmed for miR-375 showing expression in both human and mouse pancreas and enrichment in islet tissue. Although the tissue specificity was primarily restricted to pancreas, some lower level expression was observed in pulmonary tissue, testis, and the gastrointestinal tract (Erener et al. 2013). As miR-375 is elevated in some prostate cancers (Brase et al. 2011), caution should be used if it is applied as a single diagnostic test for a β cell biomarker. In vitro, when mouse islets were treated with either a cytotoxic cocktail of cytokines (IL-1β, TNF-α, and IFN-γ) or STZ, it was possible to detect miR-375 release into the media samples at 20–24 times the amount found in control samples. Additionally, the use of caspase or PARP inhibitors to block the cell death induced by cytokines or STZ showed a 2-to 4-fold reduction in miR-375 abundance in the culture media (Erener et al. 2013).

Two in vivo mouse models were employed to examine if miR-375 could be used to detect β cell death prior to appearance of overt diabetes. The first was similar to the approaches used for protein-based biomarkers: a single diabetogenic dose of STZ. In this model, a mild reduction in blood glucose was observed on day 1 (consistent with loss of β cell membrane integrity, thus releasing insulin), and hyperglycemia was observed 1 week following intraperitoneal injection of the β cell toxin (Erener et al. 2013). Peak plasma detection of miR-375 occurred within 2 h of STZ treatment (6.8-fold control values) and remained significantly elevated in one cohort of animals for a week. In a second cohort of mice, miR-375 was elevated on the first day of STZ injection, but fell back below baseline by 7 days; interestingly, a second phase of elevated circulating miR-375 appeared from day 14 that persisted to two months post-STZ. Treatment of the diabetic mice with subcutaneous insulin pellets appeared to suppress the level of circulating miR-375 in these animals. Parallel examination of miR-16.1 in this in vivo study indicated that it was unaffected by STZ treatment and hyperglycemia, and thus, this phenomenon was selective for miR-375 (Erener et al. 2013).

As human T1D manifests following a progressive decline in β cell mass over a period of years of autoimmune β cell destruction, Erener and colleagues turned to one of the most common rodent models of autoimmune diabetes, the nonobese diabetic (NOD) mouse. A subset of female NOD mice spontaneously develop a diabetic phenotype after 12 weeks of age, but insulitis can be observed during histological analysis of pancreata as early as 3 weeks (Delovitch and Singh 1997). In this experimental model, it is possible to segregate mice based upon whether or not they develop diabetes at 3–4 months of age, thus providing an internally controlled experiment. During examination of plasma miR-375 in female NOD mice, significantly elevated microRNA was detectable at least 2 weeks prior to the onset of hyperglycemia and remained raised until animals presented overt diabetes. In contrast, measurement of miR-375 in plasma from the six mice that did not develop any hyperglycemia indicated no change in circulating abundance of the microRNA (Erener et al. 2013). The potential sensitivity of miRNA detection in the circulation combined with the ease of quantitation seem to favor this class of biomarker over both protein-based and genomic DNA-based methods.

Summary and Future Perspectives

The silent and complex etiology of diabetes prior to clinical diagnosis poses an immense challenge to preventative and interventional therapeutic approaches. The use of late-stage biomarkers, such as detection of islet autoantibodies and alterations in β cell function parameters, has been the only criteria for selecting patients for immunomodulator therapy. The mixed results of clinical outcomes may stem from the late stage at which diabetics are enrolled in the trials; clearly, the earliest detection of the initial injury provides the largest window for therapy. This chapter has examined the current state of biomarkers that have been proposed to detect β cell injury in vivo. In general, all protein-based biomarkers had drawbacks in regard to sensitivity of the

assays to detect them or limitations in the pharmacokinetic properties that narrow the window of detection. Nevertheless, the utility of GAD65, DCX, and PPP1R1A may primarily lie in the optimization of islet transplantation, where a clearer relationship between the proportions of protein detected in the circulation and the degree of β cell loss is directly related. In terms of sensitivity of detection, tissue-specific miRNA and methylated DNA appear to have a great advantage; however, amplification by PCR will likely generate more false positives, and aberrant expression during cancer or other nondiabetic maladies is a concern. Until it is possible to image β cell mass by noninvasive means (reviewed in another chapter in this book), identifying the best circulating biomarker that most accurately reflects ongoing β cell death is likely our best option for early disease intervention.

Cross-References

- ▶ Current Approaches and Future Prospects for the Prevention of β-Cell Destruction in Autoimmune Diabetes
- ▶ Immunology of β-Cell Destruction
- ▶ Inflammatory Pathways Linked to β Cell Demise in Diabetes

References

Akirav EM, Lebastchi J, Galvan EM, Henegariu O, Akirav M, Ablamunits V, Lizardi PM, Herold KC (2011) Detection of β cell death in diabetes using differentially methylated circulating DNA. Proc Natl Acad Sci U S A 108:19018–19023

Babuin L, Jaffe AS (2005) Troponin: the biomarker of choice for the detection of cardiac injury. CMAJ 173:1191–1202

Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. Cell 136:215–233

Brase JC, Johannes M, Schlomm T, Falth M, Haese A, Steuber T, Beissbarth T, Kuner R, Sultmann H (2011) Circulating miRNAs are correlated with tumor progression in prostate cancer. Int J Cancer 128:608–616

Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC (2003) β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 52:102–110

Chessler SD, Lernmark A (2000) Alternative splicing of GAD67 results in the synthesis of a third form of glutamic-acid decarboxylase in human islets and other non-neural tissues. J Biol Chem 275:5188–5192

Cnop M, Welsh N, Jonas JC, Jorns A, Lenzen S, Eizirik DL (2005) Mechanisms of pancreatic β-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes 54(Suppl 2):S97–S107

Couillard-Despres S, Winner B, Schaubeck S, Aigner R, Vroemen M, Weidner N, Bogdahn U, Winkler J, Kuhn HG, Aigner L (2005) Doublecortin expression levels in adult brain reflect neurogenesis. Eur J Neurosci 21:1–14

Delovitch TL, Singh B (1997) The nonobese diabetic mouse as a model of autoimmune diabetes: immune dysregulation gets the NOD. Immunity 7:727–738

Dirkx R Jr, Thomas A, Li L, Lernmark A, Sherwin RS, De Camilli P, Solimena M (1995) Targeting of the 67-kDa isoform of glutamic acid decarboxylase to intracellular organelles is mediated by its interaction with the NH2-terminal region of the 65-kDa isoform of glutamic acid decarboxylase. J Biol Chem 270:2241–2246

Eisenbarth GS (1986) Type I diabetes mellitus. A chronic autoimmune disease. N Engl J Med 314:1360–1368

El Ouaamari A, Baroukh N, Martens GA, Lebrun P, Pipeleers D, van Obberghen E (2008) miR-375 targets 3'-phosphoinositide-dependent protein kinase-1 and regulates glucose-induced biological responses in pancreatic β-cells. Diabetes 57:2708–2717

Emamaullee JA, Shapiro AM (2006) Interventional strategies to prevent β-cell apoptosis in islet transplantation. Diabetes 55:1907–1914

Erener S, Mojibian M, Fox JK, Denroche HC, Kieffer TJ (2013) Circulating miR-375 as a biomarker of β-cell death and diabetes in mice. Endocrinology 154:603–608

Fernandez-Valverde SL, Taft RJ, Mattick JS (2011) MicroRNAs in β-cell biology, insulin resistance, diabetes and its complications. Diabetes 60:1825–1831

Gleeson JG, Lin PT, Flanagan LA, Walsh CA (1999) Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons. Neuron 23:257–271

Grady WM, Rajput A, Lutterbaugh JD, Markowitz SD (2001) Detection of aberrantly methylated hMLH1 promoter DNA in the serum of patients with microsatellite unstable colon cancer. Cancer Res 61:900–902

Hao W, Daniels T, Pipeleers DG, Smismans A, Reijonen H, Nepom GT, Lernmark A (1999) Radioimmunoassay for glutamic acid decarboxylase-65. Diabetes Technol Ther 1:13–20

Hinke SA (2007) Finding GAD: early detection of β-cell injury. Endocrinology 148:4568–4571

Hinke SA (2011) Inverse vaccination with islet autoantigens to halt progression of autoimmune diabetes. Drug Dev Res 72:788–804

Husseiny MI, Kuroda A, Kaye AN, Nair I, Kandeel F, Ferreri K (2012) Development of a quantitative methylation-specific polymerase chain reaction method for monitoring β cell death in type 1 diabetes. PLoS One 7:e47942

Jiang L, Brackeva B, Ling Z, Kramer G, Aerts JM, Schuit F, Keymeulen B, Pipeleers D, Gorus F, Martens GA (2013a) Potential of protein phosphatase inhibitor 1 as biomarker of pancreatic β-cell injury in vitro and in vivo. Diabetes 62:2683–2688

Jiang L, Brackeva B, Stange G, Verhaeghen K, Costa O, Couillard-Despres S, Rotheneichner P, Aigner L, Van Schravendijk C, Pipeleers D et al (2013b) LC-MS/MS identification of doublecortin as abundant β cell-selective protein discharged by damaged β cells in vitro. J Proteomics 80:268–280

Koberle V, Pleli T, Schmithals C, Augusto Alonso E, Haupenthal J, Bonig H, Peveling-Oberhag J, Biondi RM, Zeuzem S, Kronenberger B et al (2013) Differential stability of cell-free circulating microRNAs: implications for their utilization as biomarkers. PLoS One 8:e75184

Kredo-Russo S, Mandelbaum AD, Ness A, Alon I, Lennox KA, Behlke MA, Hornstein E (2012) Pancreas-enriched miRNA refines endocrine cell differentiation. Development 139:3021–3031

Kuroda A, Rauch TA, Todorov I, Ku HT, Al-Abdullah IH, Kandeel F, Mullen Y, Pfeifer GP, Ferreri K (2009) Insulin gene expression is regulated by DNA methylation. PLoS One 4:e6953

Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M et al (2007) A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 129:1401–1414

Lebastchi J, Herold KC (2012) Immunologic and metabolic biomarkers of β-cell destruction in the diagnosis of type 1 diabetes. Cold Spring Harb Perspect Med 2:a007708

Lebastchi J, Deng S, Lebastchi AH, Beshar I, Gitelman S, Willi S, Gottlieb P, Akirav EM, Bluestone JA, Herold KC (2013) Immune therapy and β-cell death in type 1 diabetes. Diabetes 62:1676–1680

Lilja L, Meister B, Berggren PO, Bark C (2005) DARPP-32 and inhibitor-1 are expressed in pancreatic β-cells. Biochem Biophys Res Commun 329:673–677

Makarov DV, Loeb S, Getzenberg RH, Partin AW (2009) Biomarkers for prostate cancer. Annu Rev Med 60:139–151

Mally MI, Cirulli V, Otonkoski T, Soto G, Hayek A (1996) Ontogeny and tissue distribution of human GAD expression. Diabetes 45:496–501

Martens GA, Jiang L, Verhaeghen K, Connolly JB, Geromanos SG, Stange G, Van Oudenhove L, Devreese B, Hellemans KH, Ling Z et al (2010) Protein markers for insulin-producing β cells with higher glucose sensitivity. PLoS One 5:e14214

Martens GA, Jiang L, Hellemans KH, Stange G, Heimberg H, Nielsen FC, Sand O, Van Helden J, Van Lommel L, Schuit F et al (2011) Clusters of conserved β cell marker genes for assessment of β cell phenotype. PLoS One 6:e24134

Melkman-Zehavi T, Oren R, Kredo-Russo S, Shapira T, Mandelbaum AD, Rivkin N, Nir T, Lennox KA, Behlke MA, Dor Y et al (2011) miRNAs control insulin content in pancreatic β-cells via downregulation of transcriptional repressors. EMBO J 30:835–845

Miranda TB, Jones PA (2007) DNA methylation: the nuts and bolts of repression. J Cell Physiol 213:384–390

Muller HM, Widschwendter A, Fiegl H, Ivarsson L, Goebel G, Perkmann E, Marth C, Widschwendter M (2003) DNA methylation in serum of breast cancer patients: an independent prognostic marker. Cancer Res 63:7641–7645

Neutzsky-Wulff AV, Andreassen KV, Hjuler ST, Feigh M, Bay-Jensen AC, Zheng Q, Henriksen K, Karsdal MA (2012) Future detection and monitoring of diabetes may entail analysis of both β-cell function and volume: how markers of β-cell loss may assist. J Transl Med 10:214

Nicolaou P, Rodriguez P, Ren X, Zhou X, Qian J, Sadayappan S, Mitton B, Pathak A, Robbins J, Hajjar RJ et al (2009) Inducible expression of active protein phosphatase-1 inhibitor-1 enhances basal cardiac function and protects against ischemia/reperfusion injury. Circ Res 104:1012–1020

Oliver CJ, Shenolikar S (1998) Physiologic importance of protein phosphatase inhibitors. Front Biosci 3:D961–D972

Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, Hu ZX, Lin J, Xiao JZ, Cao HB et al (1997) Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. Diabetes Care 20:537–544

Piemonti L, Guidotti LG, Battaglia M (2010) Modulation of early inflammatory reactions to promote engraftment and function of transplanted pancreatic islets in autoimmune diabetes. Adv Exp Med Biol 654:725–747

Pipeleers D, Hoorens A, Marichal-Pipeleers M, Van de Casteele M, Bouwens L, Ling Z (2001) Role of pancreatic β-cells in the process of β-cell death. Diabetes 50(Suppl 1):S52–S57

Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, Pfeffer S, Tuschl T, Rajewsky N, Rorsman P et al (2004) A pancreatic islet-specific microRNA regulates insulin secretion. Nature 432:226–230

Poy MN, Hausser J, Trajkovski M, Braun M, Collins S, Rorsman P, Zavolan M, Stoffel M (2009) miR-375 maintains normal pancreatic α- and β-cell mass. Proc Natl Acad Sci U S A 106:5813–5818

Prentki M, Nolan CJ (2006) Islet β cell failure in type 2 diabetes. J Clin Invest 116:1802–1812

Rui M, Hampe CS, Wang C, Ling Z, Gorus FK, Lernmark A, Pipeleers DG, De Pauw PE (2007) Species and epitope specificity of two 65 kDa glutamate decarboxylase time-resolved fluorometric immunoassays. J Immunol Methods 319:133–143

Schlosser M, Hahmann J, Ziegler B, Augstein P, Ziegler M (1997) Sensitive monoclonal antibody-based sandwich ELISA for determination of the diabetes-associated autoantigen glutamic acid decarboxylase GAD65. J Immunoassay 18:289–307

Schomaker S, Warner R, Bock J, Johnson K, Potter D, Van Winkle J, Aubrecht J (2013) Assessment of emerging biomarkers of liver injury in human subjects. Toxicol Sci 132:276–283

Shapiro AM, Hao EG, Lakey JR, Yakimets WJ, Churchill TA, Mitlianga PG, Papadopoulos GK, Elliott JF, Rajotte RV, Kneteman NM (2001) Novel approaches toward early diagnosis of islet allograft rejection. Transplantation 71:1709–1718

Smismans A, Ling Z, Pipeleers D (1996) Damaged rat β cells discharge glutamate decarboxylase in the extracellular medium. Biochem Biophys Res Commun 228:293–297

Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M et al (2001) Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 344:1343–1350

Vander Mierde D, Scheuner D, Quintens R, Patel R, Song B, Tsukamoto K, Beullens M, Kaufman RJ, Bollen M, Schuit FC (2007) Glucose activates a protein phosphatase-1-mediated signaling pathway to enhance overall translation in pancreatic β-cells. Endocrinology 148:609–617

Wai DH, Schaefer KL, Schramm A, Korsching E, Van Valen F, Ozaki T, Boecker W, Schweigerer L, Dockhorn-Dworniczak B, Poremba C (2002) Expression analysis of pediatric solid tumor cell lines using oligonucleotide microarrays. Int J Oncol 20:441–451

Waldrop MA, Suckow AT, Hall TR, Hampe CS, Marcovina SM, Chessler SD (2006) A highly sensitive immunoassay resistant to autoantibody interference for detection of the diabetes-associated autoantigen glutamic acid decarboxylase 65 in blood and other biological samples. Diabetes Technol Ther 8:207–218

Waldrop MA, Suckow AT, Marcovina SM, Chessler SD (2007) Release of glutamate decarboxylase-65 into the circulation by injured pancreatic islet β-cells. Endocrinology 148:4572–4578

Wallner M, Herbst A, Behrens A, Crispin A, Stieber P, Goke B, Lamerz R, Kolligs FT (2006) Methylation of serum DNA is an independent prognostic marker in colorectal cancer. Clin Cancer Res 12:7347–7352

Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, Rewers M, Eisenbarth GS, Jensen J, Davidson HW et al (2007) The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci U S A 104:17040–17045

Winter WE, Schatz DA (2011) Autoimmune markers in diabetes. Clin Chem 57:168–175

Proteomics and Islet Research

40

Meftun Ahmed

Contents

Abstract

Almost a decade has elapsed since the contemporary scientists, fascinated by the promising possibilities of proteomics, conducted extensive proteomic studies to unlock the secret of islet biology in the pathogenesis of diabetes. In recent years, proteomics has been revolutionized by the successful application of improved techniques such as 2D gel-based proteomics, mass spectrometric techniques, protein arrays, nanotechnology, and single-cell proteomics. These techniques have tremendous potential for biomarker development, target validation, diagnosis, prognosis, and optimization of treatment in medical care, especially in the field of islet and diabetes research. This chapter will highlight the contributions of proteomic technologies towards the dissection of complex network of signaling molecules regulating islet function, the identification of potential biomarkers, and the understanding of mechanisms involved in the pathogenesis of diabetes.

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_12,

Keywords
Proteomics • Islets • Two-dimensional gel electrophoresis • Mass spectrometry • Proteome • Glucotoxicity • Lipotoxicity

Introduction

Pancreatic islets, the fascinating little magic box, because of their vital performance in blood glucose regulation have long been the central focus of diabetes research. The essential illusion of these magical islets is the β-cell, a "mysterious maiden" with bags full of insulin. The search for the understanding the β-cells has given rise new ideas, imagination, and creativity in a worldwide scientific community, but till now not a single phenomenon of the β-cell has been fully understood. Every new discovery tells a tale about the previous one – a little more, but the story it seems a never ending one. In this promising journey of biomedical discovery, the completion of the human genome project has facilitated the entry of the biomedical researchers into a new dimension – the post-genomic era. This era is marked by an explosion of terms containing the suffix "omics," like the word genomics, transcriptomics, and metabolomics. One of the very stylish and trendy labels carrying the appellation "omics" is *proteomics*, which attracted the attention of contemporary scientists and offered to fill the void left by the human genome project to gain an in-depth understanding of future disease prevention and innovation of novel drug targets. The contributions of proteomic technologies towards the insights of the pathophysiology of the pancreatic islet function will be discussed in the following sections.

Proteome and Proteomics

All cells in the human body have essentially the same genetic information, and the genes possess only the information which is sequentially encoded to construct the final products – the proteins. These proteins are dynamic in nature and considered as the molecular engineers for a cell; their composition in a cell may vary at its different stages of development, whereas the genes remain as the static component of a cell. A classic example is the caterpillar and its mature form, the butterfly; they have the same genetic makeup, whereas their protein composition is quite different and it is the protein which is responsible for different shape and form of the organism. The renaissance of proteomics is due to the fact that proteins are expressed in quantities and physical forms that cannot be predicted from DNA and mRNA analysis (Anderson and Anderson 1998; Gygi et al. 1999). In addition, the diseased cells often produce proteins that healthy cells do not have and vice versa. Hence, scientists are aiming towards creating a complete catalogue of all the human proteins with an intention to uncover their interactions with one another (Anderson and Anderson 1998; Gygi et al. 1999; Anderson et al. 2001).

Their definitive goal is to discover biomarkers and to devise better drugs with fewer side effects. Significant progress has already made in biomarker discovery where several groups have announced that using proteomic techniques it is highly possible to make an accurate early diagnosis for cancers including ovarian, breast, and prostate cancer (Adam et al. 2001; Petricoin and Liotta 2004; Maurya et al. 2007).

In general, proteomics includes cataloging all the proteins present in a cell or tissue type at a specific time under specific conditions, quantitation, and functional characterization of these proteins to elucidate their relationships (protein-protein interaction networks) and functional roles and ultimately outlining their precise three-dimensional structures in order to find where the drugs might turn their activity on or off – the "Achilles heels" (Celis et al. 1998; Cahill et al. 2001; Ezzell 2002). The term proteome was coined as a linguistic equivalent to the concept of genome and first used in 1994 at the Siena 2D electrophoresis meeting (Ezzell 2002; Williams and Hochstrasser 1997; Abbott 1999). It denotes the entire PROTEin complement to a genOME, expressed by a cell or tissue type, at a specific time in the development of the organism under specific conditions (Wasinger et al. 1995; Wilkins et al. 1996). While humans are estimated to have approximately 20,000–25,000 genes, alternate RNA splicing and posttranslational modification may lead to encoding as many as 250,000–1 million individual proteins or peptides. For example, more than 22 different isoforms of α-1-antitrypsin exist in human plasma (Hoogland et al. 1999). In addition, the proteome undergoes dynamic changes as it continuously responds to autocrine, paracrine, and endocrine factors as well as exposure to any pathogen, changes in external environment, and during time course of disease and drug treatment. Various gene products, including microRNA (Bartel 2009), as well as epigenetic factors (Strohman 1994) also influence the expression levels of genes and their transcripts. As a consequence, the proteome is far more complex than the genome. Thus, the scale of protein discovery task is challenging and very large indeed. And multiple specialists from different fields must collaborate to provide a range of sophisticated tools to analyze nature's tremendous complexity. However, proteomics is still in an early stage, and at the time when mRNA expression arrays are spreading like cell phones in industry and in academic institutions, systems for large-scale protein analysis are still novelties. The commonly available proteomic technologies to date are summarized in Table 1.

Over the years, proteomics has expanded to include profiling, quantitative, functional, and structural proteomics based on a broad range of technologies. *Protein profiling* involves identifying and making a list of the proteins present in a biological sample (Figeys 2003). *Quantitative proteomics* discovers molecular physiology at the protein level and allows comparisons between samples by measuring relative changes in protein expression in response to external stimuli (Molloy et al. 2003; Domon and Broder 2004). *Functional proteomics* attempts to identify proteins in a cell, tissue, or organism that undergo changes in abundance, localization, or modification in response to a specific biological condition and discover their functions based on the presence of specific functional groups or

Table 1 Currently used proteomic technologies

Proteome profiling	**Emerging technologies**
1D gel electrophoresis	SILAC
2D gel electrophoresis	Imaging mass spectrometry (IMS)
2D-DIGE	Molecular scanner
MS-based methods	iTRAQ
SELDI-TOF	ICAT
MALDI-TOF	HysTag
CE-ESI-MS	Label-free LC-MS/MS quantitation
LC-MS	Protein chips:
Protein identification	*Spotted array based tools*:
Mass spectrometry	Forward phase arrays (FPA), e.g., antibody arrays, protein arrays
MudPIT or shotgun proteomics	Reverse phase arrays (RPA)
2D LC-MS/MS	*Microfluidic based tools*
Protein function	Single-cell proteomics
Yeast two hybrid	Nanoproteomics
Phase display	
Surface plasmon resonance analysis	
Immunoaffinity	
Structural proteomics	
X-ray crystallography	
NMR spectroscopy	
Electron tomography	
Immunoelectron microscopy	

2D-DIGE two-dimensional differential in-gel electrophoresis, *CE-ESI-MS* capillary electrophoresis electrospray ionization mass spectrometry, *ICAT* isotope-coded affinity tags, *iTRAQ* isobaric tagging for relative and absolute quantitation. *LC-MS* liquid chromatography mass spectrometry, *MudPIT* multidimensional protein identification technology, *MALDI-TOF* matrix-assisted laser desorption/ionization-time of flight, *NMR* nuclear magnetic resonance, *SELDI-TOF* surface-enhanced laser desorption/ionization-time of flight, *SILAC* stable isotope labeling by amino acids in cell culture

based on their involvement in protein-ligand interactions (Figeys 2003; Molloy et al. 2003; Domon and Broder 2004; Graves and Haystead 2003). Similarly, pathways can be characterized as a cascade of specific protein interactions required to activate cellular functions. Functional proteomics thus focuses on understanding part of the wiring diagram of a cell. *Structural proteomics* attempts to determine the three-dimensional structure of proteins, the structure of protein complexes, and the small-molecule/protein complexes. X-ray crystallography and NMR are its main approaches (Yee et al. 2002; Sali et al. 2003).

In the plethora of proteomic technologies, two-dimensional gel electrophoresis (2DGE) remains as a cornerstone of protein profiling (Lopez 2007; Gorg et al. 2004). The 2DGE separates proteins according to two independent parameters, isoelectric point (p*I*) in the first dimension and molecular mass (*M*r) in the second dimension by coupling isoelectric focusing (IEF) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Klose 1975; O'Farrell 1975). Theoretically, 2DGE is capable of resolving up to 10,000 proteins simultaneously, with approximately 2,000 proteins being routine, and detecting and

quantifying protein amounts of less than 1 ng per spot (Lopez 2007; Gorg et al. 2004). Despite the well-known limitations of the 2DGE approach, e.g., poor solubility of membrane proteins, limited dynamic range, difficulties in displaying and identifying low-abundant proteins, lack of reproducibility and automation, 2DGE will remain as a powerful and versatile tool for display and quantification of a majority of proteins in biological samples (Rogowska-Wrzesinska et al. 2013; Rabilloud 2012). The detailed technology, challenges, as well as the application potential and future of high-resolution 2DGE have been elegantly reviewed in several papers (Lopez 2007; Gorg et al. 2004, 2000; Vercauteren et al. 2006). However, gel-free high-throughput protein profiling techniques have leapt prominence and now become preferred method of choice including multidimensional protein identification technology (MudPIT) (Florens and Washburn 2006), molecular scanner (Binz et al. 2004), stable isotope labeling by amino acids in cell culture (SILAC) (Ong et al. 2002; Fenselau 2007), isotope-coded affinity tag (ICAT) (Gygi et al. 1999), isobaric tagging for relative and absolute quantitation (iTRAQ) (Aggarwal et al. 2006), protein microarrays (Zhu and Snyder 2003; Bertone and Snyder 2005; Cretich et al. 2006; Uttamchandani and Yao 2008), and HysTag reagent (Olsen et al. 2004). It should be noted that the use of these emerging techniques is limited to certain specialized and privileged laboratories. Also, the choice of a given proteomic approach depends on the type of biological question asked, since each proteomic technology is characterized by specific applications, technical advantages, and limitations. A typical gel-based proteomic workflow is schematically illustrated in Fig. 1. Peptide mass fingerprinting (PMF) and tandem mass spectrometry (peptide fragmentation to generate partial sequence; MS/MS) are commonly used for protein identification on 2D proteomic patterns (Thiede et al. 2005; Yates 1998; Aebersold and Goodlett 2001; Canas et al. 2006). The recent progress in the sensitivity of mass spectrometry analysis has significantly increased the applicability of proteomic technologies (Zhou and Veenstra 2008) as protein identification and profiling tool as well as determining protein interactions and the type and location of posttranslational modifications (Aebersold and Goodlett 2001; Aebersold and Mann 2003; Mann and Jensen 2003). Surface-enhanced laser desorption/ionization-time of flight (SELDI-TOF) is a suitable technique for high-throughput proteomic analysis of complex mixtures of proteins where proteins are retained on solid-phase chromatographic surfaces with specific properties and are subsequently ionized and detected by TOF MS (Merchant and Weinberger 2000; Issaq et al. 2002; Kiehntopf et al. 2007). However, this system is limited for profiling low-molecular-weight proteins (<20 kDa) (Issaq et al. 2002). In another protein profiling strategy, commonly referred as *bottom-up* or *shotgun proteomics* (multidimensional LC-MS/MS or MudPIT), complex protein mixtures are digested into peptides, followed by chromatographic separation of peptides prior to analysis by tandem mass spectrometry, and computer algorithms then map the peptides onto proteins to determine the original content of the mixture (Swanson and Washburn 2005). *Top-down proteomics* refers to the analysis of intact proteins, in contrast to bottom-up proteomics, which are not enzymatically digested prior to MS analysis (Angel et al. 2012; Messana et al. 2013). Applications of top-down

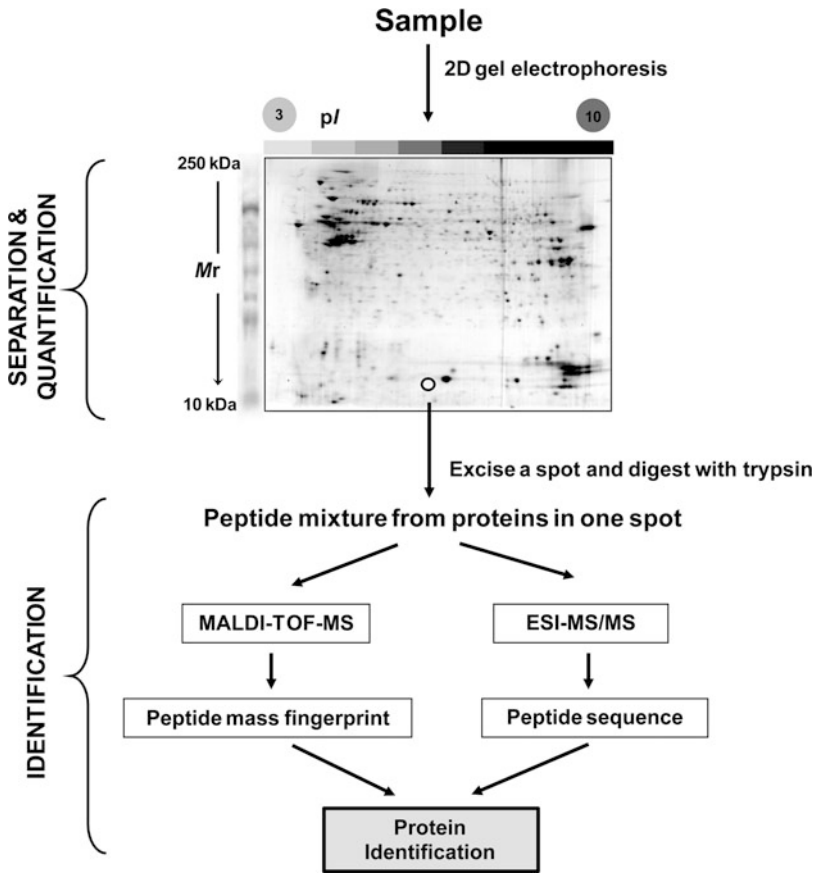

Fig. 1 A two-dimensional gel-based proteomic workflow. There are two principal steps. The *first* is separation and quantification of proteins in a sample using 2D gels. In the first dimension, proteins are separated in a pH gradient according to their molecular charge, known as isoelectric focusing. In the second dimension, the proteins are separated orthogonally by electrophoresis based on their molecular mass. The end result is a 2D gel with thousands of spots where individual spot represents a protein/peptide or a mixture. The *second* is identification of the separated proteins, typically using mass spectrometry (MS) techniques and bioinformatics. A protein spot can be excised from the 2D gel, digested with a protease and the peptides extracted. These peptides can then be analyzed using MS techniques such as matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) and electrospray ionization tandem MS (ESI-MS/MS)

proteomics include identification of protein isoforms arising from amino acid modifications, gene variants, transcript variation, and posttranslational modifications as well as proteolytic processing of proteins.

Microfluidic devices combine multiple sample preparation, purification, and separation steps in a single integrated device and emerged as an important tool for single-cell proteomics (Angel et al. 2012; Zare and Kim 2010; Chao and

Hansmeier 2013). For details of microfluidic chips, cell preparation, cell lysis, purification, and separations, readers are referred to the article by Chao and Hansmeier (2013). In a microfluidic device with microchip capillary electrophoresis (CE) platform, cells passing through an intersection are subjected to an abrupt change in solvent environment and electric field, which led to rapid lysis. Following a brief CE separation, the contents are ionized on chip and detected by MS. Several microfluidic technologies have recently been developed with the aim of creating a platform for MS-based single-cell analysis (Kelly et al. 2009, 2008; Sun et al. 2010). An emerging technology in the microfluidics field that shows perhaps the most promise for single-cell analysis involves the use of picoliter-sized aqueous droplets surrounded by an immiscible oil phase such that each droplet constitutes an individual sample vessel (Chiu 2010). While the single cell proteomics are at an early stage of development, flexibility in design of chip-based microfluidic devices, rapid analysis time, ability to automate, and successful integration of multiple functions within a single platform provide promises for high-throughput proteomic analysis at the single cell level. Nanotechnology has been used to study the dynamic concentration range of various proteins in complex biological samples, especially the low-abundance proteins (nanoproteomics) (Ray et al. 2010; Jia et al. 2013). Recently, nanomaterials have been employed for improvements of proteomic analysis especially when they are coupled with MALDI-TOF. Among the diverse classes of nanomaterials, gold nanoparticles, carbon nanotubes, silicon nanowires, and QDs are the most commonly used nanomaterials to offer several advantages in nanoproteomics such as ultralow detection, short assay time, high-throughput capability, and low sample consumption (Jia et al. 2013).

Quantifying changes in protein abundance between samples is a key goal of proteomics. Promising novel methods for high-throughput quantitation involve *label-free* approaches. Several studies have demonstrated that LC-MS peptide ions spectral peak intensities are directly proportional to the protein abundances in complex samples (America and Cordewener 2008). Another label-free method, termed spectral counting, compares the number of MS/MS spectra assigned to each protein (America and Cordewener 2008; Old et al. 2005). With controls for normalization between runs, label-free quantitation offers a simpler approach for analysis. Spectral sampling also enables ranking different proteins by their relative abundances, providing information that other methods cannot achieve (Resing and Ahn 2005).

In addition to the protein profiling and comparative proteomics, functional study of target proteins is essential in any successful proteomic study. Functional proteomic approaches are based on interactions of proteins or specific activities of proteins. Phage display is a powerful proteomic tool used to express proteins or domains of proteins (McCafferty et al. 1990; Jestin 2008). The system has played a pivotal role in mapping epitopes of monoclonal and polyclonal antibodies, defining amino acid substrate sequences, and identifying peptide ligands for drug research. Yeast two-hybrid system detects binary protein interactions by activating expression of a reporter gene upon direct binding between the two tested proteins (Fields 2005; Lalonde et al. 2008). SELDI-TOF MS has also been used to characterize

protein-protein interaction (Issaq et al. 2002). Recently, for studying the functions and interactions of proteins, protein microarrays have been developed in analogy to DNA microarrays which can also be applied for comparative studies of expression of large sets of proteins (Schweitzer et al. 2003). There are two major types of protein microarrays – forward (FPA) and reverse phase array (RPA) (Espina et al. 2003; Kikuchi and Carbone 2007). In forward protein arrays, thousands of recombinant antibodies carrying the desired specificities are arrayed on glass slides, which make it very well suited for high-throughput screening of biological samples for specific disease markers (Kusnezow and Hoheisel 2002; Wingren and Borrebaeck 2006). The BD Clontech™ Ab Microarray 500 represents a significant step in that direction. With this array, over 500 specific proteins can be assayed to detect and compare expression level of both cytosolic and membrane-bound proteins representing a broad range of biological functions, including signal transduction, cell-cycle regulation, gene transcription, and apoptosis. In contrast to using chips with immobilized antibodies to detect specific proteins, protein chips carrying the proteome of a specific organism or cell type can be made by cloning and purification of these proteins (Zhu et al. 2001). This protein microarray can then be screened on the basis of the ability of the chip to bind specific ligands or interact with specific proteins. The human ProtoArray® protein microarray (Invitrogen™) contains more than 8,000 full-length human proteins purified under native conditions. This high-content discovery tool provides highly sensitive and reproducible results enabling rapid and easy profiling of thousands of biochemical interactions. In a reverse phase microarray, tissues (Speer et al. 2005), cell lysates (Geho et al. 2005), or serum samples (Janzi et al. 2005) are spotted on the surface and probed with one antibody per analyte for a multiplex readout. Thus, this analysis evaluates the expression level of given protein in multiple samples. Both forward and reverse phase protein microarrays are novel technologies in proteomics and offer great promise for use in clinical applications.

Application of Proteomics in Islet Research

The accomplishment of human genome sequences has conferred the islet scientists with immense errands to assess the relative levels of expression of these gene products including the proteins and their posttranslational modifications in pancreatic islets. In the post-genomic era, to clarify the molecular mechanism of islet function in both normal and disease states, it is important to understand the entire gene products which regulate the phenotypes of islet cells and their ability to differentiate and secrete specific hormones. An important advantage of global protein expression profiling compared with individual gene or protein regulation studies is the ability to monitor changes in several functional groups simultaneously. It should be kept in mind that proteomics per se is not a hypothesis-driven experimental approach, but rather a hypothesis-generating *fishing expedition* where one explores the proteins that are not a priori expected to be associated with any pathophysiological conditions, which allows discovering novel proteins and

signaling networks opening new research avenues. Since its introduction in 1994, the proteomic booms continue and got considerable attention of the islet researchers as well. Improvements of the core technologies, especially advancement of protein identification by mass spectrometry and bioinformatics tools, have recently encouraged the application of proteomics to unlock the secret of islet pathophysiology. It is indeed interesting to note that the most widely used protein separation technique, the 2DGE, has been employed in 1982 for insulin granule protein profiling (Hutton et al. 1982). In those early days more than 150 protein/peptide spots were detected in a 2DG of insulin secretory granule, and some of the high-molecular-weight spots were presumed as glycoprotein. Lack of high-throughput protein identification method did not permit annotation of the granule proteins but provided an opportunity to study the functional properties of the insulin secretory granule and to dissect the molecular events of exocytosis. A similar proteomic approach has been utilized to explore the glucose-responsive granule proteins in ^{35}S-methionine-labeled rat islet and insulinoma cells, and the study showed that biosynthesis of 25 granule proteins was stimulated 15–30-fold by glucose (Guest et al. 1991). In a subsequent subproteomic study, almost after 25 years, Brunner et al. (2007) separated the INS-1E granule proteins by 1D SDS-PAGE and identified 130 different proteins by LC-MS/MS. Combining improved insulin secretory granule preparation and quantitative proteomics, Schvartz et al. (2012) have provided data on potential proteome changes during maturation of insulin secretory granule (ISG) from Golgi to the plasma membrane. They have elegantly demonstrated localization of a PC1 inhibitor, proSAAS protein in ISG, and glucose-induced modulation of proSAAS mRNA expression. It has been proposed that proSAAS may be involved in regulation of proinsulin processing in the β-cells and dysregulation of it can result in altered glucose-stimulated insulin secretion (GSIS).

Protein Profiling of Pancreatic Islets

A high-quality 2DGE reference map of the isolated pancreatic islets is essential for a 2DG-based comparative proteomic study and for generation of hypothesis. In the holy grail of protein profiling of pancreatic islets, Sanchez et al. (2001) did a pioneering work where they mapped 63 spots corresponding to 44 mouse islet protein entries. This protein map is available in the SWISS-2D database (http://us.expasy.org/ch2d/). Nicolls et al. (2003) identified 88 proteins in total from mouse islets of which 18 were already identified by Sanchez and coworkers. Continued attempts in "shooting at stars" generated another mouse islet 2DGE reference map where 124 spots corresponded to 77 distinct proteins (Ahmed and Bergsten 2005). A reference map of rat insulinoma-derived clonal INS-1E β-cell proteins has also been constructed (Fig. 2). This 2D map contains 686 valid spots, among which 118 spots corresponding to 63 different proteins have successfully identified by MALDI-TOF MS and a combination of liquid chromatography and electrospray tandem MS (LC-ESI-MS/MS). Using 2DGE and MALDI-TOF MS, the first protein map and database of human islets have been generated in 2005 where 130 spots

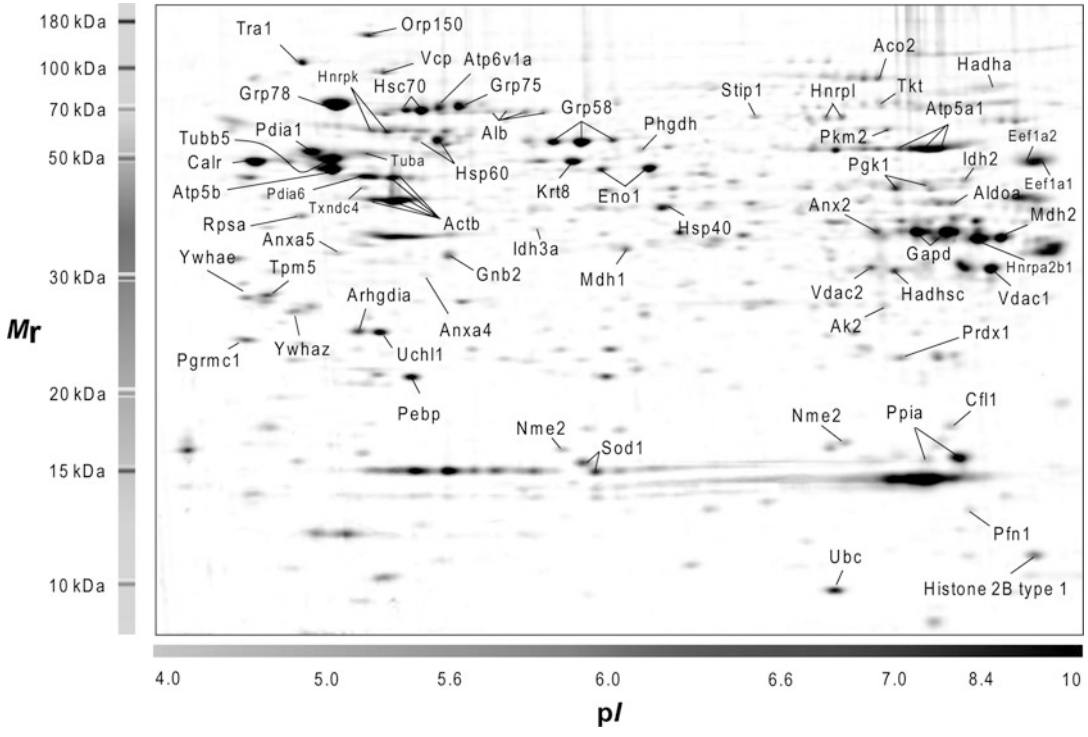

Fig. 2 2D PAGE image of INS-1E proteins. Proteins (200 µg) were loaded onto an IPG strip (pH 3–10 NL) and subsequently separated by mass on a gradient (8–16 %) SDS-PAGE gel. The gel was stained with colloidal Coomassie blue and the filtered image was generated by PDQuest software. Experimental masses and p*I*s are indicated. The gene names mark the location of the corresponding proteins on the gel. *Aco2* aconitate hydratase, mitochondrial, *Actb* β-actin, *Ak2* adenylate kinase isoenzyme 2, mitochondrial, *Alb* serum albumin, *Aldoa* fructose-bisphosphate aldolase A, *Anx2* annexin A2, *Anxa4* annexin A4, *Anxa5* annexin A5, *Arhgdia* Rho GDP dissociation inhibitor 1 (RhoGDI-α), *Atp5a1* ATP synthase subunit α, mitochondrial, *Atp6v1a* V-type proton ATPase catalytic

corresponding to 66 different protein entries were successfully identified (Ahmed et al. 2005a). A high level of reproducibility was reported among the gels, and a total of 744 protein spots were detected (Ahmed et al. 2005a). All the protein profiling studies (Sanchez et al. 2001; Nicolls et al. 2003; Ahmed and Bergsten 2005; Ahmed et al. 2005a) using 2DGE categorized the identified proteins according to cellular location and function. Any attempt to compare these studies renders déjà vu since a number of prevailing proteins were repeatedly reported and most proteins fell into the cytosolic category followed by mitochondrial and endoplasmic reticulum (reviewed by Sundsten and Ortsater 2008). In aforementioned studies a large part of the proteins have either chaperone (e.g., protein disulfide isomerase, PDI; calreticulin; 78 kDa glucose-regulated protein, GRP78; 58 kDa glucose-regulated protein, GRP58; endoplasmin) or metabolic (e.g., α-enolase, transketolase, pyruvate kinase, and hydroxyacyl-CoA dehydrogenase, SCHAD) functions. However, every laboratory blessed with the successful application of 2DGE has its own protocol for protein extraction, isoelectric focusing, and SDS-PAGE. Therefore, a reference map produced by one group cannot necessarily be useful for any other group interested in comparative islet proteomics. Moreover, since introduction in SWISS-2D database, the islet proteome map has not been updated assigning identification of more protein spots. Therefore, the technical hurdle remains for the laborious protein identification procedure even if one follows a similar protocol.

Recent advances in mass spectrometry techniques allowed the use of strong cation exchange fractionation coupled with reversed phase LC-MS/MS and

Fig. 2 (continued) subunit A, *Calr* calreticulin, *Cfl1* cofilin-1, *Eef1a1* elongation factor 1-α 1, *Eef1a2* elongation factor 1-α 2, *Eno1* α-enolase, *Gapd* glyceraldehyde-3-phosphate dehydrogenase, *Gnb2* guanine nucleotide-binding protein $G_i/G_s/G_t$ subunit β-2, *Grp58* protein disulfide-isomerase A3, *Grp75* stress-70 protein, mitochondrial (75 kDa glucose-regulated protein), *Grp78* 78 kDa glucose-regulated protein, *Hadha* trifunctional enzyme subunit α, mitochondrial, *Hadhsc* hydroxyacyl-coenzyme A dehydrogenase, mitochondrial, *Hnrpa2b1* heterogeneous nuclear ribonucleoproteins A2/B1, *Hnrpk* heterogeneous nuclear ribonucleoprotein K, *Hnrpl* heterogeneous nuclear ribonucleoprotein L, *Hsc70* Heat shock cognate 71 kDa protein (Hspa8), *Hsp40* DnaJ homolog subfamily B member 1 (heat shock 40 kDa protein 1), *Hsp60* 60 kDa heat shock protein, mitochondrial, *Idh3a* isocitrate dehydrogenase [NAD] subunit α, mitochondrial, *Ihd2* isocitrate dehydrogenase [NADP], mitochondrial, *Krt8* keratin, type II cytoskeletal 8, *Mdh1* malate dehydrogenase, cytoplasmic, *Mdh2* malate dehydrogenase, mitochondrial, *Nme2* nucleoside diphosphate kinase B, *Orp150* 150 kDa oxygen-regulated protein (hypoxia upregulated protein 1), *Pdia1* protein disulfide isomerase, *Pdia6* protein disulfide-isomerase A6, *Pebp* phosphatidylethanolamine-binding protein 1, *Pfn1* profilin-1, *Pgk1* phosphoglycerate kinase 1, *Pgrmc1* membrane-associated progesterone receptor component 1, *Phgdh* D-3-phosphoglycerate dehydrogenase, *Pkm2* pyruvate kinase isozymes M1/M2, *Ppia* peptidyl-prolyl cis-trans isomerase A (cyclophilin A), *Prdx1* peroxiredoxin-1 (thioredoxin peroxidase 2), *Rpsa* 40S ribosomal protein SA, *Sod1* superoxide dismutase [Cu-Zn], *Stip1* stress-induced-phosphoprotein 1, *Tkt* transketolase, *Tpm5* tropomyosin α-3 chain, *Tra1* endoplasmin, *Tuba* tubulin α, *Tubb5* tubulin β-5 chain, *Txndc4* thioredoxin domain-containing protein 4, *Ubc* polyubiquitin, *Uchl1* ubiquitin carboxyl-terminal hydrolase isozyme L1, *Vcp* transitional endoplasmic reticulum ATPase, *Vdac* voltage-dependent anion-selective channel protein, *Ywhae* 14-3-3 protein ε, *Ywhaz* 14-3-3 protein ζ/δ (protein kinase C inhibitor protein 1)

characterization of 2,612 proteins in the mouse islet proteome (Petyuk et al. 2008). Using nano-UPLC coupled to ESI-MS/MS, more than thousand proteins have been identified in mouse islet (unpublished data). A 2D LC-MS/MS study of the human islets characterized 3,365 proteins covering multiple signaling pathways in human islets including integrin signaling and MAP kinase, NF-κβ, and JAK/STAT pathways (Metz et al. 2006). Combined genomic and proteomic techniques have been employed for profiling of glucagon-secreting α-cells (Maziarz et al. 2005). While a total of 5,945 gene products were detected in α-cells by the gene chips alone, only 1,651 proteins were identified with high confidence using shotgun proteomics and rigorous database searching. Seven hundred sixty-two cross-mapped gene product pairs (both the gene and corresponding protein) were jointly detected by both platforms. Conversely, 126 gene products were detected exclusively by proteomics, being somehow missed by the gene chip platform (Maziarz et al. 2005). In recent years, the growing number of islet proteomic data necessitates development of bioinformatics tools for easy data handling and data mining to assign subcellular location, functional properties, molecular networks, and known potential posttranslational modifications. It is becoming essential to create a common platform for islet proteomic users integrating molecular, cellular, phenotypic, and clinical information with experimental genetic and proteomic data.

An important feature of proteomics is that protein isoforms generated by posttranslational modifications can be separated by 2DGE. Among the hundreds of different types of protein modifications, reversible protein phosphorylation is a key regulatory mechanism of cellular signaling processes (Hunter and Karin 1992; Jones and Persaud 1998; Hunter 2007). To detect global phosphoproteome profiles of islets, the advantages of the fluorescent dye Pro-Q Diamond, which is suitable for the fluorescent detection of phosphoserine-, phosphothreonine-, and phosphotyrosine-containing proteins in 2D gels directly (Steinberg et al. 2003), have been exploited, and 90 different phosphorylated proteins were detected in the 2D map (unpublished data). However, vanishingly small amounts of phosphorylated proteins in cells and lack of robotic picker in our lab precluded spot cutting and identification of most of the spots. Only a few including ATP synthase α chain, elongation factor 1-α, actin, γ-aminobutyric acid receptor α-3 subunit, and α-2-HS-glycoprotein could be successfully identified. Further isolation and purification of phosphoproteins and increasing the loading amount by pooling islet samples will possibly increase the chances for better identification for comparative studies to elucidate how posttranslational modifications regulate insulin secretion. LC-MS/MS analysis for posttranslational modifications of mouse islet proteome identified relatively abundant secretion-regulatory proteins including chromogranin A and secretogranin-2 (Petyuk et al. 2008). Then again, it's just the very minute tip of the phosphoproteome iceberg.

In recent years, several proteomic strategies have been developed/optimized for the specific enrichment and fractionation of phosphoproteins and peptides (Fila and Honys 2012; Beltran and Cutillas 2012) such as immobilized metal affinity chromatography (IMAC), metal-oxide affinity chromatography (MOAC), hydrophilic

interaction liquid chromatography (HILIC), polymer-based metal ion affinity capture (polyMAC), and strong anionic ion-exchange chromatography (SAX)/strong cationic ion-exchange chromatography (SCX). Using several strategies for sample preparation, phosphopeptide enrichment, and linear ion trap MS/MS, Han et al. (2012) have generated INS-1E phosphoproteome with 2,467 distinct phosphorylations on 1,419 phosphoproteins. They have also detected novel phosphorylation sites in several proteins including Rab proteins which may regulate insulin granule exocytosis as well as GLUT4 trafficking.

Comparative and Quantitative Islet Proteomics

The ability of the islet of Langerhans to respond with proper insulin release when the ambient glucose concentration is changed is of fundamental importance for glucose homeostasis (Rorsman 1997). In diabetes mellitus this ability is impaired with reduction in both first and second phase insulin secretion (van Haeften 2002; Scheen 2004) which leads to postprandial hyperglycemia. In the search for islet-derived factors responsible for the deranged insulin secretion, isolated islets have typically been cultured under different conditions, and it is well documented that elevated glucose concentrations (11 mM) during culture are essential for maintaining islet β-cell functions (Ling et al. 1994). Individual islets from the NOD and *ob*/*ob* mouse, which are animal models of type 1 and type 2 diabetes, respectively (Tochino 1987; Wolf 2001), have demonstrated improved glucose-stimulated insulin secretion (GSIS) after exposure to high glucose in culture medium (Bergsten and Hellman 1993; Lin et al. 1999). Such beneficial effects on GSIS have been correlated to changes in expression of individual proteins like glucokinase, glucose transporter 2, and uncoupling protein 2 (Heimberg et al. 1995; Liang et al. 1992; Chan et al. 2004). However, molecular details of the phenotypic shift in response to elevated glucose are to a large extent unknown. Since GSIS is a multifactorial event, approaches capable of determining multiple proteins simultaneously are essential for the elucidation of molecular mechanisms responsible for changes in GSIS. 2DGE and MS have been employed to characterize changes in global islet protein expressions related to exposing islets to high glucose (Ahmed and Bergsten 2005). In this proteomic study, the prohormone convertase 2 and cytokeratin 8 appeared as distinct spots on 2D gels of islets exposed to high glucose, but the proteins were barely visible on gels of freshly isolated islets (Ahmed and Bergsten 2005). The observed glucose-induced changes in global protein expression pattern suggested that enhanced insulin synthesis, restoration of insulin content and granule pools, and increased chaperone activity and antioxidants are important mechanisms underlying the augmented secretory effect of glucose in mouse islets. In comparison to other discrete hypothesis-driven studies, this report, for first time, showed orchestrated changes of multiple islet proteins that may contribute to the enhanced GSIS observed in these islets (Ahmed and Bergsten 2005). From this proteomic study, it is unclear how glucose-induced increase in cytokeratin interacts

with kinesin-microtubule system and contributes, if any, in enhanced glucose responsiveness. However, it has been conjectured that kinesin-dependent interaction of cytokeratin with microtubules is mediated by the insulin granules where cytokeratins can interact with various lipids of the insulin granules, which are anchored to microtubules through kinesin interaction. In support of this view, oligonucleotide microarray studies showed an increase in cytokeratin 19 gene in pancreatic β-cells exposed to high (25 mM) glucose compared to low glucose (5.5 mM) for 24 h (Webb et al. 2000). An increase in cytokeratin level in different cultured cells has also been reported (Alge et al. 2003; Poland et al. 2002). This type II cytoskeletal 8 protein (KRT8) has been detected in 2D maps of glucose-responding mouse islets, INS-1E cells (Ahmed et al. 2005b), and human islets (Ahmed et al. 2005a). In the search for glucose-responsive proteins, a 65 kDa protein has been detected in 2D map of mouse islets (Collins et al. 1990), and glucose-induced synthesis of this protein was blocked by D-mannoheptulose, a specific blocker of glucose phosphorylation and metabolism. However, isolation and characterization of this protein has not been performed. Among the 2,000 different islet protein spots, 1.5 % was reported to be regulated by glucose in physiological concentration range (Collins et al. 1992). In another study, depolarization-induced Ca^{2+} influx and insulin release was found to be highly correlated with phosphorylation of a 60 kDa protein (Schubart 1982). Identification of this phosphoprotein revealed an intermediate filament protein of the keratin class in hamster insulinoma cells and in pancreatic islets (Schubart and Fields 1984). This cytokeratin protein exists in both a phosphorylated and unphosphorylated state and corresponds to the gel position of KRT8 detected by Ahmed and Bergsten (2005; Ahmed et al. 2005a, b). The gel position of the unidentified glucose-responsive 65 kDa protein also matches with the KRT8. In support of the suggestion that cytokeratin may be involved in the regulation of insulin release, cytokeratins 7, 8, 18, and 19 were localized to adult endocrine pancreas and insulinoma cells by immunohistochemistry and immunoblot analysis (Schubart and Fields 1984; Kasper et al. 1991; Farina and Zedda 1992; Francini et al. 2001), and it has been well documented that disturbances in cytoskeleton of the pancreatic β-cells drastically reduced their insulin secretory function and lifetime (Blessing et al. 1993).

Comparative proteomics of glucose-responsive and glucose-nonresponsive MIN6 cells using 2D differential in-gel electrophoresis (DIGE) (Dowling et al. 2006) also contributed to the understanding of the proteins involved in GSIS. Similar to the findings of Ahmed et al. (2005), they showed that glucose-nonresponsive cells have lower ER chaperone proteins (e.g., PDI, GRP78, endoplasmin, endoplasmic reticulum protein 29) and decreased antioxidative enzymes (e.g., carbonyl reductase 3, peroxiredoxin 4, and superoxide dismutase 1), suggesting proper protein folding and protection against oxidative stress are required for glucose-stimulated insulin release from pancreatic β-cells. To dissect the molecular events associated with β-cell dysfunction and development of diabetes, Lu et al. (2008) characterized global islet protein and gene expression changes in diabetic MKR mice and compared with nondiabetic control mice. Using iTRAQ, 159 proteins were found to be differentially expressed in MKR; marked

upregulation of protein biosynthesis and endoplasmic reticulum stress pathways and parallel downregulation in insulin processing/secretion, energy utilization, and metabolism were observed. One hundred fifty-four of the differentially expressed proteins were able to be mapped to probe IDs on the microarray. In this study about 45.2 % of the differentiated proteins showed concordant changes (i.e., changes in the same direction) in mRNA, 0.6 % were discordant (i.e., having higher protein expression but lower mRNA expression), and notably 54.2 % showed changes in the proteome but not in the transcriptome. Similar approaches have been used for better understanding of the cellular and molecular functions of the signaling pathway of insulin synthesis and release in human β-cells (Jin et al. 2009). Of the 97 differentially expressed proteins involved in improved insulin release, the changes in protein and mRNA expression for 49 proteins (50.5 %) were in the same direction, while they moved oppositely for 14 proteins (14.4 %). Thirty-four of the 97 differentially expressed proteins were identified by protein expression but not by mRNA expression. The proteomic and genomic data indeed supplement each other and suggest a posttranscriptional and/or posttranslational regulation of a substantial number of differentially expressed proteins involved in islet function.

Imaging mass spectrometry (IMS) has been applied to identify differential expression of peptides in thin tissue section of pancreas of control and *ob*/*ob* mice (Minerva et al. 2008). Improvement and successful application of the IMS may lead to the discovery of new disorder-specific peptide biomarkers with potential applications in disease diagnosis. Protein expression profiling in fetal rat islets after protein restriction during gestation expanded our knowledge in the pathogenesis of type 1 and type 2 diabetes (Sparre et al. 2003).

Glucolipotoxicity and Islet Proteomics

Whereas glucose is the most important physiological stimulus for insulin secretion, chronic hyperglycemia causes desensitization and impairment of insulin release in response to glucose (Unger and Grundy 1985; Leahy et al. 1986; Eizirik et al. 1992; Purrello et al. 1996). Similarly, a high-fat intake, particularly if rich in saturated fatty acids, is associated with impaired insulin sensitivity and secretion and development of type 2 diabetes (Manco et al. 2004). It is commonly accepted that acute exposure (1–3 h) of pancreatic islets to free fatty acid leads to stimulation of GSIS both in vitro (Malaisse and Malaisse-Lagae 1968; Goberna et al. 1974; Campillo et al. 1979; Gravena et al. 2002) and in vivo (Paolisso et al. 1995; Carpentier et al. 1999; Boden and Chen 1999). However, the impact of long-term (>6 h) FFA exposure remains controversial (Ong et al. 2002; Park et al. 2009; Petricoin and Liotta 2004). The discrepancies may depend on the circulating free fatty acid levels and also on the percentage of unsaturation of the fatty acids (Dobbins et al. 2002; El-Assaad et al. 2003). It has been proposed that an increased FFA concentration alone is insufficient to induce β-cell failure and that an elevation of FFAs combined with high glucose is required to result in β-cell malfunction (Briaud et al. 2001;

Poitout and Robertson 2008), possibly as a result of accumulation of harmful lipid metabolites, e.g., ceramide in the cytoplasm (Prentki et al. 2002; Poitout 2008). This in turn is believed to interfere with the ability of the β-cells to respond to glucose with enhanced insulin secretion. Although the concept of glucolipotoxicity has become very popular and often debated, the underlying causes as well as functional consequences remain poorly defined. The main dietary fatty acids palmitate and oleate modulate the immediate early response genes, *c-fos* and *nur-77*, and a number of late genes of fatty acid metabolism including acetyl CoA carboxylase and fatty acid synthase (Roche et al. 1999). By analyzing global gene expression profiles in chronic fatty acid-treated MIN6 cells, it was found that the major groups of genes regulated by fatty acids are metabolic enzymes, transcription factors, and genes controlling distal secretory processes (Busch et al. 2002). However, in another study long-term lipid infusion in normal rats showed little influence on broad spectrum of islet-associated genes (Steil et al. 2001). A series of selected "candidate genes" have also been studied recently (Olofsson et al. 2007). The insulin (Ins1) and Glut2 transcript levels were significantly downregulated in the presence of both palmitate and oleate. Transcription of the mitochondrial acyl-CoA transporter carnitine palmitoyltransferase I (CPT1) was upregulated almost four-fold. In contrast to previous findings (Assimacopoulos-Jeannet et al. 1997; Lameloise et al. 2001; Joseph et al. 2004), the uncoupling protein UCP-2 was upregulated twofold in the presence of high glucose, but no additional effect by FFAs was detected (Olofsson et al. 2007). Therefore, it has been suggested that the failure of glucose to stimulate insulin secretion from FFA-pretreated islets is conceivably not due to increased uncoupling and reduced ATP generation (Olofsson et al. 2007). However, conflicting opinion also exists since Western blot analysis indicates that high glucose and fatty acid synergistically impaired the production of ATP in β-cells through reduction of ATP synthase β-subunit protein expression (Kohnke et al. 2007). Interestingly, we have found that the expression of ATP synthase subunit α (1.21-fold) and ATP synthase subunit β (1.16-fold) was significantly increased ($p < 0.05$) in islets isolated from high-fat-fed mice (unpublished data). In this gel-free LC-MS/MS-based proteomic study, compared to control mice, islets from high-fat-fed mice showed differential expression of 1,008 proteins. In accordance with the previous findings of fatty acid-induced inhibition of insulin gene transcription (Olofsson et al. 2007), insulin-degrading enzymes (Bhathena et al. 1985) are highly overexpressed in islets isolated from high-fat-fed mice, whereas both insulin 1 precursor and glucagon precursor are downregulated. Top 10 downregulated proteins in high-fat-diet islets include ARF (ADP ribosylation factor) GTPase-activating protein GIT1, flavin adenine dinucleotide (FAD) synthetase, CPT1, laminin subunit β2 precursor, γ-aminobutyric acid receptor subunit α-3, vesicle transport protein SEC20, reticulon 1, early endosome antigen 1, β-1,4-mannosyl-glycoprotein 4-β-N-acetylglucosaminyltransferase, and tudor domain-containing protein 5. The largely downregulated proteins include kelch-like protein 8, leucine-rich repeat-containing protein 8D, transcription factor E3, ras-related protein Rab 11B, Na^+-K^+ ATPase subunit α2 precursor, putative ATP-dependent RNA helicase DHX33, SCHAD,

F-actin capping protein subunit β, arylacetamide deacetylase, and type I inositol-3,4-bisphosphate 4-phosphatase. The vast amount of lipotoxicity proteomic data contains many novel proteins and opens new avenues for islet researchers. SELDI-TOF analysis of INS-1E cells and pancreatic islets exposed to fatty acids identified calmodulin, CPT1, and peptidylprolyl isomerase B as fatty acid-regulated proteins (Sol et al. 2009; Ortsäter et al. 2007). Using 2D-DIGE and MALDI-TOF/TOF, Maris et al. (2013) have recently demonstrated orchestrated regulation of chaperones, insulin processing and ubiquitin-related proteasomal degradation, vesicular transport and budding, as well as generation of toxic metabolites during triglyceride synthesis contribute to the fatty acid-induced β-cell dysfunction. They have shown that in the presence of high glucose, oleate and palmitate induce shunting of excess glucose and increase mitochondrial reactive oxygen species production which promote β-cell death (Schrimpe-Rutledge et al. 2012; Schubart 1982). To unravel the mechanism linking obesity to the development of T2D, Han et al. (2011) have applied iTRAQ proteomic strategy, and the differentially expressed proteins in Zucker diabetic fatty rats compared to Zucker fatty rats suggest that decreased mitochondrial oxidation, unbalance of TG/FFA cycling, and microvascular endothelial dysfunction may link obesity to T2D.

In a pioneering glucotoxicity proteomic study, Collins et al. (1992) used 2DGE of ^{35}S-methionine-labeled islet proteins that were exposed in vivo or in vitro to either low or high glucose. Approximately 2,000 protein spots were detected in 2D gels and 1.5 % and 1.6 % detectable proteins showed differential expression in response to prolonged glucose load in vitro and in vivo model, respectively. Lack of mass spectrometry did not allow protein identification of those glucose-responsive proteins. Schuit et al. (2002) purified rat β-cells and performed 2DGE of ^{35}S-methionine-labeled proteins synthesized over 4 h at 10 mM glucose after 10 days culture in low (6 mM) or high (20 mM) glucose. They distinguished two patterns of β-cell proteome change between 6 and 20 mM glucose. In one pattern two spots corresponding to proinsulin were increased almost ninefold in the presence of high glucose. Similar to this finding, on the 2DG map of INS-1E cells, proinsulin appeared as two spots. However, while one spot showed almost twofold upregulation in the presence of high glucose (25 mM), the other spot was fivefold downregulated by high glucose compared to exposure to low glucose (5.5 mM, unpublished data). The other pattern described by Schuit et al. (2002) showed suppression of translation of multiple spots close to pH 7 on 2D gels when the β-cells were exposed to 20 mM glucose. However, the identities of these protein spots were not determined. SELDI-TOF analysis of the different mitochondrial samples from INS-1E cells incubated for 5 days at 5.5, 11, 20, and 27 mM glucose showed 34 differentially expressed peaks among the samples (Nyblom et al. 2006). Such changes in expression of proteins were correlated with impairment of GSIS. Nevertheless, no identification of the differentially expressed peptides has been carried out. Comparison of INS-1E mitochondrial 2DG proteome revealed 75 spots showing twofold or more significant changes ($p < 0.05$) in relative abundance in the presence of 20 mM glucose compared to the cells exposed to 5.5 mM glucose.

Thirty-three protein spots appear only on the mitochondrial map of the INS-1E cells exposed to 5.5 mM glucose. Mitochondrial protein spots downregulated in glucotoxic conditions include ATP synthase α chain and δ chain, stress-70 protein, mitochondrial (75 kDa glucose-regulated protein; GRP75; HSPA9), malate dehydrogenase, aconitase, SCHAD, trifunctional enzyme β-subunit and NADH-cytochrome b5 reductase, and voltage-dependent anion-selective channel protein (VDAC) 2. There was upregulation of protein spots corresponding to VDAC3, trifunctional enzyme α subunit, heat shock protein 60, mitochondrial (HSP60), and 10 kDa heat shock protein, mitochondrial (HSP10). Typical to 2D map single protein appeared in multiple spots and several proteins co-migrated. For example, on the mitochondrial 2D map, five different spots corresponding to VDAC1 appeared at same molecular weight but having different p*I*. Three spots showed overexpression in response to high glucose and two other spots were downregulated. Changes in expression of a single isoform (spots) of a protein in 2DG do not necessarily signify alteration in total protein amount. Therefore, caution should be undertaken before concluding expression level of a protein on 2DG without validating the data with Western blot or other methods. In addition to the mitochondrial proteins, other differentially expressed proteins in glucotoxic condition include proinsulin; calreticulin; protein disulfide-isomerase A6 (PDIA6); PKC substrate 60.1 kDa protein; hypoxia upregulated protein 1 (ORP150); endoplasmin; heat shock cognate 71 kDa protein (HSPA8); heterogeneous nuclear ribonucleoproteins D0 and A2/B1; lamin B1; histones H2B, H3.3, and H4; and elongation factor 1-α-1. With label-free LC-MS/MS approach, 353 proteins were found to be differentially expressed in INS-1E cells exposed to 25 mM glucose compared to the cells cultured in the presence of 5.5 mM glucose (unpublished data). Ingenuity pathways analysis (IPA) revealed strong association of differentially expressed proteins with energy production, lipid metabolism, protein synthesis, DNA replication, recombination and repair, cell signaling, and metabolic disease. Using IPA we mapped biological networks affected by the differentially expressed proteins between 5.5 and 25 mM glucose-exposed INS-1E cells. Figure 3 shows the network involved in endocrine system development and function, lipid metabolism, and small-molecule biochemistry. In INS-1E cells exposed to 25 mM glucose, N-methylpurine DNA glycosylase (MPG) showed significant upregulation, while carboxypeptidase E (CPE) was fourfold downregulated. Other substantially downregulated proteins in response to high glucose exposure included chromogranin A (CGA), membrane-associated guanylate kinase (MAGI1), ubiquitin protein ligase E3 component n-recognin 5 (UBR5), and mitofusin (MFN). Although fold change is a commonly used criterion in quantitative proteomics, it does not provide an estimation of false-positive and false-negative rates that are often likely in a large-scale quantitative proteomic analysis such as in label-free LC-MS/MS quantitation. It is therefore essential for the islet researchers to adopt effective significance analysis of proteomic data which is particularly useful in the estimation of false discovery rates (Roxas and Li 2008). The proteomic data from 2DGE and LC-MS/MS analysis of the glucotoxic studies provide a comprehensive overview of the orchestrated changes in expression of

multiple proteins involved in nutrient metabolism, energy production, nucleic acid metabolism, cellular defense, glycoprotein folding, molecular transport, protein trafficking, RNA damage and repair, DNA replication, apoptosis signaling, and mDNA stability. Farnandez et al. (2008) have correlated proteomic data with metabolomic findings in glucotoxic conditions in INS-1 β-cell line. While 75 proteins showed differential expression in the presence of high glucose, only 5 of those proteins were found to be involved in the observed metabolomic alterations, suggesting allosteric regulation and/or posttranslational modifications are more important determinants of metabolite levels than enzyme expression at the protein level (Fernandez et al. 2008). Combined SELDI-TOF and 2DGE approach identified 11 different proteins coupled to altered insulin release in response to high glucose (20 mM) (Sol EM, personal communication). A recent study on human islets using 2D fractionation and LC-MS/MS (Schrimpe-Rutledge et al. 2012) has identified 256 differentially proteins in response to high glucose. This study has identified a number of glucose-regulated proteins whose functions are presently unknown in β-cells, including pleiotropic regulator 1, retinoblastoma-binding protein 6, nuclear RNA export factor 1, Bax inhibitor 1, and synaptotagmin-17.

Type 1 Diabetes and Islet Proteomics

Type 1 diabetes (T1D) is an autoimmune disorder characterized by selective destruction of insulin-producing β-cells in the pancreas resulting from the action of environmental factors on genetically predisposed individuals (Kim and Lee 2009). The prevailing view for the pathogenesis of type 1 diabetes is that an autoimmune reaction, where cytokines play an important role, causes destruction of the β-cells (Eizirik and Mandrup-Poulsen 2001). Numerous reports have demonstrated both in rodent and human islets that interleukin-1β (IL-1β) alone or in combination with interferon-γ (IFN-γ) and tumor necrosis factor α (TNF-α) affects the transcription and translation of genes, which have been implicated in β-cell destruction (Mandrup-Poulsen 2001). To search for novel proteins involved in cytokine-induced destruction of β-cells, 2DGE has been used (Andersen et al. 1995). This approach has detected upregulation of 29 proteins on 2DG image of rat islets exposed to IL-1β compared to control islets, and addition of nicotinamide reduced the upregulation of 16 IL-1β-induced proteins (Andersen et al. 1995). In a subsequent study (Andersen et al. 1997), on 2D gels of ^{35}S-methionine-labeled rat islets, 52 spots were upregulated, 47 downregulated, and 6 synthesized de novo by IL-1β. Among these 105 differentially expressed proteins, 23 protein spots were found to be significantly affected when nitric oxide (NO) production was prevented, suggesting a major role of NO-independent IL-1β-mediated regulation of gene expression (John et al. 2000). Mass spectrometric analysis allowed identification of 15 proteins, which were most profoundly altered by cytokine treatment (John et al. 2000). Also, on the transcription level similar approaches have been made to search for genes involved in the

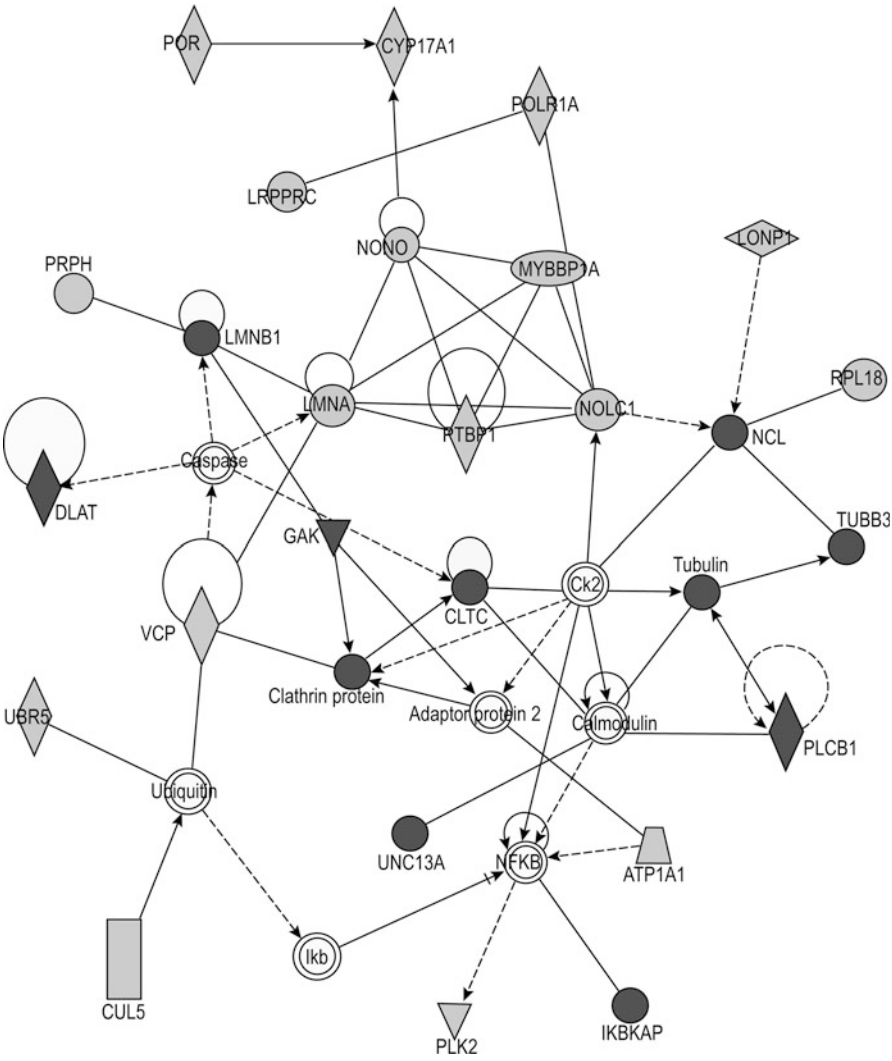

Fig. 3 Ingenuity pathway network obtained on a set of differentially regulated proteins detected in INS-1E cells exposed to 25 mM glucose compared to the cells cultured in the presence of 5.5 mM glucose. Proteins with a gray background were downregulated by high glucose, while other interacting proteins with a dark background were upregulated. *ATP1A1* ATPase, Na^+-K^+ transporting, α1 polypeptide, *CK2* casein kinase 2, *CLTC* clathrin, heavy chain, *CUL5* cullin 5, *CYP17A1* cytochrome P450, family 17, subfamily A, polypeptide 1, *DLAT* dihydrolipoamide S-acetyltransferase, *GAK* cyclin G-associated kinase, *Ikb* inhibitor of nuclear factor of κ light polypeptide gene enhancer in β-cells, β, *IKBKAP* inhibitor of kappa light polypeptide enhancer in β-cells, kinase complex-associated protein, *LMNA* lamin A/C, *LMNB1* lamin B1, *LONP1* lon peptidase 1, mitochondrial, *LRPPRC* leucine-rich PPR-motif containing, *MYBBP1A* MYB-binding protein (P160) 1a (p53-activated protein-2), *NCL* nucleolin, *NFKB* nuclear factor of κ light polypeptide gene enhancer in β-cells, *NOLC1* nucleolar and coiled-body phosphoprotein 1, *NONO* non-POU-domain-containing, octamer-binding protein, *PLCB1* phospholipase C, β

cytokine-induced alterations (Chen et al. 1999). Both these powerful approaches have yielded important information about putative genes/proteins involved in the development of the disease. Larsen et al. (2001) identified 57 different proteins from IL-1β-exposed rat islets and categorized them into several functional groups including energy transduction; glycolytic pathway; protein synthesis, chaperones, and protein folding; and signal transduction. Results of this differential expression analysis suggest that islet exposure to cytokines induces a complex pattern in β-cells comprising protective (e.g., upregulation of stress proteins) as well as deleterious (e.g., iNOS induction and NO production) events (Karlsen et al. 2001). The overall picture of the proteomic studies of type 1 diabetes is complex and does not allow us to predict which protein changes may be considered "primary" or "secondary" in importance, time, and sequence (Larsen et al. 2001). An integrative analysis method was developed combining genetic interactions using type 1 diabetes genome scan data and a high-confidence human protein interaction network (Bergholdt et al. 2007). Using this network analysis of the differentially expressed proteins in INS-1E cells exposed to cytokines, 42 of the differentially expressed proteins constituted a significant interaction network, suggesting extensive cross talk between the different proteins and the pathways in which they are involved with some proteins such as the chaperones GRP78, HSPA8, and GRP75 and the RNA synthesis/turnover proteins placed at the center of different networks. In fact all these islet proteomic studies strongly suggest a protective role of the chaperones in regulating β-cell dysfunction.

T1D is traditionally characterized by the presence of autoantibodies against β-cell proteins. However, up to 2–5 % of T1D patients exhibit no commonly detectable autoantibodies, and it has been speculated that the current T1D autoantigen panel is incomplete. To elucidate possible existence of other autoantigens in T1D, Massa et al. (2013) have applied the serological proteome analysis (SERPA) where they exploited the sera of T1D patients, who are negative for currently used T1D autoantibodies, for immunoblotting against 2DGE separated human islet proteins and detected 11 proteins by MS. Among the 11 proteins Rab GDP dissociation inhibitor β (GDIβ) is a possible novel candidate for T1D autoantigen. Proteomic approaches have also led to discover doublecortin as biomarker for β-cell injury in vitro (Jiang et al. 2013).

Fig. 3 (continued) 1, *PLK2* polo-like kinase 2, *POLR1A* polymerase (RNA) I polypeptide A, *POR* P450 (cytochrome) oxidoreductase, *PRPH* peripherin, *PTBP1* polypyrimidine tract-binding protein 1 (heterogeneous nuclear ribonucleoprotein I), *RPL18* ribosomal protein *L18*, *TUBB3* tubulin β-3, *UBR5* ubiquitin protein ligase E3 component n-recognin 5, *UNC13A* unc-13 homolog A, *VCP* valosin containing protein  = enzyme;  = peptidase;  = transporter;  = ion channel,  = transcription regulator;  = group or complex;  = kinase;  = other.  = direct interaction;  = indirect interaction;  = binding only;  = acts on; = inhibits and acts on

Pharmacoproteomics and Pancreatic Islets

A potential application of proteomics in islet research is the detection of molecular alterations in diabetes and further characterization of existing or new drug (Chapal et al. 2004). One of the prime targets for the treatment of diabetes is to enhance the insulin sensitivity so that the tissues can precisely utilize glucose and keep its plasma level within physiological limit. Rosiglitazone, a member of the thiazolidinedione (TZD) class of antidiabetic agents, improves insulin sensitivity in both liver and peripheral tissues. TZDs bind to and activate the peroxisome proliferator-activated receptor (PPARγ) and regulate the coordinated expression of multiple genes that integrate the control of energy, glucose, and lipid homeostasis and therefore contribute to increased insulin sensitivity. Rosiglitazone has been shown to prevent islet cell hyperplasia and protect islets from toxic agents (Buckingham et al. 1998; Lin et al. 2005; Kim et al. 2007). In an elegant study using 2DGE, Sanchez et al. (2002) compared protein expression profiles of pancreatic islets from obese diabetic C57BL/6J *lep*/*lep* mice and their lean littermates treated with rosiglitazone. They identified nine differentially expressed proteins between lean and obese diabetic untreated mice. The expression levels of four of those nine proteins (tropomyosin 1, profilin, profilin fragment, and fatty acid-binding protein) were significantly modulated by rosiglitazone treatment of the obese mice. In a second set of experiments designed to identify proteins potentially associated with a low islet cell mass, they compared the islet protein expression between C57BL/6J and C57BL/Ks mice. The C57BL/Ks mice have a twofold less islet cell mass as compared with the C57BL/6J (Swenne and Andersson 1984) and, as a consequence, were more susceptible to diabetes (Kaku et al. 1989; Korsgren et al. 1990). Thirty-one proteins were found to be differentially expressed between the two mouse models and two of them, tropomyosin 1 and profilin, showed the same differential pattern between C57BL/Ks and obese diabetic C57BL/6J *lep*/*lep* mice. Taken together, these results suggest that actin-binding proteins could play an important role in defective islet function. We have a long way to go for the development of novel actin-modulating drugs for treatment of diabetes similar to microtubule-interacting or microtubule-stabilizing drugs developed for cancer treatment (Trivedi et al. 2008; Anchoori et al. 2008). In a recent study, the effects of imidazolines have been tested on rat islet proteome (Jagerbrink et al. 2007) with the optimism that if it were possible to develop one of the them into a drug, this compound may be effective without risk of hypoglycemic shock in subjects with low or normal blood glucose as imidazolines increase insulin release selectively at high glucose concentrations (Efanov et al. 2001). 2DG analysis revealed 53 differentially expressed proteins between imidazoline-treated and imidazoline-nontreated islets. Of special interest among the differentially expressed proteins are those involved in protecting cells from misfolded proteins (HSP60, PDI, and calreticulin), Ca^{2+} binding (calgizzarin, calcyclin, and annexin I), and metabolism or signaling (pyruvate kinase, α-enolase, and protein kinase C inhibitor 1). However, elucidation of exact mechanism of action of imidazolines and validation of targets require further studies.

Natural medicinal plant extracts and active components have antidiabetic activity (Jung et al. 2006), and the extracellular polysaccharides (EPS) obtained from mycelia culture of *Phellinus baumii* have strong hypoglycemic activity. Proteomic study provided insights into the mechanism of antidiabetic activity of the EPS in type 1 diabetes (Kim et al. 2008). 2DGE image analysis and mass spectrometry identified 10 downregulated and 16 upregulated proteins in streptozotocin-treated diabetic mice islets. The altered level of all these differentially expressed proteins was partially or fully restored to normal level by EPS treatment. The interesting downregulated proteins in diabetic model include cholesterol esterase, PDI, and islet regenerating protein, whereas the upregulated proteins are Cu-Zn superoxide dismutase, carbonyl reductase, GRP58, hydroxymethylglutaryl-CoA synthase, and putative human mitogen-activated protein kinase activator with WD repeat-binding protein. One advantage of this study is that the proteomic data was indeed supported by transcriptomics. It would be interesting to know how alteration of certain specific protein targets modulates the development and progress of type 1 diabetes. In a recent study, using proteomic approaches it has been demonstrated that RhoGDI-α/JNK pathway might be the focus of therapeutic target for the prevention of mycophenolic acid-induced islet apoptosis (Park et al. 2009).

Conclusion

During the last decade state-of-the-art proteomic technologies including the 2DGE and label-free LC-MS/MS quantitation have been applied to dissect the pathophysiology of islet function in an increasingly manner. A vast array of proteomic data has emerged from these studies providing molecular and comprehensive snapshot of complex disease process involving the pancreatic islet cells – but just like a trace of light through an age-old dark cave, coming from the ocean of bright light. Careful analysis and powerful bioinformatics tools are still required for functional summary of the datasets and generation of new hypothesis. These proteomic studies are indeed very early steps towards better understanding of the mechanism of pathophysiology of diabetes and providing new approaches for the prevention and treatment of the disease. Almost no functional proteomics has been performed in islet research. However, improvement and easy availability of high-throughput proteomic techniques will hopefully draw the attention of more islet biologist and generate more functional data. An important feature of diabetes is that it is a progressive condition. Pancreatic β-cell function, in particular, shows a progressive decline in the prediabetic phase and in established diabetes. To clearly define islet function, therefore, we need to measure it over a period of time amalgamating multiple platforms and involving cell biologists, physiologists, geneticists, and biochemists working together with proteomic specialists. A large-scale study will allow this, together with the detection of changes in islet protein patterns, and other metabolic traits will lead to a better understanding of how susceptible gene variants and their protein products predispose to diabetes. This will also help to explore novel biomarkers to predict future diabetes, for better understanding of the

pathophysiology of diabetes, to reveal drug targets, as well as optimize the selection of molecules that interact with these targets.

Cross-References

► In vivo Biomarkers for Detection of β Cell Death

References

Abbott A (1999) A post-genomic challenge: learning to read patterns of protein synthesis. Nature 402:715–720

Adam BL, Vlahou A, Semmes OJ, Wright GL Jr (2001) Proteomic approaches to biomarker discovery in prostate and bladder cancers. Proteomics 1:1264–1270

Aebersold R, Goodlett DR (2001) Mass spectrometry in proteomics. Chem Rev 101:269–295

Aebersold R, Mann M (2003) Mass spectrometry-based proteomics. Nature 422:198–207

Aggarwal K, Choe LH, Lee KH (2006) Shotgun proteomics using the iTRAQ isobaric tags. Brief Funct Genomic Proteomic 5:112–120

Ahmed M, Bergsten P (2005) Glucose-induced changes of multiple mouse islet proteins analysed by two-dimensional gel electrophoresis and mass spectrometry. Diabetologia 48:477–485

Ahmed M, Forsberg J, Bergsten P (2005a) Protein profiling of human pancreatic islets by two-dimensional gel electrophoresis and mass spectrometry. J Proteome Res 4:931–940

Ahmed M, Bergsten P, McCarthy M, Rorsman P (2005b) Protein profiling of INS-1E cells by two-dimensional gel electrophoresis and mass spectrometry. Diabetologia 48(A):162

Alge CS, Suppmann S, Priglinger SG, Neubauer AS, May CA, Hauck S, Welge-Lussen U, Ueffing M, Kampik A (2003) Comparative proteome analysis of native differentiated and cultured dedifferentiated human RPE cells. Invest Ophthalmol Vis Sci 44:3629–3641

America AH, Cordewener JH (2008) Comparative LC-MS: a landscape of peaks and valleys. Proteomics 8:731–749

Anchoori RK, Kortenhorst MS, Hidalgo M, Sarkar T, Hallur G, Bai R, Diest PJ, Hamel E, Khan SR (2008) Novel microtubule-interacting phenoxy pyridine and phenyl sulfanyl pyridine analogues for cancer therapy. J Med Chem 51:5953–5957

Andersen HU, Larsen PM, Fey SJ, Karlsen AE, Mandrup-Poulsen T, Nerup J (1995) Two-dimensional gel electrophoresis of rat islet proteins. Interleukin 1 β-induced changes in protein expression are reduced by L-arginine depletion and nicotinamide. Diabetes 44:400–407

Andersen HU, Fey SJ, Larsen PM, Nawrocki A, Hejnaes KR, Mandrup-Poulsen T, Nerup J (1997) Interleukin-1β induced changes in the protein expression of rat islets: a computerized database. Electrophoresis 18:2091–2103

Anderson NL, Anderson NG (1998) Proteome and proteomics: new technologies, new concepts, and new words. Electrophoresis 19:1853–1861

Anderson NG, Matheson A, Anderson NL (2001) Back to the future: the human protein index (HPI) and the agenda for post-proteomic biology. Proteomics 1:3–12

Angel TE, Aryal UK, Hengel SM, Baker ES, Kelly RT, Robinson EW, Smith RD (2012) Mass spectrometry-based proteomics: existing capabilities and future directions. Chem Soc Rev 41:3912–3928

Assimacopoulos-Jeannet F, Thumelin S, Roche E, Esser V, McGarry JD, Prentki M (1997) Fatty acids rapidly induce the carnitine palmitoyltransferase I gene in the pancreatic β-cell line INS-1. J Biol Chem 272:1659–1664

Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. Cell 136:215–233

Beltran L, Cutillas PR (2012) Advances in phosphopeptide enrichment techniques for phosphoproteomics. Amino Acids 43:1009–1024

Bergholdt R, Storling ZM, Lage K, Karlberg EO, Olason PI, Aalund M, Nerup J, Brunak S, Workman CT, Pociot F (2007) Integrative analysis for finding genes and networks involved in diabetes and other complex diseases. Genome Biol 8:R253

Bergsten P, Hellman B (1993) Glucose-induced cycles of insulin release can be resolved into distinct periods of secretory activity. Biochem Biophys Res Commun 192:1182–1188

Bertone P, Snyder M (2005) Advances in functional protein microarray technology. FEBS J 272:5400–5411

Bhathena SJ, Timmers KI, Oie HK, Voyles NR, Recant L (1985) Cytosolic insulin-degrading activity in islet-derived tumor cell lines and in normal rat islets. Diabetes 34:121–128

Binz PA, Muller M, Hoogland C, Zimmermann C, Pasquarello C, Corthals G, Sanchez JC, Hochstrasser DF, Appel RD (2004) The molecular scanner: concept and developments. Curr Opin Biotechnol 15:17–23

Blessing M, Ruther U, Franke WW (1993) Ectopic synthesis of epidermal cytokeratins in pancreatic islet cells of transgenic mice interferes with cytoskeletal order and insulin production. J Cell Biol 120:743–755

Boden G, Chen X (1999) Effects of fatty acids and ketone bodies on basal insulin secretion in type 2 diabetes. Diabetes 48:577–583

Boden G, Chen X, Rosner J, Barton M (1995) Effects of a 48-h fat infusion on insulin secretion and glucose utilization. Diabetes 44:1239–1242

Briaud I, Harmon JS, Kelpe CL, Segu VB, Poitout V (2001) Lipotoxicity of the pancreatic β-cell is associated with glucose-dependent esterification of fatty acids into neutral lipids. Diabetes 50:315–321

Brunner Y, Coute Y, Iezzi M, Foti M, Fukuda M, Hochstrasser DF, Wollheim CB, Sanchez JC (2007) Proteomics analysis of insulin secretory granules. Mol Cell Proteomics 6:1007–1017

Buckingham RE, Al-Barazanji KA, Toseland CD, Slaughter M, Connor SC, West A, Bond B, Turner NC, Clapham JC (1998) Peroxisome proliferator-activated receptor-γ agonist, rosiglitazone, protects against nephropathy and pancreatic islet abnormalities in Zucker fatty rats. Diabetes 47:1326–1334

Busch AK, Cordery D, Denyer GS, Biden TJ (2002) Expression profiling of palmitate- and oleate-regulated genes provides novel insights into the effects of chronic lipid exposure on pancreatic β-cell function. Diabetes 51:977–987

Cahill DJ, Nordhoff E, O'Brien J, Klose J, Eickhoff H, Lehrach H (2001) Bridging genomics and proteomics. In: Pennington SR, Dunn MJ (eds) Proteomics: from protein sequence to function. BIOS Scientific Publishers Ltd, Oxford, pp 1–22

Campillo JE, Luyckx AS, Torres MD, Lefebvre PJ (1979) Effect of oleic acid on insulin secretion by the isolated perfused rat pancreas. Diabetologia 16:267–273

Canas B, Lopez-Ferrer D, Ramos-Fernandez A, Camafeita E, Calvo E (2006) Mass spectrometry technologies for proteomics. Brief Funct Genomic Proteomic 4:295–320

Carpentier A, Mittelman SD, Lamarche B, Bergman RN, Giacca A, Lewis GF (1999) Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. Am J Physiol 276:E1055–E1066

Celis JE, Ostergaard M, Jensen NA, Gromova I, Rasmussen HH, Gromov P (1998) Human and mouse proteomic databases: novel resources in the protein universe. FEBS Lett 430:64–72

Chan CB, Saleh MC, Koshkin V, Wheeler MB (2004) Uncoupling protein 2 and islet function. Diabetes 53(Suppl 1):S136–S142

Chao TC, Hansmeier N (2013) Microfluidic devices for high-throughput proteome analyses. Proteomics 13:467–479

Chapal N, Molina L, Molina F, Laplanche M, Pau B, Petit P (2004) Pharmacoproteomic approach to the study of drug mode of action, toxicity, and resistance: applications in diabetes and cancer. Fundam Clin Pharmacol 18:413–422

Chen MC, Schuit F, Eizirik DL (1999) Identification of IL-1β-induced messenger RNAs in rat pancreatic β cells by differential display of messenger RNA. Diabetologia 42:1199–1203
Chiu DT (2010) Interfacing droplet microfluidics with chemical separation for cellular analysis. Anal Bioanal Chem 397:3179–3183
Collins HW, Buettger C, Matschinsky F (1990) High-resolution two-dimensional polyacrylamide gel electrophoresis reveals a glucose-response protein of 65 kDa in pancreatic islet cells. Proc Natl Acad Sci U S A 87:5494–5498
Collins H, Najafi H, Buettger C, Rombeau J, Settle RG, Matschinsky FM (1992) Identification of glucose response proteins in two biological models of β-cell adaptation to chronic high glucose exposure. J Biol Chem 267:1357–1366
Cretich M, Damin F, Pirri G, Chiari M (2006) Protein and peptide arrays: recent trends and new directions. Biomol Eng 23:77–88
Dobbins RL, Szczepaniak LS, Myhill J, Tamura Y, Uchino H, Giacca A, McGarry JD (2002) The composition of dietary fat directly influences glucose-stimulated insulin secretion in rats. Diabetes 51:1825–1833
Domon B, Broder S (2004) Implications of new proteomics strategies for biology and medicine. J Proteome Res 3:253–260
Dowling P, O'Driscoll L, O'Sullivan F, Dowd A, Henry M, Jeppesen PB, Meleady P, Clynes M (2006) Proteomic screening of glucose-responsive and glucose non-responsive MIN-6 β cells reveals differential expression of proteins involved in protein folding, secretion and oxidative stress. Proteomics 6:6578–6587
Efanov AM, Zaitsev SV, Mest HJ, Raap A, Appelskog IB, Larsson O, Berggren PO, Efendic S (2001) The novel imidazoline compound BL11282 potentiates glucose-induced insulin secretion in pancreatic β-cells in the absence of modulation of K_{ATP} channel activity. Diabetes 50:797–802
Eizirik DL, Mandrup-Poulsen T (2001) A choice of death – the signal-transduction of immune-mediated β-cell apoptosis. Diabetologia 44:2115–2133
Eizirik DL, Korbutt GS, Hellerstrom C (1992) Prolonged exposure of human pancreatic islets to high glucose concentrations in vitro impairs the β-cell function. J Clin Invest 90:1263–1268
El-Assaad W, Buteau J, Peyot ML, Nolan C, Roduit R, Hardy S, Joly E, Dbaibo G, Rosenberg L, Prentki M (2003) Saturated fatty acids synergize with elevated glucose to cause pancreatic β-cell death. Endocrinology 144:4154–4163
Espina V, Mehta AI, Winters ME, Calvert V, Wulfkuhle J, Petricoin EF 3rd, Liotta LA (2003) Protein microarrays: molecular profiling technologies for clinical specimens. Proteomics 3:2091–2100
Ezzell C (2002) Proteins rule. Sci Am 286:40–47
Farina V, Zedda M (1992) On the expression of cytokeratins and their distribution in some rabbit gland tissues. Eur J Histochem 36:479–488
Fenselau C (2007) A review of quantitative methods for proteomic studies. J Chromatogr B Analyt Technol Biomed Life Sci 855:14–20
Fernandez C, Fransson U, Hallgard E, Spegel P, Holm C, Krogh M, Warell K, James P, Mulder H (2008) Metabolomic and proteomic analysis of a clonal insulin-producing β-cell line (INS-1 832/13). J Proteome Res 7:400–411
Fields S (2005) High-throughput two-hybrid analysis. The promise and the peril. FEBS J 272:5391–5399
Figeys D (2003) Proteomics in 2002: a year of technical development and wide-ranging applications. Anal Chem 75:2891–2905
Fila J, Honys D (2012) Enrichment techniques employed in phosphoproteomics. Amino Acids 43:1025–1047
Florens L, Washburn MP (2006) Proteomic analysis by multidimensional protein identification technology. Methods Mol Biol 328:159–175
Francini F, Del Zotto H, Gagliardino JJ (2001) Effect of an acute glucose overload on Islet cell morphology and secretory function in the toad. Gen Comp Endocrinol 122:130–138

Geho D, Lahar N, Gurnani P, Huebschman M, Herrmann P, Espina V, Shi A, Wulfkuhle J, Garner H, Petricoin E 3rd, Liotta LA, Rosenblatt KP (2005) Pegylated, steptavidin-conjugated quantum dots are effective detection elements for reverse-phase protein microarrays. Bioconjug Chem 16:559–566

Goberna R, Tamarit J Jr, Osorio J, Fussganger R, Tamarit J, Pfeiffer EF (1974) Action of B-hydroxy butyrate, acetoacetate and palmitate on the insulin release in the perfused isolated rat pancreas. Horm Metab Res 6:256–260

Gorg A, Obermaier C, Boguth G, Harder A, Scheibe B, Wildgruber R, Weiss W (2000) The current state of two-dimensional electrophoresis with immobilized pH gradients. Electrophoresis 21:1037–1053

Gorg A, Weiss W, Dunn MJ (2004) Current two-dimensional electrophoresis technology for proteomics. Proteomics 4:3665–3685

Gravena C, Mathias PC, Ashcroft SJ (2002) Acute effects of fatty acids on insulin secretion from rat and human islets of Langerhans. J Endocrinol 173:73–80

Graves PR, Haystead TA (2003) A functional proteomics approach to signal transduction. Recent Prog Horm Res 58:1–24

Guest PC, Bailyes EM, Rutherford NG, Hutton JC (1991) Insulin secretory granule biogenesis. Co-ordinate regulation of the biosynthesis of the majority of constituent proteins. Biochem J 274(Pt 1):73–78

Gygi SP, Rochon Y, Franza BR, Aebersold R (1999) Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 19:1720–1730

Han D, Moon S, Kim H, Choi SE, Lee SJ, Park KS, Jun H, Kang Y, Kim Y (2011) Detection of differential proteomes associated with the development of type 2 diabetes in the Zucker rat model using the iTRAQ technique. J Proteome Res 10:564–577

Han D, Moon S, Kim Y, Ho WK, Kim K, Kang Y, Jun H, Kim Y (2012) Comprehensive phosphoproteome analysis of INS-1 pancreatic β-cells using various digestion strategies coupled with liquid chromatography-tandem mass spectrometry. J Proteome Res 11:2206–2223

Heimberg H, De Vos A, Pipeleers D, Thorens B, Schuit F (1995) Differences in glucose transporter gene expression between rat pancreatic α- and β-cells are correlated to differences in glucose transport but not in glucose utilization. J Biol Chem 270:8971–8975

Hoogland C, Sanchez JC, Walther D, Baujard V, Baujard O, Tonella L, Hochstrasser DF, Appel RD (1999) Two-dimensional electrophoresis resources available from ExPASy. Electrophoresis 20:3568–3571

Hunter T (2007) The age of crosstalk: phosphorylation, ubiquitination, and beyond. Mol Cell 28:730–738

Hunter T, Karin M (1992) The regulation of transcription by phosphorylation. Cell 70:375–387

Hutton JC, Penn EJ, Peshavaria M (1982) Isolation and characterisation of insulin secretory granules from a rat islet cell tumour. Diabetologia 23:365–373

Issaq HJ, Veenstra TD, Conrads TP, Felschow D (2002) The SELDI-TOF MS approach to proteomics: protein profiling and biomarker identification. Biochem Biophys Res Commun 292:587–592

Jagerbrink T, Lexander H, Palmberg C, Shafqat J, Sharoyko V, Berggren PO, Efendic S, Zaitsev S, Jornvall H (2007) Differential protein expression in pancreatic islets after treatment with an imidazoline compound. Cell Mol Life Sci 64:1310–1316

Janzi M, Odling J, Pan-Hammarstrom Q, Sundberg M, Lundeberg J, Uhlen M, Hammarstrom L, Nilsson P (2005) Serum microarrays for large scale screening of protein levels. Mol Cell Proteomics 4:1942–1947

Jestin JL (2008) Functional cloning by phage display. Biochimie 90:1273–1278

Jia L, Lu Y, Shao J, Liang XJ, Xu Y (2013) Nanoproteomics: a new sprout from emerging links between nanotechnology and proteomics. Trends Biotechnol 31:99–107

Jiang L, Brackeva B, Stange G, Verhaeghen K, Costa O, Couillard-Despres S, Rotheneichner P, Aigner L, Van Schravendijk C, Pipeleers D, Ling Z, Gorus F, Martens GA (2013) LC-MS/MS

identification of doublecortin as abundant β cell-selective protein discharged by damaged β cells in vitro. J Proteomics 80:268–280
Jin J, Park J, Kim K, Kang Y, Park SG, Kim JH, Park KS, Jun H, Kim Y (2009) Detection of differential proteomes of human β-cells during islet-like differentiation using iTRAQ labeling. J Proteome Res 8:1393
John NE, Andersen HU, Fey SJ, Larsen PM, Roepstorff P, Larsen MR, Pociot F, Karlsen AE, Nerup J, Green IC, Mandrup-Poulsen T (2000) Cytokine- or chemically derived nitric oxide alters the expression of proteins detected by two-dimensional gel electrophoresis in neonatal rat islets of Langerhans. Diabetes 49:1819–1829
Jones PM, Persaud SJ (1998) Protein kinases, protein phosphorylation, and the regulation of insulin secretion from pancreatic β-cells. Endocrinol Rev 19:429–461
Joseph JW, Koshkin V, Saleh MC, Sivitz WI, Zhang CY, Lowell BB, Chan CB, Wheeler MB (2004) Free fatty acid-induced β-cell defects are dependent on uncoupling protein 2 expression. J Biol Chem 279:51049–51056
Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK (2006) Antidiabetic agents from medicinal plants. Curr Med Chem 13:1203–1218
Kaku K, Province M, Permutt MA (1989) Genetic analysis of obesity-induced diabetes associated with a limited capacity to synthesize insulin in C57BL/KS mice: evidence for polygenic control. Diabetologia 32:636–643
Karlsen AE, Sparre T, Nielsen K, Nerup J, Pociot F (2001) Proteome analysis – a novel approach to understand the pathogenesis of type 1 diabetes mellitus. Dis Markers 17:205–216
Kasper M, von Dorsche H, Stosiek P (1991) Changes in the distribution of intermediate filament proteins and collagen IV in fetal and adult human pancreas. I. Localization of cytokeratin polypeptides. Histochemistry 96:271–277
Kelly RT, Tang K, Irimia D, Toner M, Smith RD (2008) Elastomeric microchip electrospray emitter for stable cone-jet mode operation in the nanoflow regime. Anal Chem 80:3824–3831
Kelly RT, Page JS, Marginean I, Tang K, Smith RD (2009) Dilution-free analysis from picoliter droplets by nano-electrospray ionization mass spectrometry. Angew Chem Int Ed Engl 48:6832–6835
Kiehntopf M, Siegmund R, Deufel T (2007) Use of SELDI-TOF mass spectrometry for identification of new biomarkers: potential and limitations. Clin Chem Lab Med 45:1435–1449
Kikuchi T, Carbone DP (2007) Proteomics analysis in lung cancer: challenges and opportunities. Respirology 12:22–28
Kim HS, Lee MS (2009) Role of innate immunity in triggering and tuning of autoimmune diabetes. Curr Mol Med 9:30–44
Kim EK, Kwon KB, Koo BS, Han MJ, Song MY, Song EK, Han MK, Park JW, Ryu DG, Park BH (2007) Activation of peroxisome proliferator-activated receptor-γ protects pancreatic β-cells from cytokine-induced cytotoxicity via NFκB pathway. Int J Biochem Cell Biol 39:1260–1275
Kim SW, Hwang HJ, Baek YM, Lee SH, Hwang HS, Yun JW (2008) Proteomic and transcriptomic analysis for streptozotocin-induced diabetic rat pancreas in response to fungal polysaccharide treatments. Proteomics 8:2344–2361
Klose J (1975) Protein mapping by combined isoelectric focusing and electrophoresis of mouse tissues. A novel approach to testing for induced point mutations in mammals. Humangenetik 26:231–243
Kohnke R, Mei J, Park M, York DA, Erlanson-Albertsson C (2007) Fatty acids and glucose in high concentration down-regulates ATP synthase β-subunit protein expression in INS-1 cells. Nutr Neurosci 10:273–278
Korsgren O, Jansson L, Sandler S, Andersson A (1990) Hyperglycemia-induced B cell toxicity. The fate of pancreatic islets transplanted into diabetic mice is dependent on their genetic background. J Clin Invest 86:2161–2168
Kusnezow W, Hoheisel JD (2002) Antibody microarrays: promises and problems. Biotechniques 33(Suppl):14–23

Lalonde S, Ehrhardt DW, Loque D, Chen J, Rhee SY, Frommer WB (2008) Molecular and cellular approaches for the detection of protein-protein interactions: latest techniques and current limitations. Plant J 53:610–635

Lameloise N, Muzzin P, Prentki M, Assimacopoulos-Jeannet F (2001) Uncoupling protein 2: a possible link between fatty acid excess and impaired glucose-induced insulin secretion. Diabetes 50:803–809

Larsen PM, Fey SJ, Larsen MR, Nawrocki A, Andersen HU, Kahler H, Heilmann C, Voss MC, Roepstorff P, Pociot F, Karlsen AE, Nerup J (2001) Proteome analysis of interleukin-1-β-induced changes in protein expression in rat islets of Langerhans. Diabetes 50:1056–1063

Leahy JL, Cooper HE, Deal DA, Weir GC (1986) Chronic hyperglycemia is associated with impaired glucose influence on insulin secretion. A study in normal rats using chronic in vivo glucose infusions. J Clin Invest 77:908–915

Liang Y, Najafi H, Smith RM, Zimmerman EC, Magnuson MA, Tal M, Matschinsky FM (1992) Concordant glucose induction of glucokinase, glucose usage, and glucose-stimulated insulin release in pancreatic islets maintained in organ culture. Diabetes 41:792–806

Lin JM, Sternesjo J, Sandler S, Bergsten P (1999) Preserved pulsatile insulin release from prediabetic mouse islets. Endocrinology 140:3999–4004

Lin CY, Gurlo T, Haataja L, Hsueh WA, Butler PC (2005) Activation of peroxisome proliferator-activated receptor-γ by rosiglitazone protects human islet cells against human islet amyloid polypeptide toxicity by a phosphatidylinositol 3′-kinase-dependent pathway. J Clin Endocrinol Metab 90:6678–6686

Ling Z, Hannaert JC, Pipeleers D (1994) Effect of nutrients, hormones and serum on survival of rat islet β cells in culture. Diabetologia 37:15–21

Lopez JL (2007) Two-dimensional electrophoresis in proteome expression analysis. J Chromatogr B Analyt Technol Biomed Life Sci 849:190–202

Lu H, Yang Y, Allister EM, Wijesekara N, Wheeler MB (2008) The identification of potential factors associated with the development of type 2 diabetes: a quantitative proteomics approach. Mol Cell Proteomics 7:1434–1451

Malaisse WJ, Malaisse-Lagae F (1968) Stimulation of insulin secretion by noncarbohydrate metabolites. J Lab Clin Med 72:438–448

Manco M, Calvani M, Mingrone G (2004) Effects of dietary fatty acids on insulin sensitivity and secretion. Diabetes Obes Metab 6:402–413

Mandrup-Poulsen T (2001) β-cell apoptosis: stimuli and signaling. Diabetes 50(Suppl 1):S58–S63

Mann M, Jensen ON (2003) Proteomic analysis of post-translational modifications. Nat Biotechnol 21:255–261

Maris M, Waelkens E, Cnop M, D'Hertog W, Cunha DA, Korf H, Koike T, Overbergh L, Mathieu C (2011) Oleate-induced β cell dysfunction and apoptosis: a proteomic approach to glucolipotoxicity by an unsaturated fatty acid. J Proteome Res 10:3372–3385

Maris M, Robert S, Waelkens E, Derua R, Hernangomez MH, D'Hertog W, Cnop M, Mathieu C, Overbergh L (2013) Role of the saturated nonesterified fatty acid palmitate in β cell dysfunction. J Proteome Res 12:347–362

Mason TM, Goh T, Tchipashvili V, Sandhu H, Gupta N, Lewis GF, Giacca A (1999) Prolonged elevation of plasma free fatty acids desensitizes the insulin secretory response to glucose in vivo in rats. Diabetes 48:524–530

Massa O, Alessio M, Russo L, Nardo G, Bonetto V, Bertuzzi F, Paladini A, Iafusco D, Patera P, Federici G, Not T, Tiberti C, Bonfanti R, Barbetti F (2013) Serological Proteome Analysis (SERPA) as a tool for the identification of new candidate autoantigens in type 1 diabetes. J Proteomics 82:263–273

Maurya P, Meleady P, Dowling P, Clynes M (2007) Proteomic approaches for serum biomarker discovery in cancer. Anticancer Res 27:1247–1255

Maziarz M, Chung C, Drucker DJ, Emili A (2005) Integrating global proteomic and genomic expression profiles generated from islet α cells: opportunities and challenges to deriving reliable biological inferences. Mol Cell Proteomics 4:458–474

McCafferty J, Griffiths AD, Winter G, Chiswell DJ (1990) Phage antibodies: filamentous phage displaying antibody variable domains. Nature 348:552–554

Merchant M, Weinberger SR (2000) Recent advancements in surface-enhanced laser desorption/ionization- time of flight-mass spectrometry. Electrophoresis 21:1164–1177

Messana I, Cabras T, Iavarone F, Vincenzoni F, Urbani A, Castagnola M (2013) Unraveling the different proteomic platforms. J Sep Sci 36:128–139

Metz TO, Jacobs JM, Gritsenko MA, Fontes G, Qian WJ, Camp DG 2nd, Poitout V, Smith RD (2006) Characterization of the human pancreatic islet proteome by two-dimensional LC/MS/MS. J Proteome Res 5:3345–3354

Minerva L, Clerens S, Baggerman G, Arckens L (2008) Direct profiling and identification of peptide expression differences in the pancreas of control and *ob*/*ob* mice by imaging mass spectrometry. Proteomics 8:3763–3774

Molloy MP, Brzezinski EE, Hang J, McDowell MT, VanBogelen RA (2003) Overcoming technical variation and biological variation in quantitative proteomics. Proteomics 3:1912–1919

Nicolls MR, D'Antonio JM, Hutton JC, Gill RG, Czwornog JL, Duncan MW (2003) Proteomics as a tool for discovery: proteins implicated in Alzheimer's disease are highly expressed in normal pancreatic islets. J Proteome Res 2:199–205

Nyblom HK, Thorn K, Ahmed M, Bergsten P (2006) Mitochondrial protein patterns correlating with impaired insulin secretion from INS-1E cells exposed to elevated glucose concentrations. Proteomics 6:5193–5198

O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250:4007–4021

Old WM, Meyer-Arendt K, Aveline-Wolf L, Pierce KG, Mendoza A, Sevinsky JR, Resing KA, Ahn NG (2005) Comparison of label-free methods for quantifying human proteins by shotgun proteomics. Mol Cell Proteomics 4:1487–1502

Olofsson CS, Collins S, Bengtsson M, Eliasson L, Salehi A, Shimomura K, Tarasov A, Holm C, Ashcroft F, Rorsman P (2007) Long-term exposure to glucose and lipids inhibits glucose-induced insulin secretion downstream of granule fusion with plasma membrane. Diabetes 56:1888–1897

Olsen JV, Andersen JR, Nielsen PA, Nielsen ML, Figeys D, Mann M, Wisniewski JR (2004) HysTag – a novel proteomic quantification tool applied to differential display analysis of membrane proteins from distinct areas of mouse brain. Mol Cell Proteomics 3:82–92

Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, Mann M (2002) Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Mol Cell Proteomics 1:376–386

Ortsäter H, Sundsten T, Lin JM, Bergsten P (2007) Evaluation of the SELDI-TOF MS technique for protein profiling of pancreatic islets exposed to glucose and oleate. Proteomics 7:3105–3115

Paolisso G, Gambardella A, Amato L, Tortoriello R, D'Amore A, Varricchio M, D'Onofrio F (1995) Opposite effects of short- and long-term fatty acid infusion on insulin secretion in healthy subjects. Diabetologia 38:1295–1299

Park YJ, Ahn HJ, Chang HK, Kim JY, Huh KH, Kim MS, Kim YS (2009) The RhoGDI-α/JNK signaling pathway plays a significant role in mycophenolic acid-induced apoptosis in an insulin-secreting cell line. Cell Signal 21:356–364

Petricoin EF, Liotta LA (2004) SELDI-TOF-based serum proteomic pattern diagnostics for early detection of cancer. Curr Opin Biotechnol 15:24–30

Petyuk VA, Qian WJ, Hinault C, Gritsenko MA, Singhal M, Monroe ME, Camp DG 2nd, Kulkarni RN, Smith RD (2008) Characterization of the mouse pancreatic islet proteome and comparative analysis with other mouse tissues. J Proteome Res 7:3114–3126

Poitout V (2008) Glucolipotoxicity of the pancreatic β-cell: myth or reality. Biochem Soc Trans 36:901–904

Poitout V, Robertson RP (2008) Glucolipotoxicity: fuel excess and β-cell dysfunction. Endocrinol Rev 29:351–366

Poland J, Sinha P, Siegert A, Schnolzer M, Korf U, Hauptmann S (2002) Comparison of protein expression profiles between monolayer and spheroid cell culture of HT-29 cells revealed fragmentation of CK18 in three-dimensional cell culture. Electrophoresis 23:1174–1184

Prentki M, Joly E, El-Assaad W, Roduit R (2002) Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in β-cell adaptation and failure in the etiology of diabetes. Diabetes 51 (Suppl 3):S405–S413

Purrello F, Rabuazzo AM, Anello M, Patane G (1996) Effects of prolonged glucose stimulation on pancreatic β cells: from increased sensitivity to desensitization. Acta Diabetol 33:253–256

Rabilloud T (2012) The whereabouts of 2D gels in quantitative proteomics. Methods Mol Biol 893:25–35

Ray S, Chandra H, Srivastava S (2010) Nanotechniques in proteomics: current status, promises and challenges. Biosens Bioelectron 25:2389–2401

Resing KA, Ahn NG (2005) Proteomics strategies for protein identification. FEBS Lett 579:885–889

Roche E, Buteau J, Aniento I, Reig JA, Soria B, Prentki M (1999) Palmitate and oleate induce the immediate-early response genes *c-fos* and *nur-77* in the pancreatic β-cell line INS-1. Diabetes 48:2007–2014

Rogowska-Wrzesinska A, Le Bihan MC, Thaysen-Andersen M, Roepstorff P (2013) 2D gels still have a niche in proteomics. J Proteomics 88:4–13

Rorsman P (1997) The pancreatic β-cell as a fuel sensor: an electrophysiologist's viewpoint. Diabetologia 40:487–495

Roxas BA, Li Q (2008) Significance analysis of microarray for relative quantitation of LC/MS data in proteomics. BMC Bioinforma 9:187

Sali A, Glaeser R, Earnest T, Baumeister W (2003) From words to literature in structural proteomics. Nature 422:216–225

Sanchez JC, Chiappe D, Converset V, Hoogland C, Binz PA, Paesano S, Appel RD, Wang S, Sennitt M, Nolan A, Cawthorne MA, Hochstrasser DF (2001) The mouse SWISS-2D PAGE database: a tool for proteomics study of diabetes and obesity. Proteomics 1:136–163

Sanchez JC, Converset V, Nolan A, Schmid G, Wang S, Heller M, Sennitt MV, Hochstrasser DF, Cawthorne MA (2002) Effect of rosiglitazone on the differential expression of diabetes-associated proteins in pancreatic islets of C57Bl/6 *lep/lep* mice. Mol Cell Proteomics 1:509–516

Scheen AJ (2004) Pathophysiology of insulin secretion. Ann Endocrinol (Paris) 65:29–36

Schrimpe-Rutledge AC, Fontes G, Gritsenko MA, Norbeck AD, Anderson DJ, Waters KM, Adkins JN, Smith RD, Poitout V, Metz TO (2012) Discovery of novel glucose-regulated proteins in isolated human pancreatic islets using LC-MS/MS-based proteomics. J Proteome Res 11:3520–3532

Schubart UK (1982) Regulation of protein phosphorylation in hamster insulinoma cells. Identification of Ca^{2+}-regulated cytoskeletal and cAMP-regulated cytosolic phosphoproteins by two-dimensional electrophoresis. J Biol Chem 257:12231–12238

Schubart UK, Fields KL (1984) Identification of a calcium-regulated insulinoma cell phosphoprotein as an islet cell keratin. J Cell Biol 98:1001–1009

Schuit F, Flamez D, De Vos A, Pipeleers D (2002) Glucose-regulated gene expression maintaining the glucose-responsive state of β-cells. Diabetes 51(Suppl 3):S326–S332

Schvartz D, Brunner Y, Coute Y, Foti M, Wollheim CB, Sanchez JC (2012) Improved characterization of the insulin secretory granule proteomes. J Proteomics 75:4620–4631

Schweitzer B, Predki P, Snyder M (2003) Microarrays to characterize protein interactions on a whole-proteome scale. Proteomics 3:2190–2199

Sol ER, Hovsepyan M, Bergsten P (2009) Proteins altered by elevated levels of palmitate or glucose implicated in impaired glucose-stimulated insulin secretion. Proteome Sci 7:24

Sparre T, Reusens B, Cherif H, Larsen MR, Roepstorff P, Fey SJ, Mose Larsen P, Remacle C, Nerup J (2003) Intrauterine programming of fetal islet gene expression in rats – effects of maternal protein restriction during gestation revealed by proteome analysis. Diabetologia 46:1497–1511

Speer R, Wulfkuhle JD, Liotta LA, Petricoin EF 3rd (2005) Reverse-phase protein microarrays for tissue-based analysis. Curr Opin Mol Ther 7:240–245

Steil GM, Trivedi N, Jonas JC, Hasenkamp WM, Sharma A, Bonner-Weir S, Weir GC (2001) Adaptation of β-cell mass to substrate oversupply: enhanced function with normal gene expression. Am J Physiol Endocrinol Metab 280:E788–E796

Steinberg TH, Agnew BJ, Gee KR, Leung WY, Goodman T, Schulenberg B, Hendrickson J, Beechem JM, Haugland RP, Patton WF (2003) Global quantitative phosphoprotein analysis using multiplexed proteomics technology. Proteomics 3:1128–1144

Strohman R (1994) Epigenesis: the missing beat in biotechnology. Biotechnology (N Y) 12:156–164

Sun X, Kelly RT, Tang K, Smith RD (2010) Ultrasensitive nanoelectrospray ionization-mass spectrometry using poly (dimethylsiloxane) microchips with monolithically integrated emitters. Analyst (Cambridge, U K) 135:2296–2302

Sundsten T, Ortsater H (2008) Proteomics in diabetes research. Mol Cell Endocrinol 24:148

Swanson SK, Washburn MP (2005) The continuing evolution of shotgun proteomics. Drug Discov Today 10:719–725

Swenne I, Andersson A (1984) Effect of genetic background on the capacity for islet cell replication in mice. Diabetologia 27:464–467

Thiede B, Hohenwarter W, Krah A, Mattow J, Schmid M, Schmidt F, Jungblut PR (2005) Peptide mass fingerprinting. Methods 35:237–247

Tochino Y (1987) The NOD mouse as a model of type I diabetes. Crit Rev Immunol 8:49–81

Trivedi M, Budihardjo I, Loureiro K, Reid TR, Ma JD (2008) Epothilones: a novel class of microtubule-stabilizing drugs for the treatment of cancer. Futur Oncol 4:483–500

Unger RH, Grundy S (1985) Hyperglycaemia as an inducer as well as a consequence of impaired islet cell function and insulin resistance: implications for the management of diabetes. Diabetologia 28:119–121

Uttamchandani M, Yao SQ (2008) Peptide microarrays: next generation biochips for detection, diagnostics and high-throughput screening. Curr Pharm Des 14:2428–2438

van Haeften TW (2002) Early disturbances in insulin secretion in the development of type 2 diabetes mellitus. Mol Cell Endocrinol 197:197–204

Vercauteren FG, Arckens L, Quirion R (2006) Applications and current challenges of proteomic approaches, focusing on two-dimensional electrophoresis. Amino Acids 33:405

Wasinger VC, Cordwell SJ, Cerpa-Poljak A, Yan JX, Gooley AA, Wilkins MR, Duncan MW, Harris R, Williams KL, Humphery-Smith I (1995) Progress with gene-product mapping of the Mollicutes: *Mycoplasma genitalium*. Electrophoresis 16:1090–1094

Webb GC, Akbar MS, Zhao C, Steiner DF (2000) Expression profiling of pancreatic β cells: glucose regulation of secretory and metabolic pathway genes. Proc Natl Acad Sci USA 97:5773–5778

Wilkins MR, Pasquali C, Appel RD, Ou K, Golaz O, Sanchez JC, Yan JX, Gooley AA, Hughes G, Humphery-Smith I, Williams KL, Hochstrasser DF (1996) From proteins to proteomes: large scale protein identification by two-dimensional electrophoresis and amino acid analysis. Biotechnology (N Y) 14:61–65

Williams KL, Hochstrasser DF (1997) Introduction to the proteome. In: Wilkins MR, Williams KL, Appel RD, Hochstrasser DF (eds) Proteome research: new frontiers in functional genomics. Springer, New York, pp 1–12

Wingren C, Borrebaeck CA (2006) Antibody microarrays: current status and key technological advances. Omics 10:411–427

Wolf G (2001) Insulin resistance associated with leptin deficiency in mice: a possible model for noninsulin-dependent diabetes mellitus. Nutr Rev 59:177–179
Yates JR III (1998) Mass spectrometry and the age of the proteome. J Mass Spectrom 33:1–19
Yee A, Chang X, Pineda-Lucena A, Wu B, Semesi A, Le B, Ramelot T, Lee GM, Bhattacharyya S, Gutierrez P, Denisov A, Lee CH, Cort JR, Kozlov G, Liao J, Finak G, Chen L, Wishart D, Lee W, McIntosh LP, Gehring K, Kennedy MA, Edwards AM, Arrowsmith CH (2002) An NMR approach to structural proteomics. Proc Natl Acad Sci U S A 99:1825–1830
Zare RN, Kim S (2010) Microfluidic platforms for single-cell analysis. Annu Rev Biomed Eng 12:187–201
Zhou M, Veenstra T (2008) Mass spectrometry: m/z 1983–2008. Biotechniques 44:667–668, 670
Zhu H, Snyder M (2003) Protein chip technology. Curr Opin Chem Biol 7:55–63
Zhu H, Bilgin M, Bangham R, Hall D, Casamayor A, Bertone P, Lan N, Jansen R, Bidlingmaier S, Houfek T, Mitchell T, Miller P, Dean RA, Gerstein M, Snyder M (2001) Global analysis of protein activities using proteome chips. Science 293:2101–2105

Advances in Clinical Islet Isolation

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Contents

Abstract

Currently, islet transplantation is continuing to emerge as a viable treatment strategy for selected patients with type 1 diabetes who suffer from severe hypoglycemia and glycemic instability. Subsequent to the initial report, in 2000 from Edmonton, of insulin independence in seven out of seven consecutive recipients, there has been an immense expansion in clinical islet transplantation in specialized centers worldwide. The challenge now is to avoid the observed islet graft attrition over time. Isolating high-quality human islets, which survive and function indefinitely, will undoubtedly contribute to the further improvements in long-term clinical outcome. This chapter outlines the criteria

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_20,

for selecting appropriate donors for islet isolation and transplantation, describes the processes involved in islet isolation, and discusses the scope for areas of further improvements.

Keywords
Culture • Islet purification • Organ preservation • Pancreas dissociation

Abbreviations

BMI	Body mass index
CA	Catalytic antioxidants
CBD	Collagen binding domains
CI	Class I collagenase
CII	Class II collagenase
DCD	Donation after cardiac death
FDA	Fluorescein diacetate
HbA_{1c}	Hemoglobin A_{1c}
HTK	Histidine-tryptophan-ketoglutarate
IEs	Islet equivalents
OCR	Oxygen consumption rate
PI	Propidium iodide
TLM	Two-layer method
UW	University of Wisconsin

Introduction

The transplantation of pancreatic tissue in either the form of whole organ or isolated islets of Langerhans has become an attractive alternative clinical option to daily insulin injection to achieve a more physiological means for precise restoration of glucose homeostasis in patients with type 1 diabetes. Successful β-cell replacement through transplantation can offer the recipient the advantage of achieving normal carbohydrate control, eliminate the need for exogenous insulin, restore normal physiological level of hemoglobin A_{1c} (HbA_{1c}), minimize micro- and macro-disease, reduce diabetes-induced organ dysfunction, and ameliorate the life-threatening risk of severe hypoglycemia often associated with exogenous insulin therapy. The abrogation of these primary and secondary complications is often accompanied by an overall improvement in the quality of life for the transplant recipient.

Pancreas transplantation is a highly successful and well-established treatment for selected cases of type 1 diabetes; however, it is associated with significant surgical morbidity. Conversely, islet transplantation offers advantages as stated above and has a low morbidity, but has historically been considered investigational and experimental because of limited success rates. Nevertheless, recent advances in islet isolation technology have opened the door for the reinstitution and development of new clinical islet transplantation programs around the globe, which have

reported increasing success. In 2000, the Edmonton group attained insulin independence in all of seven patients by using freshly isolated islets from multiple donors while utilizing a steroid-free antirejection therapy, a procedure now known as the "Edmonton protocol" (Shapiro et al. 2000; Ryan et al. 2001). This protocol has set the standard worldwide for islet transplantation allowing other groups to achieve similar successes (Hering et al. 2004; Markmann et al. 2003; Warnock et al. 2005).

While it is clear that major advances have been made in achieving more consistent insulin independence following islet transplantation, it is also evident that the majority of islet recipients experience graft attrition over time, with an insulin independence rate of only 10 % at 5 years posttransplant (Ryan et al. 2005). The chronic decay in islet graft function is likely impacted by subclinical allograft rejection and recurrent autoimmunity. In light of the multiple pathways known to be involved in β-cell dysfunction as well as the alloresponse to foreign antigens, it is unlikely that a monotherapy will further optimize clinical islet transplantation and lead to single-donor recipients, indicating a need for refined immunosuppression protocols (Gala-Lopez et al. 2013). However, experimental studies in the absence of specific immunological destruction have indicated slowly progressive dysfunction of transplanted islets with time in nonhuman primates (Gray et al. 1986; Koulmanda et al. 2006), dogs (Alejandro et al. 1986; Kaufman et al. 1990; Levy et al. 2002; Warnock et al. 1988), and rodents (Hiller et al. 1991; Keymeulen et al. 1997; Orloff et al. 1988). In addition, clinical studies in autotransplantation show that patients experience a gradual decrease in islet graft function after a sustained period of graft function, despite the absence of graft-specific immunity (Oberholzer et al. 1999, 2000; Robertson et al. 2001). The autografts function well, with 85 % of patients retaining some function 2 years posttransplant. However, the insulin independence rate 1-year post-autotransplantation is approximately 32 %, indicating nonimmunological factors may hinder islet engraftment and function (Sutherland et al. 2008). Diminution of long-term autograft function may be attributed to a combination of low islet yield, suboptimal islet vascularization, engraftment site, and innate immune reactions such as the instant blood-mediated inflammatory reaction (Bennet et al. 2000). In contrast to allografts, autografts are routinely conducted in the absence of factors such as extended cold ischemia, alloimmunity, lack of diabetic immunity, and the toxicity associated with immunosuppressive drugs. Therefore, the gradual allograft attrition can be partially attributed to nonimmunological factors, emphasizing the need for further optimization of islet transplantation strategies to improve overall patient outcomes.

Islets are subjected to numerous stresses prior to transplantation. The process of islet attrition appears to begin at the time of donor brain death and continues during the islet isolation procedure. Despite many advances in technical aspects of human islet isolation, it still remains a technically demanding procedure, with several different factors influencing isolation outcome (Harlan et al. 2009). In addition, it is difficult to isolate a sufficient number of viable islets with any regularity. Providing high-quality human islets that survive and function for a longer period will no doubt contribute to further improvement of long-term clinical outcome.

The entire process of islet preparation comprises a number of steps. Among these steps are donor selection, pancreas preservation, enzymatic digestion of the pancreas, islet purification, islet culture, and islet assessment prior to transplantation. We outline each of these steps and provide the rationale for continued efforts in islet isolation. This chapter has been updated and partially adapted from the previously published version (Kin 2010).

Donor Selection

Identifying donor-based specific markers of islet isolation success may indeed provide a means of improving the success rates of the subsequent islet transplant. Previous single-center retrospective studies have identified several donor-related variables affecting islet isolation outcome, including but not limited to donor age, cause of death, body mass index (BMI), cold ischemia time, length of hospitalization, use of vasopressors, and blood glucose levels (Kin 2010; Goto et al. 2004; Ihm et al. 2006; Lakey et al. 1995, 1996; Matsumoto et al. 2004; Nano et al. 2005; Zeng et al. 1994). Pancreas weight has not been considered as a donor selection criterion because a value cannot be obtained prior to organ procurement. In most cases, a larger pancreas contains a larger islet mass (Nano et al. 2005; Kin et al. 2006a). Thus, pancreas size can serve as a surrogate parameter for donor islet mass on its own. One study developed a formula to predict pancreas weight, by analyzing data from 345 deceased donors (Kin et al. 2006a). Key observations made by this group are (1) males have a larger pancreas than females; (2) pancreas weight increases with age, reaching plateau in the fourth decade; and (3) BMI correlates with pancreas weight, but body surface area is a better predictor of pancreas weight than BMI. The finding that males have a larger pancreas size is consistent with the observations from other such studies (de la Grandmaison et al. 2001; Saisho et al. 2007). It has been reported that male donors provided a higher probability of yielding adequate islets (Hanley et al. 2008; Ponte et al. 2007). As for donor age, a positive correlation between age and islet yield is well documented (Lakey et al. 1996; Nano et al. 2005). A juvenile donor pancreas is often difficult to obtain an adequate number of islets (Balamurugan et al. 2006; Socci et al. 1993), which in part can be explained by its small size. Regarding BMI, several groups have indicated that BMI positively affects islet yield (Brandhorst et al. 1995a), leading many to consider BMI as an important donor factor influencing islet isolation outcome (Lakey et al. 1996; Matsumoto et al. 2004; Nano et al. 2005). However, this view has led to the misconception that an obese donor is a good candidate for successful islet isolation and transplantation. Supporting this notion is a recent report by Berney and Johnson who conclude that islet mass transplanted does not unequivocally correlate with islet graft function and therefore argue that donor selection criteria for islet transplantation, and hence allocation rules (pancreas for whole organ or islet transplant), may need to be redefined (Berney and Johnson 2010).

Donors with type 2 diabetes are considered unsuitable for islet isolation and transplantation because β-cell mass (Butler et al. 2003; Yoon et al. 2003) and

function (Deng et al. 2004) may be decreased in type 2 diabetes. Type 2 diabetes is clinically insidious and can remain undiagnosed for many years. A negative medical history of diabetes obtained from the next of kin does not necessarily indicate the absence of glucose intolerance. Thus, it is not unexpected that a large proportion of organ donors for islets may have undiagnosed type 2 diabetes. In fact, a pancreas from an older donor with a higher BMI is not likely used for a whole organ transplant, but is preferred for islet isolation and transplantation (Ris et al. 2004; Stegall et al. 2007). Such a donor may be on the spectrum toward type 2 diabetes. Previous studies indicated that high glycemic values during donor management were detrimental to islet recovery after isolation (Lakey et al. 1996; Nano et al. 2005; Zeng et al. 1994). However, blood glucose levels are far too unreliable to use for the assessment of the donors' glucose metabolism in light of the pathophysiology of brain death and the pharmacology of drugs administered during the management of brain death. Although HbA_{1c} itself is not a diagnostic criterion for diabetes mellitus, its measurement in potential donors would provide useful information, since it has a high degree of specificity for detecting chronic hyperglycemia. Our islet isolation laboratory at the University of Alberta has implemented the routine measurement of donor HbA_{1c} levels prior to islet isolation. Our current practice is that donors with $HbA_{1c} > 7$ % are excluded for clinical islet transplantation (Koh et al. 2008).

O'Gorman and colleagues developed a scoring system based on donor characteristics that can predict islet isolation outcomes (O'Gorman et al. 2005). This scoring system has proven to be effective in assessing whether a pancreas should be processed for islet isolation (Witkowski et al. 2006). It also allows for better management of the islet processing facility, as the cost of islet isolations is high. However, using a donor score of 79 as the most appropriate cutoff value, the sensitivity and specificity for predicting successful islet isolations were only 43 % and 82 %, respectively. Moreover, it is not clear about the actual impact of donor score on transplantation outcome, because the scoring system was developed solely based on islet isolation outcome. Similarly, other published studies dealing with donor factors do not take transplant outcome into consideration (Kin 2010; Goto et al. 2004; Lakey et al. 1995, 1996; Matsumoto et al. 2004; Nano et al. 2005; Zeng et al. 1994). An older donor with a higher BMI may be a better donor with respect to successful islet isolation, but probably is not ideal for islet transplantation when the biology of islets derived from such a donor is considered. An improved scoring system which takes both the islet isolation and transplantation outcomes into consideration would be more appropriate.

Donation after cardiac death (DCD) has also been used for islet transplantation. In experimental settings, islet yield and function derived from DCD pancreases seem to be comparable with those from their brain dead counterparts (Ris et al. 2004). However, in clinical settings, the results are not entirely promising. Japan has one of the most extensive experiences using DCD donors for organ transplant. They have been using optimized retrieval in these type of donors, as well as the Kyoto preservation solution and the two-layer method (TLM) (Nagata et al. 2006). Their most recent report for islet transplantation from this source shows

that overall graft survival was 76.5 %, 47.1 %, and 33.6 % at 1, 2, and 3 years, respectively, whereas corresponding graft survival after multiple transplantations was 100 %, 80.0 %, and 57.1 %, respectively. All recipients remained free of severe hypoglycemia, while three achieved insulin independence for 14, 79, and 215 days (Saito et al. 2010). This is a clear indication of the potential benefits of DCD as an alternative source if used under strict releasing criteria, particularly in countries where heart-beating donors may not be readily available.

Pancreas Preservation Prior to Islet Isolation

According to a report from the National Islet Cell Resource Center Consortium in the USA, University of Wisconsin (UW) solution is currently the standard preservation solution prior to islet isolation. Recently, more pancreata are stored in histidine-tryptophan-ketoglutarate (HTK) solution, while TLM is decreasingly employed for pancreas preservation, at least in the USA.

HTK solution, originally developed for use as a cardioplegia solution, has long been used for abdominal organ preservation in Europe (de Boer et al. 1999). In 1995, Brandhorst and colleagues compared HTK and UW solutions in pancreas preservation for human islet isolation for the first time (Brandhorst et al. 1995b). They observed that both solutions were comparable in terms of islet yield, in vitro functional viability of islets, and in vivo islet function in a mouse transplant model. Similar findings were subsequently reported by Salehi and colleagues (Salehi et al. 2006). In an experimental model performed in pigs, Stadlbauer and colleagues did not find any differences in frequency of apoptotic islet cells between pancreata preserved in UW versus those in HTK (Stadlbauer et al. 2003). At the present time, there is no evidence that HTK solution is superior to UW regarding islet isolation outcome. However, cost advantages in utilization of HTK may see further increased use of this solution for organ preservation.

The reason for decreased utilization of TLM is not clear but might be explained by recent observations in 166 and 200 human pancreata indicating no beneficial effect of TLM (Caballero-Corbalan et al. 2007; Kin et al. 2006b). TLM was developed in the 1980s by Kuroda, who focused on organ protection from hypoxia by supplying oxygen via perfluorocarbon during cold preservation (Kuroda et al. 1988). Maintenance of adenosine triphosphate production in pancreata stored at the interface between perfluorocarbon and UW solution was observed as a result of oxygenation (Morita et al. 1993). Tanioka and colleagues applied for the first time TLM prior to islet isolation in a canine model (Tanioka et al. 1997). Subsequently many centers introduced TLM prior to clinical islet isolation and reported improvement in islet isolation outcomes for pancreata preserved in TLM, when compared with pancreata stored in UW alone (Hering et al. 2002; Ricordi et al. 2003; Tsujimura et al. 2002). Of note, most of the initial studies employed a short period of TLM with continuous oxygenation at the islet isolation facilities. In an attempt to enhance the beneficial effect of TLM, our center at the University of Alberta introduced TLM for an entire period of pancreas preservation using

pre-oxygenated perfluorocarbon. However, in contrast to the expectation, no advantages of TLM over UW were observed in terms of pancreatic adenosine triphosphate level, islet yield, in vitro functional viability, and in vivo function after clinical transplantation (Kin et al. 2006b). These findings were subsequently confirmed by other groups (Ponte et al. 2007; Caballero-Corbalan et al. 2007). In a recent, meta-analysis study comparing pancreas preservation with the TLM versus UW for islet transplantation, the research concluded that the TLM was indeed beneficial for pancreata with prolonged cold ischemia prior to human islet isolation; however, benefits of the TLM for short-term preservation were inconclusive (Qin et al. 2011). Thus, there remains much work to be done to optimize pancreas preservation methods. One such strategy for optimization of pancreas preservation is the formulation of novel solution. The Matsumoto group has developed a Kyoto solution that contains trehalose and ulinastatin as distinct components (Noguchi et al. 2006). This solution and modification of it have demonstrated favorable results in human islet isolation outcomes, such that it has become the standard preservation solution for Baylor's clinical islet transplant program (Noguchi et al. 2010, 2012a).

Recently, hypothermic machine perfusion has been gaining increasing acceptance as a preservation method mainly for marginal donor kidneys (Sung et al. 2008). Hypothermic machine perfusion has several advantages over static cold storage. First, preservation solution can be continuously supplied directly to all cells. In addition, machine perfusion permits ex vivo pharmacologic manipulation of the graft. Moreover, real-time assessment of graft quality can be done by analysis of perfusate. Toledo-Pereyra and colleagues reported a canine islet autotransplantation study with 60 % and 40 % animal survival following hypothermic machine perfusion for 24 and 48 h, respectively (Toledo-Pereyra et al. 1980). In porcine islet isolation, Taylor and colleagues showed that machine perfusion improved islet isolation outcomes when compared with static UW preservation (Taylor et al. 2008). Our center at the University of Alberta performed machine perfusion in 12 human pancreata using a LifePort^{TM} Kidney Transporter (Organ Recovery Systems, Des Plaines, IL, USA) (Kin et al. 2006c). The first four pancreata were placed on the machine, after 10 h of static preservation in UW, for up to 24 h; metabolic and histologic changes of pancreata were assessed. It was found that tissue energy charge was maintained during the first 3 h in the machine perfusion and thereafter it gradually decreased. Histologic analysis revealed that tissue edema became evident at 24 h. The next eight pancreata were processed for islet isolation after 6 h of machine perfusion. Islet recovery and viability tended to be higher in pancreata preserved with the machine perfusion than in matched pancreata stored in static UW. These results are in accordance with the work of Leeser and colleagues who showed a feasibility of pump perfusion of human pancreata prior to islet isolation (Leeser et al. 2004).

Another recent advancement in organ preservation is the use of hypothermic persufflation, gaseous oxygen perfusion, as a surrogate to machine liquid perfusion (Suszynski et al. 2013, 2012; Nagai et al. 2013; Srinivasan et al. 2012; Tolba et al. 2006; Minor et al. 2000). In a preclinical study, persufflation of the pancreas prior to islet isolation demonstrated improved yields in viable islets and extended the duration of preservation compared to the TLM (Scott et al. 2010). Currently the

utility and efficacy of persufflation for clinical islet transplantation is being actively investigated at the University of Alberta's Clinical Islet Transplant Program.

Pancreas Dissociation and Enzyme

A critical component to successful islet transplantation is the enzymatic dissociation of insulin-producing islets of Langerhans, from the surrounding pancreatic exocrine tissue. To facilitate the isolation of the islets, enzyme blends are delivered to the islet-exocrine interface, of which collagen is the major structural protein constituting this interface (Hughes et al. 2006; Van Deijnen et al. 1994). As a result of its dense structure and mechanical strength, collagen is not generally degraded by ordinary protease; however, it can be efficiently degraded with high specificity by collagenase (Watanabe 2004). As such, collagenase is a key component of an enzyme product for isolating islets; however, the use of collagenase alone results in an inadequate tissue digestion (Wolters et al. 1992, 1995). It is apparent that the presence of non-collagenase impurities is required for enhanced pancreas dissociation. Prior to the 1990s, crude collagenase, a fermentation product derived from *Clostridium histolyticum*, was exclusively used for isolating islets. This crude preparation contains two different classes of collagenase: class I collagenase (CI) and class II collagenase (CII) in addition to non-collagenolytic enzymes including but not limited to amylase, cellulase, pectinase, chitinase, sialidase, hyaluronidase, lipase, and phospholipase (Johnson et al. 1996; Matsushita and Okabe 2001; Soru and Zaharia 1972). Unfortunately, a major limitation to successful islet isolation is the exceedingly variable composition and activity of crude collagenase preparations that exists between lots of commercially available products (Johnson et al. 1996; Kin et al. 2007a). In the late 1990s a purified enzyme blend became available from Roche (Roche Applied Science, Indianapolis, IN). This enzyme blend, Liberase HI, is comprised of CI and CII, in addition to a non-collagenolytic enzyme, thermolysin, derived from *Bacillus thermoproteolyticus*. The introduction of Liberase HI had a profound impact on the field as it helped reduce a significant portion of the lot-to-lot enzyme variability. Subsequent to Liberase HI's inception into clinical and experimental islet isolations, enhanced islet yield and function in the human and animal models, compared to the historical use of crude collagenase, were immediately observed (Berney et al. 2001; Lakey et al. 1998; Linetsky et al. 1997; Olack et al. 1999). However several studies showed that Liberase is no more effective than crude collagenase in certain models, such as neonatal rat (Hyder 2005) and fetal pig (Georges et al. 2002) pancreata, and induces functional damage to rat (Vargas et al. 2001) and human (Balamurugan et al. 2005) islets. Moreover, this enzyme blend is not immune to a degree of lot-to-lot variations (Barnett et al. 2005; Kin et al. 2007b).

While the use of non-collagenolytic enzyme components has been shown to enhance pancreas dissociation, excessive exposure of these enzyme is found to decrease islet yields through islet fragmentation and disintegration (Wolters et al. 1992; Bucher et al. 2004) and to reduce islet viability (Brandhorst et al. 2005).

This is a major limitation of traditional products as fine tuning the narrow dose range is not possible by the user. A recently developed Collagenase NB1 (Serva Electrophoresis, Heidelberg, Germany) contains only CI and CII, which can be blended with separately packaged Neutral Protease NB (Serva Electrophoresis) as a non-collagenolytic component. This type of product has several potential advantages over traditional enzyme blends. First, the ratio between non-collagenolytic activity and collagenase activity can be adjusted accordingly to further optimize the isolation procedure. Moreover, separate storage of the individual enzyme components would improve the overall stability of each enzyme activity. Finally and importantly, the non-collagenolytic component can be sequentially administered after intraductal collagenase distention, in an attempt to avoid excessive exposure of islets to non-collagenolytic enzyme (Kin et al. 2009).

Clostridium histolyticum possesses two homologous but distinct genes, ColG and ColH. The former encodes CI and the later encodes CII (Matsushita et al. 1999; Yoshihara et al. 1994). It is important to know similarities and differences between the two enzymes. CI and CII are quite different in their primary and secondary structures, but the catalytic machinery of the two enzymes is essentially identical (Mookhtiar and Van Wart 1992). Both enzymes have a similar segmental structure consisting of three different segments: catalytic domain, spacing domain, and binding domain (Kin et al. 2007a; Matsushita et al. 2001). CI has tandem collagen binding domains (CBD) but CII possesses a single CBD (Matsushita et al. 2001). Tandem CBDs of CI may have advantages for binding to collagens in the pancreas; because tandem-repeated binding domains are generally considered useful for stabilization of bindings (Linder et al. 1996). Kinetic studies evaluating the hydrolysis of collagens by CI or CII indicate a higher catalytic efficiency of CI on collagen (Mallya et al. 1992). On the other hand, CII is characterized by the ability to attack synthetic peptide substrates at a much greater rate than CI (Bond and Van Wart 1984). Wolters and colleagues showed that rat pancreas digestion was more effective when both classes were used together instead of CI or CII alone (Wolters et al. 1995). van Wart and colleagues found a synergistic effect of the two enzymes on collagen degradation (Van Wart and Steinbrink 1985). Wolters and colleagues concluded that CII plays a predominant role in rat pancreas dissociation, whereas CI is minor in comparison (Wolters et al. 1995). Several investigators (Antonioli et al. 2007) and manufacturers have emphasized the view that CII is a key player in pancreas dissociation. Indeed, manufacturers have measured only CII activity in their product specification and CI activity has been ignored so far. However, the importance of CII has been challenged by a recent study demonstrating that neither CI nor CII alone is able to release islets from a rat pancreas (Brandhorst et al. 2008). Findings from human studies are in conflict with the classical view, too. Barnett and colleagues showed that the stability of intact CI is of great importance to the quality of the blend (Barnett et al. 2005). It is also demonstrated that a better enzyme performance is ascribed to a higher proportion of CI rather than a higher proportion of CII (Kin et al. 2007b). It is further shown that excessive CII is not effective to release islets from a human pancreas and rather a balanced CII/CI ratio is of paramount importance (Kin et al. 2008a).

Cross and colleagues performed extensive immunohistological studies on binding of collagenase to collagen (Cross et al. 2008), suggesting that collagenase perfused through the duct binds to collagen inside the pancreas. Their findings also suggest that collagenase can bind to collagen without help of non-collagenolytic enzyme activation and that low temperature does not inhibit binding of collagenase to collagen which is in line with a previous report (Matsushita et al. 1998). Another important finding from their studies is that collagenase binds to collagen located inside the islets as a result of intraductal perfusion with collagenase. This may result in islet fragmentation when the enzyme is activated.

Understanding of the structure of the islet-exocrine interface, and the nature of substrate at this interface, will be exploited to optimize pancreas dissociation. Previous studies have described the distribution of collagen types in the human pancreas. Type IV collagen is present in basement membranes associated with ducts and acini (Kadono et al. 2004). Collagen subtypes identified in the islet-exocrine interface are I, III, IV, V, and VI (Van Deijnen et al. 1994; Hughes et al. 2005). Recently, Hughes and colleagues found that type VI collagen is one of the major collagen subtypes at the islet-exocrine interface of the adult human pancreas (Hughes et al. 2006). Type VI collagen has a high disulfide content which serves to protect the molecule from bacterial collagenase digestion (Heller-Harrison and Carter 1984). It is also known that type VI collagen does not form banded collagen fibrils and is extensively glycosylated (Aumailley and Gayraud 1998). Regarding the amount of collagen, it is well known that the total collagen content increases with age in most tissues (Akamatsu et al. 1999; Clausen 1963; Gomes et al. 1997; Sobel and Marmorston 1956). In addition, pancreatic collagen is affected by the normal aging process. Bedossa and colleagues found significantly higher collagen content in pancreata from patients over the age of 50, as compared to younger subjects (Bedossa et al. 1989). The importance of pancreatic ultrastructure has been pointed out and discussed over the past two decades (van Deijnen et al. 1992); unfortunately, there has been little progress in this field. A better understanding of the differences in biomatrix among donor pancreata, for example, older versus younger donors, will help to improve pancreas dissociation.

In early 2007, the islet transplant community was notified of the potential risk of prion disease transmission, when using Liberase, due to the manufacturing of the product, which evolves a bovine neural component. As a result many islet isolation centers switched to Serva collagenase, as it was considered a safer option; however, this conversion had a considerable impact on the field in terms of islet yield and quality. In two recent retrospective studies, the researchers compared the human islet isolation outcomes between pancreata processed by Liberase HI and Collagenase NB1. Despite reporting no difference with respect to pre-purification, post-purification, or post-culture islet mass or percent recovery between the two groups, they concluded that islets prepared with Liberase HI were significantly more viable than those isolated with Collagenase NB1 (Iglesias et al. 2012; Misawa et al. 2012). In a biochemical study, the collagenases from Roche (Liberase MTF), Serva (Collagenase NB1), and VitaCyte (CIzyme Collagenase HA) (Indianapolis, IN) were analyzed by analytical high-performance liquid chromatography and collagen

degradation activity, an assay that preferentially detects intact CI; the researchers demonstrate that the highest collagen degradation activity was found in the VitaCyte product followed by the Roche and Serva enzyme products. Furthermore, this group successfully used the VitaCyte product in 14 human islet isolations (Balamurugan et al. 2010). Of note, the biochemical analysis of the purified Collagenase NB1 consistently showed that these products contain primarily truncated CI, resulting in a molecular form that has lost one of the two CBDs (Balamurugan et al. 2010). This may in part explain the finding by O'Gorman and colleague, which demonstrated favorable clinical islet isolation outcomes with the new Liberase MTF, compared to Serva NB1 (O'Gorman et al. 2010).

In light of this significant setback, some centers successively adapted Serva enzyme blend with minimal modification of their preexisting islet isolation protocol (Szot et al. 2009; Sabek et al. 2008). The University of California San Francisco group achieved a high rate of islet isolation success using ~1,600 units of Serva collagenase and ~200 units of neutral protease for younger donor pancreata (Szot et al. 2009). A recent in-depth report by Balamurugan and colleagues from the University of Minnesota detailed the evaluation of eight different enzyme combinations in an attempt to improve islet yield for both autologous and allogeneic human islet transplantation. This comprehensive study included 249 clinical islet isolations consisting of enzyme combinations including purified, intact, or truncated CI and CII and thermolysin from *Bacillus thermoproteolyticus rokko* or neutral protease from *Clostridium histolyticum*. Based on the biochemical characteristics of enzymes and observations of enzyme behavior during isolations, they blended intact CI/CII and neutral protease from *Clostridium histolyticum* instead of thermolysin into a new enzyme mixture (Balamurugan et al. 2012). This new enzyme mixture consistently produced significantly higher islet yields from both pancreatitis and deceased donor pancreata compared to standard enzyme combinations, while retaining islet potency and function (Balamurugan et al. 2012).

Now considerable research efforts are being directed to experimentation with nonenzymatic pancreas dissociation methodologies. Some of these novel modalities include but are not limited to in situ cryopreservation of islets and selective destruction of acinar tissue (Taylor and Baicu 2011), as well as utilizing dielectrophoresis as a noncontact technique for isolating islets of Langerhans (Burgarella et al. 2013). Whether these and other enzymatic and nonenzymatic isolation strategies can be translated to clinical islet isolation and improve outcomes has yet to be elucidated.

Islet Purification

Following the enzymatic digestion of an average-size human pancreas (~90 g), the total packed volume of digested tissue is typically greater than 40 mL. While it is known that human liver has a capacity for adaptation and revascularization in the context of portal vein occlusion (Casey et al. 2002), the liver cannot accommodate 40 mL of tissue consisting of particles on the 100-μm scale. Furthermore, the substantial evidence of liver embolism, thrombosis, damage, and even death are

documented in clinical settings immediately after intraportal infusion of a large amount of tissue (Mehigan et al. 1980; Mittal et al. 1981; Walsh et al. 1982; Toledo-Pereyra et al. 1984; Shapiro et al. 1995). Notably, there is the need to reduce tissue volume with minimum loss of islets for the safer intraportal infusion as well as to accommodate islet graft transplanted into extrahepatic sites. This can be achieved by a procedure called "islet purification."

Density-dependent separation of islets from exocrine tissue is the most simple and effective approach for islet purification. This methodology is based on the principle that, during centrifugation, tissue will migrate and settle to the density that is equal to its own density. Using this technique, separation can be achieved based on intrinsic differences in density between islet tissue (~1.059 g/mL) and exocrine tissue (1.059–1.074 g/mL) (London et al. 1992). Theoretically, a larger difference in cellular density between the two tissues could result more efficacious separation. The ideal separation would be expected when the islets are free from exocrine tissue and the density of exocrine tissue is well preserved. In contrast, the worst scenario would happen when the majority of islets are entrapped in the exocrine tissue (thereby a higher density of tissue) and the density of exocrine tissue is decreased due to exocrine enzyme discharge or tissue swelling. Thus, there is a trade-off between purity of islets and islet mass recovered. Obtaining an extremely high purity of islets requires sacrificing a less pure layer, which contains a considerable amount of islets. Nearly 100 % of islets can be recovered if a less pure layer with a large amount of exocrine tissue is included to the final preparation, but this turns in a lower purity and may be deleterious to the subsequent islet transplant.

Purification of large numbers of human islets has advanced rapidly with the introduction of the COBE 2991 (COBE Laboratories Inc., Lakewood, CO, USA) (Lake et al. 1989). The COBE 2991 cell processor, originally developed for producing blood cell concentrates, is an indispensable equipment in human islet processing facilities. It allows processing of a large volume of tissue in an enclosed sterile system. Moreover, it offers decreased operating time with an ease of generating continuous density gradients in conjunction with a two-chamber gradient maker.

Various gradient media have been developed and tested for islet purification. One of the most commonly used media is a synthetic polymer of sucrose (Ficoll; Amersham, Uppsala, Sweden)-based media. Scharp and colleagues reported that islet recovery was improved when Ficoll was dialyzed before centrifugation, to remove the low molecular weight osmotically active contaminants (Scharp et al. 1973). Olack and colleagues used Euro-Collins, an organ preservation solution, as the vehicle for dissolving the Ficoll powder (Olack et al. 1991). Hypertonic density solutions such as Euro-Collins/Ficoll prevent edema of the exocrine tissue at low temperature and result in improved separation of islets from the exocrine tissue, when compared with standard Ficoll solution (London et al. 1992). More recently, purification with iodixanol (OptiPrep) has been recently reported in islet transplant series with successful clinical outcomes in addition to significantly reducing the secretion of proinflammatory cytokine/chemokines from the islet, in comparison to Ficoll-based density gradients (Mita et al. 2010). Of note, in a

comparative preclinical study of Ficoll-, histopaque-, dextran-, or iodixanol-based gradients, histopaque led to the isolation of viable and functional islets with a reduced cost as compared to a standard Ficoll gradient (McCall et al. 2011).

UW solution has been used for storing the pancreatic digest prior to density gradient centrifugation in an attempt to reduce acinar tissue swelling (Chadwick et al. 1994; Robertson et al. 1992; van der Burg et al. 1990). The beneficial effect of UW storage is ascribed to the presence of the osmotic effective substances lactobionate, raffinose, and hydroxyethyl starch. To extend this beneficial action, Huang and colleagues have introduced a mixture of UW and Ficoll-sodium-diatrizoate (Biocoll; Biochrom, Berlin, Germany) for density gradient separation (Huang et al. 2004). They showed that their new gradient medium improved post-purification islet yield when compared with the standard medium. The UW-Bicoll purification method has been further refined by Barbaro and colleagues, who recovered 85 % of islets after purification using a narrow range of density gradients (Barbaro et al. 2007).

Ichii and colleagues performed discontinuous density gradient purification to recover islets from the exocrine-enriched fraction obtained after the initial purification procedure (Ichii et al. 2005a). This supplemental purification, so-called rescue purification, contributed to the increase in the number of islet preparations suitable for transplantation. In a subsequent report, however, the same group showed no benefit of rescue purification on isolation success (Ponte et al. 2007).

Among islet processing laboratories, islet recovery rates vary from 50 % to 85 %, depending on purification methods and the quality of the pancreas, summarized in Table 1 (Ihm et al. 2006; Nagata et al. 2006; Kin et al. 2007b, 2008a; Mita et al. 2010; Barbaro et al. 2007; Brandhorst et al. 2003; Wang et al. 2007; Yamamoto et al. 2007; Noguchi et al. 2009), clearly indicating that more research is warranted. Some of the strategies include the avoidance of the high shear forces associated with COBE 2991 cell processor-based purification methods. Shimoda and colleagues reported such a method with their top loading large bottle purification technique in conjunction with low viscosity ET-Kyoto and iodixanol gradient solutions, resulting in improved islet yield and viability compared to traditional isolation techniques (Shimoda et al. 2012). Furthermore, others are investigating the utility of magnetic separation of either encapsulated (Mettler et al. 2013) or nonencapsulated islets, completely avoiding density gradient separation all together. Despite promising preclinical results using porcine pancreata, this technology translation to the clinical setting is yet to be demonstrated.

Islet Culture

Clinical islet transplantations rely on successful isolation of the human islets from donors followed by the in vitro culturing of the cells to maintain functionality until transplantation can be performed. Although there is debate as to whether freshly isolated islets are superior to cultured islets in experimental transplantation (Ihm et al. 2009; King et al. 2005; Olsson and Carlsson 2005), preservation of human

Table 1 Islet recovery rate after purification

Author [reference]	Density gradient	n	Pre-purification islet yield (IE)	Post-purification islet yield (IE)	Recovery rate (%)[a]
Brandhorst et al. (2003)	HBSS-Ficoll	76	463,872	245,889	53.0
Nagata et al. (2006)	Ficoll	8[b]	660,770	444,426	67.3
Ihm et al. (2006)	Iodixanol or Biocoll	110	356,745	244,034	68.4
Barbaro et al. (2007)	Biocoll	32	359,425	194,022	64.5[c]
	UW-Biocoll	132	370,682	310,607	84.9[c]
Yamamoto et al. (2007)	Ficoll or ficoll followed by rescue[d]	169	454,049	306,728	67.6
Kin et al. (2007b)	Ficoll	251	348,794	227,832	65.3
Wang et al. (2007)	Biocoll	23	373,350	184,284	49.4
Kin et al. (2008a)	UW-Biocoll	21	394,619	303,905	77.0
Mita et al. (2010)	Iodixanol	5[e]	264,533	120,268	52.9
	Ficoll	5[e]	270,361	132,560	49.4
Noguchi et al. (2009)	Iodixanol-Kyoto	11	699,780	594,136	84.9
	Ficoll	19	670,939	377,230	55.6

[a]Mean post-purification IE/mean pre-purification IE × 100
[b]Nonheart beating donors
[c]Mean of each isolation's recovery rate
[d]See Ichii et al. (2005a) regarding "rescue" purification
[e]Pancreatic digest equally divided into two group

islets for a certain period of time by means of culture provides many benefits to clinical islet transplantation. First, islet culture allows travel time for patients living away from transplant centers, as most of these procedures are conducted in specialized centers. Pre-transplant culture can moreover provide attainment of therapeutic levels of immunosuppression before islet infusion. During the culture period additional quality control testing can be undertaken, including microbiological and pyrogenic tests. In addition to these practical advantages, modification or treatment of islets through culture provides a strategic opportunity to promote islet survival after transplantation. Surface modification of islets by bioconjunction during culture is one of the examples (Cabric et al. 2007; Totani et al. 2008). Thus, a strong rationale exists for culturing islets prior to transplantation. A number of issues will be discussed below, including culture temperature, base media, and risk of islet loss during culture.

In 1977, Kedinger and colleagues transplanted cultured or fresh allo-islets into the liver of diabetic rats (Kedinger et al. 1977). Rats receiving fresh islets returned to diabetic state in 8 days after transplantation. In contrast, when islets were cultured for 3–4 days prior to transplantation, graft survival was prolonged to 90 days without immunosuppression. Culture temperature is not described in the report, but this was the first attempt to alter or reduce immunogenicity of islets by in vitro culture. A few years later, Lacy and colleagues adapted room temperature

for culturing islets in an attempt to prolong allograft survival (Lacy et al. 1979a). They reported that culture of rat islets at 24 °C resulted in a prolonged islet allograft survival in immunosuppressed recipients. Their idea of culturing islets at 24 °C was based on a study performed by Opelz and Terasaki (Opelz and Terasaki 1974), who found that lymphocytes being placed in culture at room temperature lost their ability to stimulate allogeneic cells when tested in subsequent mixed lymphocyte culture. The results of Lacy's experiments support the theory that passenger leukocytes are involved in rejection of the allografts. However, the study did not demonstrate culture temperature at 24 °C per se contributed to altering immunogenicity of islets. To investigate the influence of temperature, they further compared allograft survival in immunosuppressed rats receiving islets cultured at 24 °C versus 37 °C (Lacy et al. 1979b). Culture of islets at 24 °C resulted in a longer allograft survival as compared to 37 °C culture. When recipients were not immunosuppressed, however, culture at 24 °C did not prolong graft survival as compared to fresh islets. Following the initial study performed by Lacy and colleagues (Lacy et al. 1979a), many investigators have set up a culture system at 22–24 °C prior to transplantation (Horcher et al. 1995; Kamei et al. 1989; Lacy and Finke 1991; Lacy et al. 1989; Mandel and Koulmanda 1985; Morsiani and Lacy 1990; Ryu and Yasunami 1991; Scharp et al. 1987; Yasunami et al. 1994). There are, however, little published studies specifically showing immunological superiority of 22–24 °C culture over 37 °C culture in an islet transplant model. A group from Germany reported a marked prolongation of rat islet allograft survival by culture at 22 °C comparing to 37 °C culture (Woehrle et al. 1989). Interestingly, the prolongation of graft survival was observed only when islets were transplanted under the renal subcapsular space; islets cultured at 22 °C were rapidly destroyed at the liver. They further confirmed a similar effect in a rat-to-mouse xenotransplantation model (Jaeger et al. 1994). It is uncertain if the strategy used in the animal models will be as satisfactory in the clinical situation.

Another possible benefit of low-temperature culture is that the structural and functional integrity of islets is well preserved, likely due to a lowered metabolic rate at temperatures below 37 °C. For instance, Ono and colleagues reported that rat islets cultured at 37 °C possessed a higher rate of central necrosis than islets cultured at 22 °C (Ono et al. 1979). Lakey and colleagues assessed human islet recovery after 24 h culture and they described a mean recovery rate of 73 % at 22 °C compared to 55 % at 37 °C (Lakey et al. 1994). Similarly, inadequate recovery rate at 37 °C was observed in pig islets after culture compared to 22 °C culture (Brandhorst et al. 1999). On the other hand, investigators have asserted that low-temperature culture results in impaired insulin production (Escolar et al. 1990) and more apoptotic cells in islets (Ilieva et al. 1999). In clinical settings, there is no consensus regarding culture temperature. Some centers have employed culture temperature at 37 °C followed by culture at 22 °C prior to clinical transplantation (Hering et al. 2004). Initially our center at the University of Alberta adapted this approach but consistently found significant loss of islets after culture (unpublished observation). Since 2003, our center has been using only 22 °C during the entire period prior to transplantation (Kin et al. 2008b).

Connaught Medical Research Laboratory 1066, originally designed for use with fibroblasts and kidney epithelial cells, appears to be the most widely used base medium for islet culture. Other base media used for clinical transplantation include Ham's F10 (Keymeulen et al. 2006) and M199 (Bertuzzi et al. 2002). Regardless of whichever base medium is employed, supplementation of medium seems to be a routine practice. Because serum contains many components that have a beneficial effect on cell survival, animal serum such as fetal calf serum is traditionally added to culture media in experimental settings. However, when islets are destined for clinical transplantation, use of animal sera has been considered unacceptable because of potential risk associated with viral or prion-related disease transmission (Will et al. 1996). Other potential problems of animal sera are evoking immune or inflammatory reactions in host against animal proteins (Johnson et al. 1991; Mackensen et al. 2000; Meyer et al. 1982), which cannot be diminished even by several washing steps (Spees et al. 2004). Therefore, adding human serum albumin as an alternative is the current standard in clinical islet culture.

One of the major concerns with culturing islets is the uncertainty of islet recovery rate after culture. There is ample evidence of a reduction in the islet mass during culture. Bottino and colleagues reported that there was at most 80 % reduction in DNA content in islet preparations following 24 h culture (Bottino et al. 2002). Another report showed only 18 % recovery rate in islet mass after 48 h culture (Zhang et al. 2004). A retrospective study on 104 islet preparations for clinical transplantation has identified several factors associated with risk of islet loss (Kin et al. 2008b). These include longer cold ischemia time prior to islet isolation, TLM for pancreas preservation, lower islet purity, and higher proportion of larger islets in the pre-cultured preparation. One may wonder if a longer culture period leads to a greater decrease in islet mass. The retrospective study does not support this caution probably because most of the islet preparations were cultured for a short period (20 h, median) with a narrow range (14 h, interquartile range). Most islet processing centers employ short period culture (up to 3 days) prior to transplantation (Hering et al. 2004; Warnock et al. 2005; Ryan et al. 2005; Kin et al. 2008b). A recent study from Baylor All Saints Medical Center concluded that fresh human islets (less than 6 h of culture) are more potent compared to cultured islets based on their in vitro and in vivo data. As such, this group's current clinical islet transplantation protocol implements fresh islet transplantation without culture (Noguchi et al. 2012b). Of note, the Brussels group cultured islets derived from several donors for up to 1 month until a critical islet mass is reached and then infused all islet preparations into a recipient as a single transplant procedure (Keymeulen et al. 1998). The impact of prolonged culture period on islet loss seems to be significant as islets were combined from as many as nine donors. Importantly, two of the seven recipients became insulin independent after transplantation of islets cultured for long periods.

Multiple experimentations have occurred during the design of an optimal culture media for islets. However, protocols for islet culture have not completely been standardized and practice may vary between centers. Optimal culture conditions for isolated human islet preparations should aim at providing sufficient oxygen and

nutrients to allow for recovery from isolation-induced damage while maintaining tridimensional structure of the clusters and preventing islet mass loss (Ichii et al. 2007). The simplest and most investigated approach to islet preservation and culture in vitro is through alteration in culture conditions, including temperature and media composition (Daoud et al. 2010a). Several studies have also involved the administration of various growth factors and compounds in order to enhance the suspension culture of islets such as glutamine compounds, human albumin, insulin, and sericin (Daoud et al. 2010a). These investigations have been the focus of many laboratories for the purpose of short-term culture for transplantation as well as long-term culture in vitro testing. The use of slightly impure islet preparations and co-culture with extracellular matrix components such as collagen has shown to enhance the viability and function of isolated islets (Daoud et al. 2010b). In addition, supplementation of culture medium with small intestinal submucosa has also shown to improve islet functioning and viability (Lakey et al. 2001). Media composition, seeding density, and temperature play a significant role (Ichii et al. 2007).

A very important area of investigation is the use of compounds capable of improving the islet health during culture and enhancing their function after transplant. Very promising results have been obtained with antioxidants, which seem to successfully preserve islet integrity during culture. When using these agents the functional behavior and phenotypic cell characteristics of treated islets were preserved, as was the capacity to normalize diabetic mice, even when a marginal mass of islets was transplanted (Bottino et al. 2004). Similar results were obtained when using a catalytic antioxidant (CA) formulation with mice and human with clear benefits during transplant (Sklavos et al. 2010). Another area of investigation is methods for reducing anoxia during culture. Isolated islets are especially susceptible to damage from anoxia due to their large size relative to single cells, high oxygen consumption rate, and low levels of enzymes necessary for energy production under anaerobic conditions (Papas et al. 2005; Lau et al. 2009). Islets cultured at high surface densities in standard T-flasks also exhibit low viable tissue recovery, viability, and potency, due to anoxic conditions. These effects have been prevented by culturing islets in gas-permeable devices, which increase oxygen availability to islets with clear benefits for clinical islet culture and shipment (Papas et al. 2005). Finally, several new biomaterials have also been used to improve islet viability during culture. Some of them provide a surrogate extracellular matrix for isolated islets as a way to recreate the native islet microenvironment (Daoud et al. 2010b). Some of the most promising advances include the use of ECM-coated surfaces, islet encapsulation, scaffolding techniques, and bioreactors, among others.

Assessment of Islet Preparations

Unlike rodent pancreata, substantial heterogeneity in islet size exists within a human pancreas. In order to accurately and consistently quantify the islets within an isolation preparation, both the number of islets and size should be taken into

consideration. Historically, it has been difficult to accurately establish the amount of total islet equivalents (IEs) (or total islet volume) in the human pancreas, namely, due to the fact that islets are scattered in a large exocrine gland of which they represent only small percent in volume. As such there is some controversy as to how many islets are present in the average human pancreas. Korsgern and colleagues (Korsgren et al. 2005) estimated the number of IEs in a normal pancreas is about 500,000 IEs based on seven autopsy cases reporting islet volume of 0.5–1.3 mL (Saito et al. 1978). Others estimated that the total islet volume is 2.4 mL in a normal pancreas, which is corresponding to 1,300,000 IEs (Colton et al. 2006). In Table 2, islet volume data from autopsy studies are listed (Maclean and Ogilvie 1955; Rahier et al. 1983; Sakuraba et al. 2002; Westermark and Wilander 1978). Calculated total IEs varies from 500,000 to 1,000,000 IEs, depending on the size of pancreas studied. One Japanese study (Sakuraba et al. 2002) reported a mean pancreas weight of 122 g, which is the highest among other studies. Consequently, islet mass reported in the study is remarkably large, resulting in a calculated IE of >1,000,000 IEs. In contrast, the pancreas weight in Maclean's study is only 50 % of that reported in the Japanese study (Maclean and Ogilvie 1955). Accordingly, the calculated IE from Maclean's data is almost half of the number from the Japanese study. Based on all data listed in the table, it would be reasonable to say that the average number of IEs in a normal 90-g human pancreas (Kin et al. 2006a) is about 800,000 IEs.

In addition to the quantity of islets, the functional viability of an islet preparation is critical in predicting the success of islet transplantation. To date, there lacks a consensus within the islet transplantation field as to which assays accurately assess islet potency prior to transplantation and predicts their subsequent function posttransplant. The viability of an islet preparation is currently assessed with the use of fluorescent stains based on dye exclusion polarity. For example, fluorescein diacetate (FDA) is a nonpolar dye and passes through the plasma membrane of living cells, whereas propidium iodide (PI) can only enter cells that have a compromised membrane. Using these two dyes together, the proportion of viable (green, FDA-positive) versus dead (red, PI-positive) cells can be assessed. FDA/PI is currently a widely used method for viability determination of the islet preparation prior to transplantation. These tests can be rapidly performed and are less labor intensive, making them attractive for use just prior to transplantation. However, there are several problems, making them of limited value. The main problem is that membrane integrity tests cannot distinguish between islets and non-islets. Another problem with the tests is the difficulty in assessing live/dead cells within a three-dimensional structure. In addition these tests fail to measure the metabolic capacity of the islet preparation. Nevertheless, it is important to acknowledge that viability estimated by membrane integrity tests is predictive of some outcome measurements in clinical transplantation, according to an annual report from Collaborative Islet Transplant Registry (2008).

A potentially more efficacious marker to determine the islet functional capacity is assaying for mitochondrial activity. Mitochondrial integrity is central to islet quality because mitochondria play a crucial role for glucose-stimulated insulin

Table 2 Estimation of total islet equivalents in a pancreas

Author [reference]	Mean age (range) year	*n*	Pancreas size (range) g or mL	Islet mass g	Islet volume mL	Calculated islet equivalents IE
Sakuraba et al. (2002)	51.7 (27–69)	15	122 g (75–170)	2.03	1.92[a]	1,085,295
Westermark and Wilander (1978)	74.9 (66–88)	15	76 mL	NR	1.60	905,874
Rahier et al. (1983)	54 (18–86)	8	83 g (67–110)	1.395	1.32[a]	745,806
Maclean and Ogilvie (1955)	56.1 (15–81)	30	61.7 g (38.9–99.2)	1.06	1.00[a]	566,706

Adapted from Kin (2010)
[a]Calculated assuming that islet density is 1.059 g/mL London et al. (1992)
NR not reported

secretion (Maechler 2002) and islet cell apoptosis (Aikin et al. 2004). Mitochondrial activity can be evaluated using a variety of methods. These include oxygen consumption rate (OCR), detection of mitochondrial membrane potential using dyes, release of cytochrome c, and measurement of redox state. Papas and colleagues assessed OCR of human islet preparations; they also measured DNA content of the preparations in order to normalize the OCR (Papas et al. 2007). They showed that OCR/DNA assay predicted efficacy of human islets grafted into mice. Furthermore, other groups have also demonstrated the utility of modified oxygen consumption rates as a predictor of islet in vivo function (Pepper et al. 2012). Despite beginning able to dynamically ascertain cellular potency, a drawback for this assay is that it cannot offer islet specificity, as all cells in the islet preparation will consume oxygen. Like membrane integrity test, the purity of the islet preparations significantly influences the assays precision. To circumvent this limitation, Sweet and colleagues developed a flow culture system (Sweet et al. 2002) that allows one to measure the OCR response, in human islets, against glucose stimulation. They demonstrated that glucose-stimulated changes in OCR were well correlated with in vivo function of human islet grafts (Sweet et al. 2005, 2008). They also showed glucose stimulation hardly increased OCR in non-islet tissue (Sweet et al. 2008). Given the fact that a clinical islet preparation contains a considerable amount of non-endocrine tissue, their approach would be logical and practical.

Since β cells within the islet are the only cells with the capacity to secrete insulin, it could be argued that the viability of β cells is probably most important to the outcome of transplantation. Ichii and colleagues reported a method for quantitating β-cell-specific viability (Ichii et al. 2005b). They dissociated islets into single cells and then stained the cells with a zinc specific dye, Newport Green (Molecular Probes, Eugene, OR, USA), and with a mitochondrial dye, tetramethylrhodamine ethyl ester. The double positive cells were quantified on a flow cytometer after dead cells were excluded using a DNA-binding dye. They showed that the β-cell-specific

viability of human islet preparations was a useful marker of the outcome of a mouse transplant assay. The major limitation of this method is that the dispersed single cells are not likely representative of the original islets because a substantial fraction of cells is lost during dissociation. This dissociation of the islet also may contribute to β-cell death, resulting in a false-negative outcome. In addition, necrotic cells or late-stage apoptotic cells were not counted as nonviable cells, thereby leading to overestimation. Finally, several recent studies brought into question the use of Newport Green for detection of β cells because of its low quantum yield and poor selectivity to zinc (Gee et al. 2002).

Cytoprotective Strategies During Islet Isolation

During the isolation procedure the islets are exposed to numerous types of stress induced by nonphysiological stimuli. These include ischemic stress during organ procurement, preservation and islet isolation, mechanical and enzymatic stress during digestion, and osmotic stress during purification. A number of investigators have explored strategies to confer islet resistance to stress-induced damage. Most investigations have centered on treatment of the islets during culture. Some are focusing on modification in the isolation procedure to protect islets.

The use of antioxidants during islet isolation to protect islets from oxidative cell injury is a rational approach, because islet cells harbor poor endogenous antioxidant defense systems (Tiedge et al. 1997). Oxidative stress is a mechanism associated with disease states marked by inflammatory processes, including, but not limited to, autoimmune diseases such as type 1 diabetes and islet graft dysfunction (Tse et al. 2004; Toyokuni 1999; McCord 2000). Oxidative stress is initiated by the excessive production of reactive oxygen species, which are potent inducers of proinflammatory stress responses often marked by proinflammatory cytokines and chemokine synthesis (Tse et al. 2004; Adler et al. 1999).

Avila and colleagues delivered glutamine to the human pancreas via the duct prior to pancreas dissociation (Avila et al. 2005). They found that glutamine treatment reduced islet cell apoptosis and improved islet yield and function. Similarly, Bottino and colleagues perfused human pancreata with a mimetic superoxide dismutase, a novel class of chemical antioxidant compounds (Bottino et al. 2002). Islet yield immediately after isolation from a treated pancreas was similar to those from a non-treated pancreas. However, in vitro islet survival was significantly improved when islets were further treated with this compound during subsequent culture.

The ability to catalytically modulate oxidation-reduction reactions within a cell may control signaling cascades necessary for generating inflammation and provide therapeutic benefit targeted at downregulation of the immune response. The metalloporphyrin-based CAs have been demonstrated to scavenge a broad range of oxidants (Day et al. 1995, 1997, 1999; Ferrer-Sueta et al. 2003; Batinic-Haberle et al. 1998). The utility of these CAs to ameliorate other inflammatory-mediated disease processes has been demonstrated in a type 1 diabetes model of adoptive

transfer, apoptosis, and blocking of hydrogen peroxide-induced mitochondrial DNA damage and partial rescue of a lethal phenotype in a manganese superoxide dismutase knockout mouse (Melov et al. 1998; Piganelli et al. 2002). Furthermore, Piganelli and colleagues have demonstrated that redox modulation protects islets from both the stresses involved in the isolation procedure and transplant-related injury (Bottino et al. 2004; Sklavos et al. 2010). It is conceivable that islet-sparing agents (i.e., CAs), which decrease the production of free radicals and, therefore, inflammatory cytokines may have a positive impact on islet function posttransplant and reduced the prevalence of primary nonfunction, increase the incidences of insulin independence from single islet infusions. In addition CA may allow for the utilization of islet isolated from expanded-criteria donors and DCD donors potentially increase the number of patients that can receive an islet transplant.

Conclusions

In recent years, the results of clinical islet transplantation have improved dramatically; such that the 5-year insulin independence rates now match the results of whole organ pancreas transplantation. This reality has become possible through the implementation of new more potent immunosuppressive agents while avoiding corticosteroids. Not to be forgotten are the substantial advancements in human islet isolation technology that have provided critical contributions to the steady evolution of this therapy. The goal now should be to sharply focus on routinely obtaining a large number of viable islets that provide full functional survival for the long term. Once met, this goal will undoubtedly enhance the long-term rates of insulin independence from single-donor recipients in the clinical setting. Indeed, much work remains to be done to achieve this goal, but it is clear that there is scope and tangible path for significant improvements that will permit islet transplantation to be a practical therapy for all patients with type 1 diabetes.

Acknowledgments To members of the Clinical Islet Laboratory at the University of Alberta for technical help in islet preparation, to the organ procurement organizations across Canada for identifying donors, and to our colleagues in the Human Organ Procurement and Exchange program in Edmonton for assistance in organ procurement. The Clinical Islet Transplant Program at the University of Alberta receives funding from the Juvenile Diabetes Research Foundation and the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health and charitable donations administered through the Diabetes Research Institute Foundation Canada.

Cross-References

► Human Islet Autotransplantation
► Islet Isolation from Pancreatitis Pancreas for Islet Autotransplantation
► Mouse Islet Isolation
► Successes and Disappointments with Clinical Islet Transplantation

References

Adler V, Yin Z, Tew KD, Ronai Z (1999) Role of redox potential and reactive oxygen species in stress signaling. Oncogene 18:6104–6111

Aikin R, Rosenberg L, Paraskevas S, Maysinger D (2004) Inhibition of caspase-mediated PARP-1 cleavage results in increased necrosis in isolated islets of Langerhans. J Mol Med (Berl) 82:389–397

Akamatsu FE, De-Souza RR, Liberti EA (1999) Fall in the number of intracardiac neurons in aging rats. Mech Ageing Dev 109:153–161

Alejandro R, Cutfield RG, Shienvold FL, Polonsky KS, Noel J, Olson L et al (1986) Natural history of intrahepatic canine islet cell autografts. J Clin Invest 78:1339–1348

Antonioli B, Fermo I, Cainarca S, Marzorati S, Nano R, Baldissera M et al (2007) Characterization of collagenase blend enzymes for human islet transplantation. Transplantation 84:1568–1575

Aumailley M, Gayraud B (1998) Structure and biological activity of the extracellular matrix. J Mol Med (Berl) 76:253–265

Avila J, Barbaro B, Gangemi A, Romagnoli T, Kuechle J, Hansen M et al (2005) Intra-ductal glutamine administration reduces oxidative injury during human pancreatic islet isolation. Am J Transplant 5:2830–2837

Balamurugan AN, He J, Guo F, Stolz DB, Bertera S, Geng X et al (2005) Harmful delayed effects of exogenous isolation enzymes on isolated human islets: relevance to clinical transplantation. Am J Transplant 5:2671–2681

Balamurugan AN, Chang Y, Bertera S, Sands A, Shankar V, Trucco M et al (2006) Suitability of human juvenile pancreatic islets for clinical use. Diabetologia 49:1845–1854

Balamurugan AN, Breite AG, Anazawa T, Loganathan G, Wilhelm JJ, Papas KK et al (2010) Successful human islet isolation and transplantation indicating the importance of class 1 collagenase and collagen degradation activity assay. Transplantation 89:954–961

Balamurugan AN, Loganathan G, Bellin MD, Wilhelm JJ, Harmon J, Anazawa T et al (2012) A new enzyme mixture to increase the yield and transplant rate of autologous and allogeneic human islet products. Transplantation 93:693–702

Barbaro B, Salehi P, Wang Y, Qi M, Gangemi A, Kuechle J et al (2007) Improved human pancreatic islet purification with the refined UIC-UB density gradient. Transplantation 84:1200–1203

Barnett MJ, Zhai X, LeGatt DF, Cheng SB, Shapiro AM, Lakey JR (2005) Quantitative assessment of collagenase blends for human islet isolation. Transplantation 80:723–728

Batinic-Haberle I, Benov L, Spasojevic I, Fridovich I (1998) The ortho effect makes manganese (III) meso-tetrakis(*N*-methylpyridinium-2-yl)porphyrin a powerful and potentially useful superoxide dismutase mimic. J Biol Chem 273:24521–24528

Bedossa P, Lemaigre G, Bacci J, Martin E (1989) Quantitative estimation of the collagen content in normal and pathologic pancreas tissue. Digestion 44:7–13

Bennet W, Groth CG, Larsson R, Nilsson B, Korsgren O (2000) Isolated human islets trigger an instant blood mediated inflammatory reaction: implications for intraportal islet transplantation as a treatment for patients with type 1 diabetes. Ups J Med Sci 105:125–133

Berney T, Johnson PR (2010) Donor pancreata: evolving approaches to organ allocation for whole pancreas versus islet transplantation. Transplantation 90:238–243

Berney T, Molano RD, Cattan P, Pileggi A, Vizzardelli C, Oliver R et al (2001) Endotoxin-mediated delayed islet graft function is associated with increased intra-islet cytokine production and islet cell apoptosis. Transplantation 71:125–132

Bertuzzi F, Grohovaz F, Maffi P, Caumo A, Aldrighetti L, Nano R et al (2002) Successful transplantation of human islets in recipients bearing a kidney graft. Diabetologia 45:77–84

Bond MD, Van Wart HE (1984) Characterization of the individual collagenases from Clostridium histolyticum. Biochemistry 23:3085–3091

Bottino R, Balamurugan AN, Bertera S, Pietropaolo M, Trucco M, Piganelli JD (2002) Preservation of human islet cell functional mass by anti-oxidative action of a novel SOD mimic compound. Diabetes 51:2561–2567

Bottino R, Balamurugan AN, Tse H, Thirunavukkarasu C, Ge X, Profozich J et al (2004) Response of human islets to isolation stress and the effect of antioxidant treatment. Diabetes 53:2559–2568

Brandhorst H, Brandhorst D, Hering BJ, Federlin K, Bretzel RG (1995a) Body mass index of pancreatic donors: a decisive factor for human islet isolation. Exp Clin Endocrinol Diabetes 103(Suppl 2):23–26

Brandhorst H, Hering BJ, Brandhorst D, Hiller WF, Gubernatis G, Federlin K et al (1995b) Comparison of Histidine-Tryptophane-Ketoglutarate (HTK) and University of Wisconsin (UW) solution for pancreas perfusion prior to islet isolation, culture and transplantation. Transplant Proc 27:3175–3176

Brandhorst D, Brandhorst H, Hering BJ, Bretzel RG (1999) Long-term survival, morphology and in vitro function of isolated pig islets under different culture conditions. Transplantation 67:1533–1541

Brandhorst H, Brandhorst D, Hesse F, Ambrosius D, Brendel M, Kawakami Y et al (2003) Successful human islet isolation utilizing recombinant collagenase. Diabetes 52:1143–1146

Brandhorst H, Brendel MD, Eckhard M, Bretzel RG, Brandhorst D (2005) Influence of neutral protease activity on human islet isolation outcome. Transplant Proc 37:241–242

Brandhorst H, Raemsch-Guenther N, Raemsch C, Friedrich O, Huettler S, Kurfuerst M, Korsgren O, Brandhorst D (2008) The ratio between collagenase class I and class II influences the efficient islet release from the rat pancreas. Transplantation 35:456–461

Bucher P, Bosco D, Mathe Z, Matthey-Doret D, Andres A, Kurfuerst M et al (2004) Optimization of neutral protease to collagenase activity ratio for islet of Langerhans isolation. Transplant Proc 36:1145–1146

Burgarella S, Merlo S, Figliuzzi M, Remuzzi A (2013) Isolation of Langerhans islets by dielectrophoresis. Electrophoresis 34:1068–1075

Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC (2003) β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 52:102–110

Caballero-Corbalan J, Eich T, Lundgren T, Foss A, Felldin M, Kallen R et al (2007) No beneficial effect of two-layer storage compared with UW-storage on human islet isolation and transplantation. Transplantation 84:864–869

Cabric S, Sanchez J, Lundgren T, Foss A, Felldin M, Kallen R et al (2007) Islet surface heparinization prevents the instant blood-mediated inflammatory reaction in islet transplantation. Diabetes 56:2008–2015

Casey JJ, Lakey JR, Ryan EA, Paty BW, Owen R, O'Kelly K et al (2002) Portal venous pressure changes after sequential clinical islet transplantation. Transplantation 74:913–915

Chadwick DR, Robertson GS, Contractor HH, Rose S, Johnson PR, James RF et al (1994) Storage of pancreatic digest before islet purification. The influence of colloids and the sodium to potassium ratio in University of Wisconsin-based preservation solutions. Transplantation 58:99–104

Clausen B (1963) Influence of age on chondroitin sulfates and collagen of human aorta, myocardium, and skin. Lab Invest 12:538–542

Collaborative Islet Transplant Registry: Annual Report (2008) Available from https://web.emmes.com/study/isl/reports/reports.htm. Accessed 20 Oct 2013

Colton CK, Papas KK, Pisania A, Rappel MJ, Powers DE, O'Neil JJ et al (2006) Characterization of islet preparations. In: Halberstadt CD, Emerich DF (eds) Cellular transplantation: from laboratory to clinic. Elsevier, New York, pp 85–133

Cross SE, Hughes SJ, Partridge CJ, Clark A, Gray DW, Johnson PR (2008) Collagenase penetrates human pancreatic islets following standard intraductal administration. Transplantation 86:907–911

Daoud J, Rosenberg L, Tabrizian M (2010a) Pancreatic islet culture and preservation strategies: advances, challenges, and future outlook. Cell Transplant 19:1523–1535

Daoud J, Petropavlovskaia M, Rosenberg L, Tabrizian M (2010b) The effect of extracellular matrix components on the preservation of human islet function in vitro. Biomaterials 31:1676–1682

Day BJ, Shawen S, Liochev SI, Crapo JD (1995) A metalloporphyrin superoxide dismutase mimetic protects against paraquat-induced endothelial cell injury, in vitro. J Pharmacol Exp Ther 275:1227–1232

Day BJ, Fridovich I, Crapo JD (1997) Manganic porphyrins possess catalase activity and protect endothelial cells against hydrogen peroxide-mediated injury. Arch Biochem Biophys 347:256–262

Day BJ, Batinic-Haberle I, Crapo JD (1999) Metalloporphyrins are potent inhibitors of lipid peroxidation. Free Radic Biol Med 26:730–736

de Boer J, De Meester J, Smits JM, Groenewoud AF, Bok A, van der Velde O et al (1999) Eurotransplant randomized multicenter kidney graft preservation study comparing HTK with UW and Euro-Collins. Transpl Int 12:447–453

de la Grandmaison GL, Clairand I, Durigon M (2001) Organ weight in 684 adult autopsies: new tables for a Caucasoid population. Forensic Sci Int 119:149–154

Deng S, Vatamaniuk M, Huang X, Doliba N, Lian MM, Frank A et al (2004) Structural and functional abnormalities in the islets isolated from type 2 diabetic subjects. Diabetes 53:624–632

Escolar JC, Hoo-Paris R, Castex C, Sutter BC (1990) Effect of low temperatures on glucose-induced insulin secretion and glucose metabolism in isolated pancreatic islets of the rat. J Endocrinol 125:45–51

Ferrer-Sueta G, Vitturi D, Batinic-Haberle I, Fridovich I, Goldstein S, Czapski G et al (2003) Reactions of manganese porphyrins with peroxynitrite and carbonate radical anion. J Biol Chem 278:27432–27438

Gala-Lopez B, Pepper AR, Shapiro AM (2013) Biologic agents in islet transplantation. Curr Diab Rep 13:713–722

Gee KR, Zhou ZL, Ton-That D, Sensi SL, Weiss JH (2002) Measuring zinc in living cells. A new generation of sensitive and selective fluorescent probes. Cell Calcium 31:245–251

Georges P, Muirhead RP, Williams L, Holman S, Tabiin MT, Dean SK et al (2002) Comparison of size, viability, and function of fetal pig islet-like cell clusters after digestion using collagenase or liberase. Cell Transplant 11:539–545

Gomes OA, de Souza RR, Liberti EA (1997) A preliminary investigation of the effects of aging on the nerve cell number in the myenteric ganglia of the human colon. Gerontology 43:210–217

Goto M, Eich TM, Felldin M, Foss A, Kallen R, Salmela K et al (2004) Refinement of the automated method for human islet isolation and presentation of a closed system for in vitro islet culture. Transplantation 78:1367–1375

Gray DW, Warnock GL, Sutton R, Peters M, McShane P, Morris PJ (1986) Successful autotransplantation of isolated islets of Langerhans in the cynomolgus monkey. Br J Surg 73:850–853

Hanley SC, Paraskevas S, Rosenberg L (2008) Donor and isolation variables predicting human islet isolation success. Transplantation 85:950–955

Harlan DM, Kenyon NS, Korsgren O, Roep BO (2009) Current advances and travails in islet transplantation. Diabetes 58:2175–2184

Heller-Harrison RA, Carter WG (1984) Pepsin-generated type VI collagen is a degradation product of GP140. J Biol Chem 259:6858–6864

Hering BJ, Matsumoto I, Sawada T, Nakano M, Sakai T, Kandaswamy R et al (2002) Impact of two-layer pancreas preservation on islet isolation and transplantation. Transplantation 74:1813–1816

Hering BJ, Kandaswamy R, Harmon JV, Ansite JD, Clemmings SM, Sakai T et al (2004) Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. Am J Transplant 4:390–401

Hiller WF, Klempnauer J, Luck R, Steiniger B (1991) Progressive deterioration of endocrine function after intraportal but not kidney subcapsular rat islet transplantation. Diabetes 40:134–140

Horcher A, Siebers U, Bretzel RG, Federlin K, Zekorn T (1995) Transplantation of microencapsulated islets in rats: influence of low temperature culture before or after the encapsulation procedure on the graft function. Transplant Proc 27:3232–3233

Huang GC, Zhao M, Jones P, Persaud S, Ramracheya R, Lobner K et al (2004) The development of new density gradient media for purifying human islets and islet-quality assessments. Transplantation 77:143–145

Hughes SJ, McShane P, Contractor HH, Gray DW, Clark A, Johnson PR (2005) Comparison of the collagen VI content within the islet-exocrine interface of the head, body, and tail regions of the human pancreas. Transplant Proc 37:3444–3445

Hughes SJ, Clark A, McShane P, Contractor HH, Gray DW, Johnson PR (2006) Characterisation of collagen VI within the islet-exocrine interface of the human pancreas: implications for clinical islet isolation? Transplantation 81:423–426

Hyder A (2005) Effect of the pancreatic digestion with liberase versus collagenase on the yield, function and viability of neonatal rat pancreatic islets. Cell Biol Int 29:831–834

Ichii H, Pileggi A, Molano RD, Baidal DA, Khan A, Kuroda Y et al (2005a) Rescue purification maximizes the use of human islet preparations for transplantation. Am J Transplant 5:21–30

Ichii H, Inverardi L, Pileggi A, Molano RD, Cabrera O, Caicedo A et al (2005b) A novel method for the assessment of cellular composition and β-cell viability in human islet preparations. Am J Transplant 5:1635–1645

Ichii H, Pileggi A, Khan A, Fraker C, Ricordi C (2007) Culture and transportation of human islets between centers. In: Shapiro AMJ, Shaw J (eds) Islet transplantation and β cell replacement therapy. Informa Healthcare, New York, p 251

Iglesias I, Valiente L, Shiang KD, Ichii H, Kandeel F, Al-Abdullah IH (2012) The effects of digestion enzymes on islet viability and cellular composition. Cell Transplant 21:649–655

Ihm SH, Matsumoto I, Sawada T, Nakano M, Zhang HJ, Ansite JD et al (2006) Effect of donor age on function of isolated human islets. Diabetes 55:1361–1368

Ihm SH, Matsumoto I, Zhang HJ, Ansite JD, Hering BJ (2009) Effect of short-term culture on functional and stress-related parameters in isolated human islets. Transpl Int 22:207–216

Ilieva A, Yuan S, Wang R, Duguid WP, Rosenberg L (1999) The structural integrity of the islet in vitro: the effect of incubation temperature. Pancreas 19:297–303

Jaeger C, Wohrle M, Bretzel RG, Federlin K (1994) Effect of transplantation site and culture pretreatment on islet xenograft survival (rat to mouse) in experimental diabetes without immunosuppression of the host. Acta Diabetol 31:193–197

Johnson LF, deSerres S, Herzog SR, Peterson HD, Meyer AA (1991) Antigenic cross-reactivity between media supplements for cultured keratinocyte grafts. J Burn Care Rehabil 12:306–312

Johnson PR, White SA, London NJ (1996) Collagenase and human islet isolation. Cell Transplant 5:437–452

Kadono G, Ishihara T, Yamaguchi T, Kato K, Kondo F, Naito I et al (2004) Immunohistochemical localization of type IV collagen α chains in the basement membrane of the pancreatic duct in human normal pancreas and pancreatic diseases. Pancreas 29:61–66

Kamei T, Yasunami Y, Terasaka R, Konomi K (1989) Importance of transplant site for prolongation of islet xenograft survival (rat to mouse) after low-temperature culture and cyclosporin A. Transplant Proc 21:2693–2694

Kaufman DB, Morel P, Field MJ, Munn SR, Sutherland DE (1990) Purified canine islet autografts. Functional outcome as influenced by islet number and implantation site. Transplantation 50:385–391

Kedinger M, Haffen K, Grenier J, Eloy R (1977) In vitro culture reduces immunogenicity of pancreatic endocrine islets. Nature 270:736–738

Keymeulen B, Anselmo J, Pipeleers D (1997) Length of metabolic normalization after rat islet cell transplantation depends on endocrine cell composition of graft and on donor age. Diabetologia 40:1152–1158

Keymeulen B, Ling Z, Gorus FK, Delvaux G, Bouwens L, Grupping A et al (1998) Implantation of standardized β-cell grafts in a liver segment of IDDM patients: graft and recipients characteristics in two cases of insulin-independence under maintenance immunosuppression for prior kidney graft. Diabetologia 41:452–459

Keymeulen B, Gillard P, Mathieu C, Movahedi B, Maleux G, Delvaux G et al (2006) Correlation between β cell mass and glycemic control in type 1 diabetic recipients of islet cell graft. Proc Natl Acad Sci U S A 103:17444–17449

Kin T (2010) Islet isolation for clinical transplantation. Adv Exp Med Biol 654:683–710

Kin T, Murdoch TB, Shapiro AM, Lakey JR (2006a) Estimation of pancreas weight from donor variables. Cell Transplant 15:181–185

Kin T, Mirbolooki M, Salehi P, Tsukada M, O'Gorman D, Imes S et al (2006b) Islet isolation and transplantation outcomes of pancreas preserved with University of Wisconsin solution versus two-layer method using preoxygenated perfluorocarbon. Transplantation 82:1286–1290

Kin T, Mirbolooki M, Brassil J, Shapiro AM, Lakey J (2006) Machine perfusion for prolonged pancreas preservation prior to islet isolation (Abstract). In: Annual scientific meeting, Canadian Society of Transplantation

Kin T, Johnson PR, Shapiro AM, Lakey JR (2007a) Factors influencing the collagenase digestion phase of human islet isolation. Transplantation 83:7–12

Kin T, Zhai X, Murdoch TB, Salam A, Shapiro AM, Lakey JR (2007b) Enhancing the success of human islet isolation through optimization and characterization of pancreas dissociation enzyme. Am J Transplant 7:1233–1241

Kin T, Zhai X, O'Gorman D, Shapiro AM (2008a) Detrimental effect of excessive collagenase class II on human islet isolation outcome. Transpl Int 21:1059–1065

Kin T, Senior P, O'Gorman D, Richer B, Salam A, Shapiro AM (2008b) Risk factors for islet loss during culture prior to transplantation. Transpl Int 21:1029–1035

Kin T, O'Gorman D, Zhai X, Pawlick R, Imes S, Senior P, Shapiro AM (2009) Nonsimultaneous administration of pancreas dissociation enzymes during islet isolation. Transplantation 87:1700–1705

King A, Lock J, Xu G, Bonner-Weir S, Weir GC (2005) Islet transplantation outcomes in mice are better with fresh islets and exendin-4 treatment. Diabetologia 48:2074–2079

Koh A, Kin T, Imes S, Shapiro AM, Senior P (2008) Islets isolated from donors with elevated HbA1c can be successfully transplanted. Transplantation 86:1622–1624

Korsgren O, Nilsson B, Berne C, Felldin M, Foss A, Kallen R et al (2005) Current status of clinical islet transplantation. Transplantation 79:1289–1293

Koulmanda M, Smith RN, Qipo A, Weir G, Auchincloss H, Strom TB (2006) Prolonged survival of allogeneic islets in cynomolgus monkeys after short-term anti-CD154-based therapy: nonimmunologic graft failure? Am J Transplant 6:687–696

Kuroda Y, Kawamura T, Suzuki Y, Fujiwara H, Yamamoto K, Saitoh Y (1988) A new, simple method for cold storage of the pancreas using perfluorochemical. Transplantation 46:457–460

Lacy PE, Finke EH (1991) Activation of intraislet lymphoid cells causes destruction of islet cells. Am J Pathol 138:1183–1190

Lacy PE, Davie JM, Finke EH (1979a) Prolongation of islet allograft survival following in vitro culture (24 degrees C) and a single injection of ALS. Science 204:312–313

Lacy PE, Davie JM, Finke EH (1979b) Induction of rejection of successful allografts of rat islets by donor peritoneal exudate cells. Transplantation 28:415–420

Lacy PE, Ricordi C, Finke EH (1989) Effect of transplantation site and α L3T4 treatment on survival of rat, hamster, and rabbit islet xenografts in mice. Transplantation 47:761–766

Lake SP, Bassett PD, Larkins A, Revell J, Walczak K, Chamberlain J et al (1989) Large-scale purification of human islets utilizing discontinuous albumin gradient on IBM 2991 cell separator. Diabetes 38(Suppl 1):143–145

Lakey JR, Warnock GL, Kneteman NM, Ao Z, Rajotte RV (1994) Effects of pre-cryopreservation culture on human islet recovery and in vitro function. Transplant Proc 26:820

Lakey JR, Rajotte RV, Warnock GL, Kneteman NM (1995) Human pancreas preservation prior to islet isolation. Cold ischemic tolerance. Transplantation 59:689–694

Lakey JR, Warnock GL, Rajotte RV, Suarez-Alamazor ME, Ao Z, Shapiro AM et al (1996) Variables in organ donors that affect the recovery of human islets of Langerhans. Transplantation 61:1047–1053

Lakey JR, Cavanagh TJ, Zieger MA, Wright M (1998) Evaluation of a purified enzyme blend for the recovery and function of canine pancreatic islets. Cell Transplant 7:365–372
Lakey JR, Woods EJ, Zieger MA, Avila JG, Geary WA, Voytik-Harbin SL et al (2001) Improved islet survival and in vitro function using solubilized small intestinal submucosa. Cell Tissue Bank 2:217–224
Lau J, Henriksnas J, Scennson J, Carlsson PO (2009) Oxygenation of islets and its role in transplantation. Curr Opin Organ Transpl 14:688–693
Leeser DB, Bingaman AW, Poliakova L, Shi Q, Gage F, Bartlett ST et al (2004) Pulsatile pump perfusion of pancreata before human islet cell isolation. Transplant Proc 36:1050–1051
Levy MM, Ketchum RJ, Tomaszewski JE, Naji A, Barker CF, Brayman KL (2002) Intrathymic islet transplantation in the canine: I. Histological and functional evidence of autologous intrathymic islet engraftment and survival in pancreatectomized recipients. Transplantation 73:842–852
Linder M, Salovuori I, Ruohonen L, Teeri TT (1996) Characterization of a double cellulose-binding domain. Synergistic high affinity binding to crystalline cellulose. J Biol Chem 271:21268–21272
Linetsky E, Bottino R, Lehmann R, Alejandro R, Inverardi L, Ricordi C (1997) Improved human islet isolation using a new enzyme blend, liberase. Diabetes 46:1120–1123
London NJ, James RF, Bell PR (1992) Islet purification. In: Ricordi C (ed) Pancreatic islet transplantation. Landes, Austin, pp 113–123
Mackensen A, Drager R, Schlesier M, Mertelsmann R, Lindemann A (2000) Presence of IgE antibodies to bovine serum albumin in a patient developing anaphylaxis after vaccination with human peptide-pulsed dendritic cells. Cancer Immunol Immunother 49:152–156
Maclean N, Ogilvie RF (1955) Quantitative estimation of the pancreatic islet tissue in diabetic subjects. Diabetes 4:367–376
Maechler P (2002) Mitochondria as the conductor of metabolic signals for insulin exocytosis in pancreatic β-cells. Cell Mol Life Sci 59:1803–1818
Mallya SK, Mookhtiar KA, Van Wart HE (1992) Kinetics of hydrolysis of type I, II, and III collagens by the class I and II Clostridium histolyticum collagenases. J Protein Chem 11:99–107
Mandel TE, Koulmanda M (1985) Effect of culture conditions on fetal mouse pancreas in vitro and after transplantation in syngeneic and allogeneic recipients. Diabetes 34:1082–1087
Markmann JF, Deng S, Huang X, Desai NM, Velidedeoglu EH, Lui C et al (2003) Insulin independence following isolated islet transplantation and single islet infusions. Ann Surg 237:741–749
Matsumoto I, Sawada T, Nakano M, Sakai T, Liu B, Ansite JD et al (2004) Improvement in islet yield from obese donors for human islet transplants. Transplantation 78:880–885
Matsushita O, Okabe A (2001) Clostridial hydrolytic enzymes degrading extracellular components. Toxicon 39:1769–1780
Matsushita O, Jung CM, Minami J, Katayama S, Nishi N, Okabe A (1998) A study of the collagen-binding domain of a 116-kDa *Clostridium histolyticum* collagenase. J Biol Chem 273:3643–3648
Matsushita O, Jung CM, Katayama S, Minami J, Takahashi Y, Okabe A (1999) Gene duplication and multiplicity of collagenases in *Clostridium histolyticum*. J Bacteriol 181:923–933
Matsushita O, Koide T, Kobayashi R, Nagata K, Okabe A (2001) Substrate recognition by the collagen-binding domain of *Clostridium histolyticum* class I collagenase. J Biol Chem 276:8761–8770
McCall MD, Maciver AH, Pawlick R, Edgar R, Shapiro AM (2011) Histopaque provides optimal mouse islet purification kinetics: comparison study with Ficoll, iodixanol and dextran. Islets 3:144–149
McCord J (2000) The evolution of free radicals and oxidative stress. Am J Med 108:652–659
Mehigan DG, Bell WR, Zuidema GD, Eggleston JC, Cameron JL (1980) Disseminated intravascular coagulation and portal hypertension following pancreatic islet autotransplantation. Ann Surg 191:287–293

Melov S, Schneider JA, Day BJ, Hinerfeld D, Coskun P, Mirra SS et al (1998) A novel neurological phenotype in mice lacking mitochondrial manganese superoxide dismutase. Nat Genet 18:159–163

Mettler E, Trenkler A, Feilen PJ, Wiegand F, Fottner C, Ehrhart F et al (2013) Magnetic separation of encapsulated islet cells labeled with superparamagnetic iron oxide nano particles. Xenotransplantation 20:219–226

Meyer AA, Manktelow A, Johnson M, deSerres S, Herzog S, Peterson HD (1982) Antibody response to xenogeneic proteins in burned patients receiving cultured keratinocyte grafts. J Trauma 28:1054–1059

Minor T, Tolba R, Akbar S, Dombrowski T, Muller A (2000) The suboptimal donor: reduction of ischemic injury in fatty livers by gaseous oxygen persufflation. Transplant Proc 32:10

Misawa R, Ricordi C, Miki A, Barker S, Molano RD, Khan A et al (2012) Evaluation of viable β-cell mass is useful for selecting collagenase for human islet isolation: comparison of collagenase NB1 and liberase HI. Cell Transplant 21:39–47

Mita A, Ricordi C, Messinger S, Miki A, Misawa R, Barker S et al (2010) Antiproinflammatory effects of iodixanol (OptiPrep)-based density gradient purification on human islet preparations. Cell Transplant 19:1537–1546

Mittal VK, Toledo-Pereyra LH, Sharma M, Ramaswamy K, Puri VK, Cortez JA et al (1981) Acute portal hypertension and disseminated intravascular coagulation following pancreatic islet autotransplantation after subtotal pancreatectomy. Transplantation 31:302–304

Mookhtiar KA, Van Wart HE (1992) *Clostridium histolyticum* collagenases: a new look at some old enzymes. Matrix Suppl 1:116–126

Morita A, Kuroda Y, Fujino Y, Tanioka Y, Ku Y, Saitoh Y (1993) Assessment of pancreas graft viability preserved by a two-layer (University of Wisconsin solution/perfluorochemical) method after significant warm ischemia. Transplantation 55:667–669

Morsiani E, Lacy PE (1990) Effect of low-temperature culture and L3T4 antibody on the survival of pancreatic islets xenograft (fish to mouse). Eur Surg Res 22:78–85

Nagai K, Yagi S, Afify M, Bleilevens C, Uemoto S, Tolba RH (2013) Impact of venous-systemic oxygen persufflation with nitric oxide gas on steatotic grafts after partial orthotopic liver transplantation in rats. Transplantation 95:78–84

Nagata H, Matsumoto S, Okitsu T et al (2006) Procurement of the human pancreas for pancreatic islet transplantation from marginal cadaver donors. Transplantation 82:327–331

Nano R, Clissi B, Melzi R, Calori G, Maffi P, Antonioli B et al (2005) Islet isolation for allotransplantation: variables associated with successful islet yield and graft function. Diabetologia 48:906–912

Noguchi H, Ueda M, Nakai Y, Iwanaga Y, Okitsu T, Nagata H et al (2006) Modified two-layer preservation method (M-Kyoto/PFC) improves islet yields in islet isolation. Am J Transplant 6:496–504

Noguchi H, Ikemoto T, Naziruddin B, Jackson A, Shimoda M, Fujita Y et al (2009) Iodixanol-controlled density gradient during islet purification improves recovery rate in human islet isolation. Transplantation 87:1629–1635

Noguchi H, Naziruddin B, Onaca N, Jackson A, Shimoda M, Ikemoto T et al (2010) Comparison of modified Celsior solution and M-kyoto solution for pancreas preservation in human islet isolation. Cell Transplant 19:751–758

Noguchi H, Naziruddin B, Jackson A, Shimoda M, Fujita Y, Chujo D et al (2012a) Comparison of ulinastatin, gabexate mesilate, and nafamostat mesilate in preservation solution for islet isolation. Cell Transplant 21:509–516

Noguchi H, Naziruddin B, Jackson A, Shimoda M, Ikemoto T, Fujita Y et al (2012b) Fresh islets are more effective for islet transplantation than cultured islets. Cell Transplant 21:517–523

Oberholzer J, Triponez F, Lou J, Morel P (1999) Clinical islet transplantation: a review. Ann N Y Acad Sci 875:189–199

Oberholzer J, Triponez F, Mage R, Andereggen E, Buhler L, Cretin N et al (2000) Human islet transplantation: lessons from 13 autologous and 13 allogeneic transplantations. Transplantation 69:1115–1123

O'Gorman D, Kin T, Murdoch T, Richer B, McGhee-Wilson D, Ryan E et al (2005) The standardization of pancreatic donors for islet isolations. Transplantation 80:801–806

O'Gorman D, Kin T, Imes S, Pawlick R, Senior P, Shapiro AM (2010) Comparison of human islet isolation outcomes using a new mammalian tissue-free enzyme versus collagenase NB-1. Transplantation 90:255–259

Olack B, Swanson C, McLear M, Longwith J, Scharp D, Lacy PE (1991) Islet purification using Euro-Ficoll gradients. Transplant Proc 23:774–776

Olack BJ, Swanson CJ, Howard TK, Mohanakumar T (1999) Improved method for the isolation and purification of human islets of langerhans using liberase enzyme blend. Hum Immunol 60:1303–1309

Olsson R, Carlsson PO (2005) Better vascular engraftment and function in pancreatic islets transplanted without prior culture. Diabetologia 48:469–476

Ono J, Lacy PE, Michael HE, Greider MH (1979) Studies of the functional and morphologic status of islets maintained at 24 C for four weeks in vitro. Am J Pathol 97:489–503

Opelz G, Terasaki PI (1974) Lymphocyte antigenicity loss with retention of responsiveness. Science 184:464–466

Orloff MJ, Macedo A, Greenleaf GE, Girard B (1988) Comparison of the metabolic control of diabetes achieved by whole pancreas transplantation and pancreatic islet transplantation in rats. Transplantation 45:307–312

Papas KK, Avgoustiniatos ES, Tempelman LA, Weir GC, Colton CK, Pisania A et al (2005) High-density culture of human islets on top of silicone rubber membranes. Transplant Proc 37:3412–3414

Papas KK, Colton CK, Nelson RA, Rozak PR, Avgoustiniatos ES, Scott WE 3rd et al (2007) Human islet oxygen consumption rate and DNA measurements predict diabetes reversal in nude mice. Am J Transplant 7:707–713

Pepper AR, Hasilo CP, Melling CW, Mazzuca DM, Vilk G, Zou G et al (2012) The islet size to oxygen consumption ratio reliably predicts reversal of diabetes posttransplant. Cell Transplant 21:2797–2804

Piganelli JD, Flores SC, Cruz C, Koepp J, Batinic-Haberle I, Crapo J et al (2002) A metalloporphyrin-based superoxide dismutase mimic inhibits adoptive transfer of autoimmune diabetes by a diabetogenic T-cell clone. Diabetes 51:347–355

Ponte GM, Pileggi A, Messinger S, Alejandro A, Ichii H, Baidal DA et al (2007) Toward maximizing the success rates of human islet isolation: influence of donor and isolation factors. Cell Transplant 16:595–607

Qin H, Matsumoto S, Klintmalm GB, De vol EB (2011) A meta-analysis for comparison of the two-layer and university of Wisconsin pancreas preservation methods in islet transplantation. Cell Transplant 20:1127–1137

Rahier J, Goebbels RM, Henquin JC (1983) Cellular composition of the human diabetic pancreas. Diabetologia 24:366–371

Ricordi C, Fraker C, Szust J, Al-Abdullah I, Poggioli R, Kirlew T et al (2003) Improved human islet isolation outcome from marginal donors following addition of oxygenated perfluorocarbon to the cold-storage solution. Transplantation 75:1524–1527

Ris F, Toso C, Veith FU, Majno P, Morel P, Oberholzer J (2004) Are criteria for islet and pancreas donors sufficiently different to minimize competition? Am J Transplant 4:763–766

Robertson GS, Chadwick D, Contractor H, Rose S, Chamberlain R, Clayton H et al (1992) Storage of human pancreatic digest in University of Wisconsin solution significantly improves subsequent islet purification. Br J Surg 79:899–902

Robertson RP, Lanz KJ, Sutherland DE, Kendall DM (2001) Prevention of diabetes for up to 13 years by autoislet transplantation after pancreatectomy for chronic pancreatitis. Diabetes 50:47–50

Ryan EA, Lakey JR, Rajotte RV, Korbutt GS, Kin T, Imes S et al (2001) Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. Diabetes 50:710–719
Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM et al (2005) Five-year follow-up after clinical islet transplantation. Diabetes 54:2060–2069
Ryu S, Yasunami Y (1991) The necessity of differential immunosuppression for prevention of immune rejection by FK506 in rat islet allografts transplanted into the liver or beneath the kidney capsule. Transplantation 52:599–605
Sabek OM, Cowan P, Fraga DW, Gaber AO (2008) The effect of isolation methods and the use of different enzymes on islet yield and in vivo function. Cell Transplant 17:785–792
Saisho Y, Butler AE, Meier JJ, Monchamp T, Allen-Auerbach M, Rizza RA et al (2007) Pancreas volumes in humans from birth to age one hundred taking into account sex, obesity, and presence of type-2 diabetes. Clin Anat 20:933–942
Saito K, Iwama N, Takahashi T (1978) Morphometrical analysis on topographical difference in size distribution, number and volume of islets in the human pancreas. Tohoku J Exp Med 124:177–186
Saito T, Gotoh M, Satomi S, Uemoto S, Kenmochi T, Itoh T et al (2010) Islet transplantation using donors after cardiac death: report of the Japan Islet Transplantation Registry. Transplantation 90:740–747
Sakuraba H, Mizukami H, Yagihashi N, Wada R, Hanyu C, Yagihashi S (2002) Reduced β-cell mass and expression of oxidative stress-related DNA damage in the islet of Japanese Type II diabetic patients. Diabetologia 45:85–96
Salehi P, Hansen MA, Avila JG, Barbaro B, Gangemi A, Romagnoli T et al (2006) Human islet isolation outcomes from pancreata preserved with Histidine-Tryptophan Ketoglutarate versus University of Wisconsin solution. Transplantation 82:983–985
Scharp DW, Kemp CB, Knight MJ, Ballinger WF, Lacy PE (1973) The use of ficoll in the preparation of viable islets of langerhans from the rat pancreas. Transplantation 16:686–689
Scharp DW, Lacy PE, Finke E, Olack B (1987) Low-temperature culture of human islets isolated by the distention method and purified with Ficoll or Percoll gradients. Surgery 102:869–879
Scott WE 3rd, O'Brien TD, Ferrer-Fabrega J, Avgoustiniatos ES, Weegman BP, Anazawa T et al (2010) Persufflation improves pancreas preservation when compared with the two-layer method. Transplant Proc 42:2016–2019
Shapiro AM, Lakey JR, Rajotte RV, Warnock GL, Friedlich MS, Jewell LD et al (1995) Portal vein thrombosis after transplantation of partially purified pancreatic islets in a combined human liver/islet allograft. Transplantation 59:1060–1063
Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL et al (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 343:230–238
Shimoda M, Itoh T, Iwahashi S, Takita M, Sugimoto K, Kanak MA et al (2012) An effective purification method using large bottles for human pancreatic islet isolation. Islets 4:398–404
Sklavos MM, Bertera S, Tse HM, Bottino R, He J, Beilke JN et al (2010) Redox modulation protects islets from transplant-related injury. Diabetes 59:1731–1738
Sobel H, Marmorston J (1956) The possible role of the gel-fiber ratio of connective tissue in the aging process. J Gerontol 11:2–7
Socci C, Davalli AM, Vignali A, Pontiroli AE, Maffi P, Magistretti P et al (1993) A significant increase of islet yield by early injection of collagenase into the pancreatic duct of young donors. Transplantation 55:661–663
Soru E, Zaharia O (1972) *Clostridium histolyticum* collagenase. II. Partial characterization. Enzymologia 43:45–55
Spees JL, Gregory CA, Singh H, Tucker HA, Peister A, Lynch PJ et al (2004) Internalized antigens must be removed to prepare hypoimmunogenic mesenchymal stem cells for cell and gene therapy. Mol Ther 9:747–756

Srinivasan PK, Yagi S, Doorschodt B, Nagai K, Afify M, Uemoto S et al (2012) Impact of venous systemic oxygen persufflation supplemented with nitric oxide gas on cold-stored, warm ischemia-damaged experimental liver grafts. Liver Transpl 18:219–225

Stadlbauer V, Schaffellner S, Iberer F, Lackner C, Liegl B, Zink B et al (2003) Occurrence of apoptosis during ischemia in porcine pancreas islet cells. Int J Artif Organs 26:205–210

Stegall MD, Dean PG, Sung R, Guidinger MK, McBride MA, Sommers C et al (2007) The rationale for the new deceased donor pancreas allocation schema. Transplantation 83:1156–1161

Sung RS, Christensen LL, Leichtman AB, Greenstein SM, Distant DA, Wynn JJ et al (2008) Determinants of discard of expanded criteria donor kidneys: impact of biopsy and machine perfusion. Am J Transplant 8:783–792

Suszynski TM, Rizzari MD, Scott WE 3rd, Tempelman LA, Taylor MJ, Papas KK (2012) Persufflation (or gaseous oxygen perfusion) as a method of organ preservation. Cryobiology 64:125–143

Suszynski TM, Rizzari MD, Scott WE, Eckman PM, Fonger JD, John R et al (2013) Persufflation (gaseous oxygen perfusion) as a method of heart preservation. J Cardiothorac Surg 8:105

Sutherland DE, Gruessner AC, Carlson AM, Blondet JJ, Balamurugan AN, Reigstad KF et al (2008) Islet autotransplant outcomes after total pancreatectomy: a contrast to islet allograft outcomes. Transplantation 86:1799–1802

Sweet IR, Khalil G, Wallen AR, Steedman M, Schenkman KA, Reems JA et al (2002) Continuous measurement of oxygen consumption by pancreatic islets. Diabetes Technol Ther 4:661–672

Sweet IR, Gilbert M, Jensen R, Sabek O, Fraga DW, Gaber AO et al (2005) Glucose stimulation of cytochrome C reduction and oxygen consumption as assessment of human islet quality. Transplantation 80:1003–1011

Sweet IR, Gilbert M, Scott S, Todorov I, Jensen R, Nair I et al (2008) Glucose-stimulated increment in oxygen consumption rate as a standardized test of human islet quality. Am J Transplant 8:183–192

Szot GL, Lee MR, Tavakol MM, Lang J, Dekovic F, Kerlan RK et al (2009) Successful clinical islet isolation using a GMP-manufactured collagenase and neutral protease. Transplantation 88:753–756

Tanioka Y, Sutherland DE, Kuroda Y, Gilmore TR, Asaheim TC, Kronson JW et al (1997) Excellence of the two-layer method (University of Wisconsin solution/perfluorochemical) in pancreas preservation before islet isolation. Surgery 122:435–441

Taylor MJ, Baicu S (2011) Cryo-isolation: a novel method for enzyme-free isolation of pancreatic islets involving in situ cryopreservation of islets and selective destruction of acinar tissue. Transplant Proc 43:3181–3183

Taylor MJ, Baicu S, Leman B, Greene E, Vazquez A, Brassil J (2008) Twenty-four hour hypothermic machine perfusion preservation of porcine pancreas facilitates processing for islet isolation. Transplant Proc 40:480–482

Tiedge M, Lortz S, Drinkgern J, Lenzen S (1997) Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes 46:1733–1742

Tolba RH, Schildberg FA, Schnurr C, Glatzel U, Decker D, Minor T (2006) Reduced liver apoptosis after venous systemic oxygen persufflation in non-heart-beating donors. J Invest Surg 19:219–227

Toledo-Pereyra LH, Valgee KD, Castellanos J, Chee M (1980) Hypothermic pulsatile perfusion: its use in the preservation of pancreases for 24 to 48 hours before islet cell transplantation. Arch Surg 115:95–98

Toledo-Pereyra LH, Rowlett AL, Cain W, Rosenberg JC, Gordon DA, MacKenzie GH (1984) Hepatic infarction following intraportal islet cell autotransplantation after near-total pancreatectomy. Transplantation 38:88–89

Totani T, Teramura Y, Iwata H (2008) Immobilization of urokinase on the islet surface by amphiphilic poly(vinyl alcohol) that carries alkyl side chains. Biomaterials 29:2878–2883

Toyokuni S (1999) Reactive oxygen species-induced molecular damage and its application in pathology. Pathol Int 49:91–102

Tse HM, Milton MJ, Piganelli JD (2004) Mechanistic analysis of the immunomodulatory effects of a catalytic antioxidant on antigen-presenting cells: implication for their use in targeting oxidation-reduction reactions in innate immunity. Free Radic Biol Med 36:233–247

Tsujimura T, Kuroda Y, Kin T, Avila JG, Rajotte RV, Korbutt GS et al (2002) Human islet transplantation from pancreases with prolonged cold ischemia using additional preservation by the two-layer (UW solution/perfluorochemical) cold-storage method. Transplantation 74:1687–1691

van Deijnen JH, Hulstaert CE, Wolters GH, van Schilfgaarde R (1992) Significance of the peri-insular extracellular matrix for islet isolation from the pancreas of rat, dog, pig, and man. Cell Tissue Res 267:139–146

Van Deijnen JH, Van Suylichem PT, Wolters GH, Van Schilfgaarde R (1994) Distribution of collagens type I, type III and type V in the pancreas of rat, dog, pig and man. Cell Tissue Res 277:115–121

van der Burg MP, Gooszen HG, Ploeg RJ, Guicherit OR, Scherft JP, Terpstra JL et al (1990) Pancreatic islet isolation with UW solution: a new concept. Transplant Proc 22:2050–2051

Van Wart HE, Steinbrink DR (1985) Complementary substrate specificities of class I and class II collagenases from *Clostridium histolyticum*. Biochemistry 24:6520–6526

Vargas F, Julian JF, Llamazares JF, Garcia-Cuyas F, Jimenez M, Pujol-Borrell R et al (2001) Engraftment of islets obtained by collagenase and liberase in diabetic rats: a comparative study. Pancreas 23:406–413

Walsh TJ, Eggleston JC, Cameron JL (1982) Portal hypertension, hepatic infarction, and liver failure complicating pancreatic islet autotransplantation. Surgery 91:485–487

Wang W, Upshaw L, Zhang G, Strong DM, Reems JA (2007) Adjustment of digestion enzyme composition improves islet isolation outcome from marginal grade human donor pancreata. Cell Tissue Bank 8:187–194

Warnock GL, Cattral MS, Rajotte RV (1988) Normoglycemia after implantation of purified islet cells in dogs. Can J Surg 31:421–426

Warnock GL, Meloche RM, Thompson D, Shapiro RJ, Fung M, Ao Z et al (2005) Improved human pancreatic islet isolation for a prospective cohort study of islet transplantation vs best medical therapy in type 1 diabetes mellitus. Arch Surg 140:735–744

Watanabe K (2004) Collagenolytic proteases from bacteria. Appl Microbiol Biotechnol 63:520–526

Westermark P, Wilander E (1978) The influence of amyloid deposits on the islet volume in maturity onset diabetes mellitus. Diabetologia 15:417–421

Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A et al (1996) A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 347:921–925

Witkowski P, Liu Z, Cernea S, Guo Q, Poumian-Ruiz E, Herold K et al (2006) Validation of the scoring system for standardization of the pancreatic donor for islet isolation as used in a new islet isolation center. Transplant Proc 38:3039–3040

Woehrle M, Beyer K, Bretzel RG, Federlin K (1989) Prevention of islet allograft rejection in experimental diabetes of the rat without immunosuppression of the host. Transplant Proc 21:2705–2706

Wolters GH, Vos-Scheperkeuter GH, van Deijnen JH, van Schilfgaarde R (1992) An analysis of the role of collagenase and protease in the enzymatic dissociation of the rat pancreas for islet isolation. Diabetologia 35:735–742

Wolters GH, Vos-Scheperkeuter GH, Lin HC, van Schilfgaarde R (1995) Different roles of class I and class II *Clostridium histolyticum* collagenase in rat pancreatic islet isolation. Diabetes 44:227–233

Yamamoto T, Ricordi C, Messinger S, Sakuma Y, Miki A, Rodriguez R et al (2007) Deterioration and variability of highly purified collagenase blends used in clinical islet isolation. Transplantation 84:997–1002

Yasunami Y, Ryu S, Ueki M, Arima T, Kamei T, Tanaka M et al (1994) Donor-specific unresponsiveness induced by intraportal grafting and FK506 in rat islet allografts: importance of low temperature culture and transplant site on induction and maintenance. Cell Transplant 3:75–82

Yoon KH, Ko SH, Cho JH, Lee JM, Ahn YB, Song KH et al (2003) Selective β-cell loss and α-cell expansion in patients with type 2 diabetes mellitus in Korea. J Clin Endocrinol Metab 88:2300–2308

Yoshihara K, Matsushita O, Minami J, Okabe A (1994) Cloning and nucleotide sequence analysis of the colH gene from *Clostridium histolyticum* encoding a collagenase and a gelatinase. J Bacteriol 176:6489–6496

Zeng Y, Torre MA, Karrison T, Thistlethwaite JR (1994) The correlation between donor characteristics and the success of human islet isolation. Transplantation 57:954–958

Zhang G, Matsumoto S, Hyon SH, Qualley SA, Upshaw L, Strong DM et al (2004) Polyphenol, an extract of green tea, increases culture recovery rates of isolated islets from nonhuman primate pancreata and marginal grade human pancreata. Cell Transplant 13:145–152

Islet Isolation from Pancreatitis Pancreas for Islet Autotransplantation

42

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_48,

Abstract

For patients suffering from intractable chronic pancreatitis, surgical removal of the pancreas may be recommended. While pancreatectomy has the potential to alleviate suffering and prolong life, the induction of iatrogenic diabetes, through the loss of insulin-producing islet cells, becomes an immediate threat to the postoperative patient. Since the procedure was first performed in 1977 at the University of Minnesota, autologous islet transplantation has become the best treatment option to restore a patient's ability to endogenously regulate their blood sugar. Autologous islet isolation starts with a specific procurement and packaging of the pancreas, which is then transported to a specialized clean-room facility. The pancreas is distended with tissue dissociation enzymes that digest the extracellular matrix of the pancreas, freeing the embedded cells, which are combined into a single tube. If necessary, this tissue is purified by density gradient and the islets transferred to a transplant bag for intraportal infusion back into the patient. The most critical factor for a positive metabolic outcome from this procedure is the islet mass transplanted, making total isolation yield of particular concern. While the pancreas contains an abundance of islets, even the best isolation techniques capture only half of these. Furthermore, patient-donor characteristics and tissue conditions, like fibrosis and cell atrophy, can further diminish islet yields. Our goal at the University of Minnesota has been to research the underlying factors that influence islet yield and viability and to propose specific procedures designed to optimize isolation success regardless of tissue condition. The purpose of this review is to describe our basic protocol as well as to highlight our system of flexible techniques that can be adapted based on an ongoing evaluation of each individual pancreas and the procedural progress itself.

Keywords

Chronic pancreatitis • Pancreatectomy • Autograft • Allograft • Human islets • Autologous islet isolation • Transplantation • Insulin independence • Collagenase digestion

Abbreviations	
ATGS	Analytical test gradient system
ATP	Adenosine triphosphate
cGMP	Current good manufacturing practices
CIT	Clinical islet transplantation
CP	Chronic pancreatitis
ECM	Extracellular matrix
FDA	Fluorescein diacetate
HBSS	Hanks balanced salt solution
HSA	Human serum albumin
HTK	Histidine-tryptophan-ketoglutarate
IAT	Islet autotransplantation
IEQ	Islet equivalents
NEM	New enzyme mixture
PI	Propidium iodide
TLM	Two-layer method
TP	Total pancreatectomy
TP/IAT	Total pancreatectomy and islet autotransplantation
UW	University of Wisconsin (solution)

Introduction

Chronic pancreatitis (CP) is a severe, debilitating, and progressive inflammatory disease that results in irreversible destruction of the pancreatic structure (DiMagno and DiMagno 2013; Braganza et al. 2011). CP is characterized by disabling abdominal pain and a gradual loss of both exocrine and endocrine function. Various therapies are available to manage pain or slow disease progression, but when a patient presents with refractory disease or signs of pancreatic cancer, surgery is often deemed necessary (D'Haese et al. 2013; Sah et al. 2013). Drainage/decompression and denervation operations are typically considered first while pancreatic resection, or pancreatectomy, is usually indicated after other procedures fail to alleviate pain (Gachago and Draganov 2008). Pancreas resection may be partial, involving the tail and body of the organ (distal pancreatectomy) or the head as well as the neighboring duodenum and gall bladder (pancreaticoduodenectomy, or Whipple's procedure). The most effective option for CP is a total or near-total pancreatectomy (TP) in which at least 95 % of the pancreas is removed (Blondet et al. 2007).

Total pancreatectomy necessarily results in surgical (iatrogenic) diabetes, due to a loss of insulin-producing pancreatic islet cells, but the risks of this condition can be effectively mitigated through simultaneous islet isolation and autograft transplantation via intraportal infusion (Fig. 1) (Sutherland et al. 2012). Unfortunately, while TP operations are relatively common, conjunctive islet autotransplantation (IAT) is not always available as the cost and difficulty of establishing an experienced and cGMPs (current good manufacturing practices) compliant islet isolation

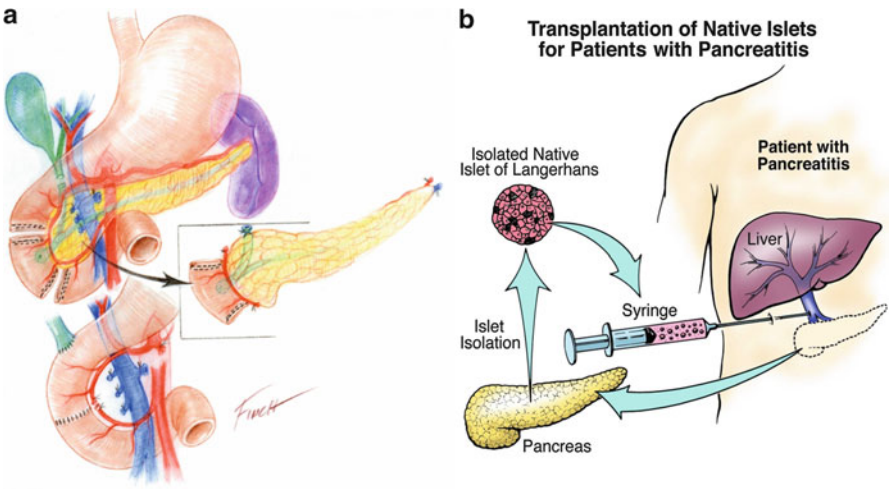

Fig. 1 (**a**) Surgical technique for total pancreatectomy. Total pancreatectomy and pylorus- and distal-sparing duodenectomy with orthotopic reconstruction by means of duodenostomy and choledochoduodenostomy (Adapted from Farney AC, Najarian JS, Nakhleh RE, et al. Autotransplantation of dispersed pancreatic islet tissue combined with total or near-total pancreatectomy for treatment of chronic pancreatitis. Surgery 1991; 110(2):427–37 [discussion: 437–9]; with permission. Copyright © 1991, Elsevier). (**b**) Sequence of events to preserve β cell mass in patients undergoing a total pancreatectomy for benign disease. The resected pancreas is dispersed by collagenase digestion followed by islet isolation. Autologous islets are then embolized to the patient's liver by means of the portal vein (From Blondet et al. 2007)

laboratory have limited the number of facilities capable of performing such a procedure (Rabkin et al. 1999a). For the best results, the resected pancreas should be processed immediately and may require 4–8 h for a trained team to isolate and package an islet preparation for transplant (Gruessner et al. 2004). Nevertheless, the importance of IAT for pancreatectomized patients is realized from the fact that iatrogenic diabetes is more brittle and difficult to manage than other forms of diabetes, with increased insulin sensitivity and more frequent episodes of severe hypoglycemia, which elevates the immediate danger to the postoperative patient (Sutherland et al. 2008; Morrison et al. 2002; Watkins et al. 2003).

The first IAT was performed in 1977 by Dr. Najarian and Dr. Sutherland at the University of Minnesota following a CP total pancreatectomy (Najarian et al. 1979). Apart from CP, however, IAT can be used in other scenarios (Berney et al. 2004; Forster et al. 2004; Alsaif et al. 2006; Garraway et al. 2009) where a pancreatic resection is performed, so long as the precipitating condition does not damage the islets themselves. Another indication for IAT is pancreatic trauma, following blunt or penetrating injury that requires a large (80 % or 90 %) pancreatic resection (Garraway et al. 2009). The basic principles of IAT can also be used to isolate islets from brain-dead or cadaver donors to transplant into patients with type I diabetes, whose islets have been lost. These allograft transplants come with their

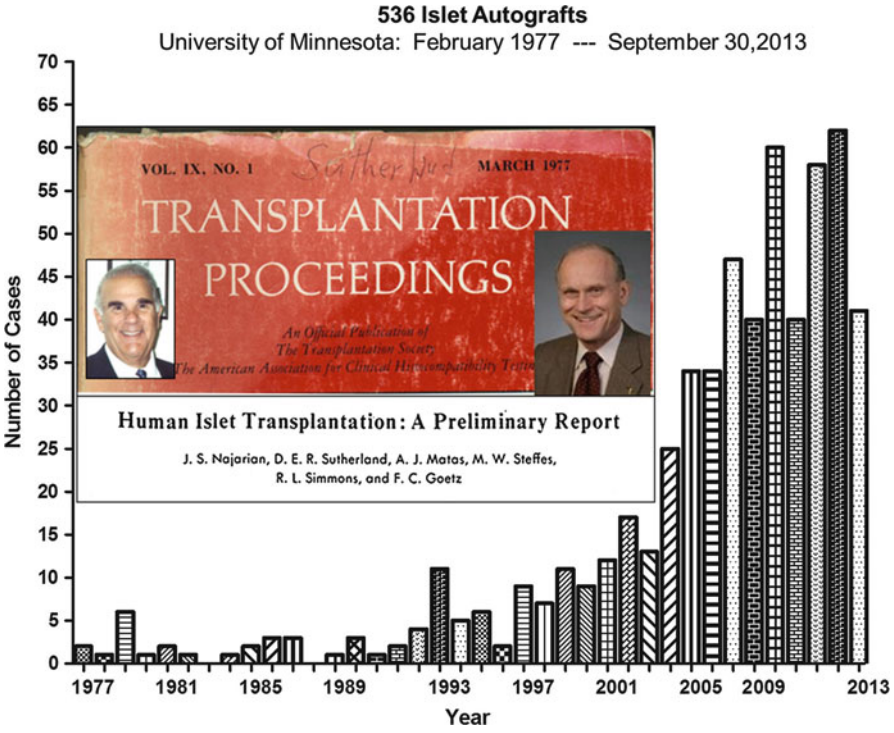

Fig. 2 Islet autograft experience at the University of Minnesota by year, in 536 patients (adults and children) from February 1977 to September 30, 2013. Dr. Najarian and Dr. Sutherland performed the world's first islet autotransplantation at the University of Minnesota, in 1977

own set of problems, however, namely, the need for prophylactic immunosuppression (Hering et al. 2005). In our institute, we have successfully completed more than 536 islet autotransplantations (Fig. 2).

The single most critical factor for optimal survival and function of transplanted islets is the size of the islet mass acquired from the isolation procedure. Sutherland et al. have reported up to 2 years of insulin independence in 71 % of patients who received an IAT of >300,000 islet equivalents (IEQ) (Sutherland et al. 2008, 2012; Bellin et al. 2008, 2011). A healthy human pancreas contains more than sufficient one to two million islets but a typical isolation recovers only 30–50 % of these (Lakey et al. 2003; Balamurugan et al. 2012). The efficacy of enzyme digestion is limited by the heterogeneous composition of the extracellular matrix (ECM), which is composed of a honeycomb of reticular fibers, adhesive proteins, large molecules, and multiple isoforms of collagen. A successful islet isolation protocol must break down the ECM to release the cells and separate endocrine from exocrine tissue without degrading the bonds that hold the individual islet cells together. Furthermore, disease pathology, acute trauma, previous surgical intervention, and each donor's unique biological characteristics result in variable tissue conditions that can

dramatically impact islet yield and viability (Bellin et al. 2012a; Kobayashi et al. 2011).

To achieve consistent success in clinical outcome, an experienced isolation team must learn to recognize key variables, such as fibrosis and donor age, and to understand the principles of the isolation procedure sufficiently to adapt it for each individual organ. Thus, while a seemingly simple procedure, IAT requires highly specialized and flexible techniques in order to obtain an adequate number of healthy islets from a variety of donor types (Morrison et al. 2002). This report aims to describe the technical aspects of pancreas processing and islet isolation that are critical for achieving successful islet autotransplantations.

Islet Cell Isolation

Human islet isolation is a time-sensitive procedure that requires the trained attention and cooperation of, minimally, a three- to four-person team led by an experienced supervisor. The role of each individual must be carefully considered to avoid errors, identify and correct problems, and ensure the efficiency of the procedure to achieve the best possible yield of healthy islets for transplant (Lakey et al. 2003).

Adequate laboratory preparation prior to the isolation procedure is important so that the team is ready to begin as soon as the pancreas arrives to the clean-room facility. Using sterile technique, two people should set up the biological safety cabinets (or laminar flow hood) with materials needed for pancreas trimming, cannulation, distension, digestion, recombination, purification, and transplant bag preparation. Simultaneously, the remaining individuals prepare media and other in-use solutions that will be needed during isolation. Team members should also ensure that all necessary instrumentation, such as centrifuges and thermal probes, are turned on and functioning within normal parameters.

Pancreatectomy and Pancreas Transport

For autologous isolations, the pancreas is dissected and immersed immediately in a 1 L Nalgene container filled with cold preservation solution. Excess fat, connective tissue and duodenal tissue are removed before packing on ice, and pancreas is delivered to the islet isolation lab. During pancreatectomy, some transplant centers recommend flushing the ductal system with chilled preservation solution, containing a trypsin inhibitor, to minimize warm ischemia time and to maintain optimal ductal integrity for enzyme delivery and distension (Naziruddin et al. 2012; Shimoda et al. 2012a). A recent study, however, found that the more common vascular flush technique was just as effective as a ductal injection (Nakanishi et al. 2012). We have found these methods unnecessary for short transport of the pancreas, but for allograft isolations, where ischemia time is longer and donor conditions less ideal, we require en bloc pancreatic harvest with arterial flush (Kin 2010; Kin and Shapiro 2010).

Preservation Solution

Cold storage preservation relies on hypothermia and carefully tailored solutions to slow metabolism inhibit endogenous enzyme activity and support critical cellular processes despite the loss of an oxygenated blood supply. Organ packaging methods and solution ingredients have been designed to address several key problems associated with hypothermic ischemia followed by reperfusion including cellular swelling, ionic imbalance, acidosis, calcium accumulation, and the production of reactive oxygen species.

University of Wisconsin (UW) solution was developed in 1986, specifically for pancreas cold storage preservation (Wahlberg et al. 1986). UW contains phosphate as a pH buffer against anaerobic lactic acidosis, the large molecule saccharide raffinose and the anionic lactobionate as membrane-impermeant osmotic balancers, allopurinol and glutathione to scavenge free radicals, adenosine as an ATP substrate, and a high, intracellular-mimicking K^+/Na^+ ratio that was originally thought to retard cell-damaging Na^+ absorption and K^+ efflux ('tHart et al. 2002). Lactobionic acid is also a calcium chelator that prevents this cation from activating degenerative phopholipases during reperfusion ('tHart et al. 2002). While UW has become the standard organ transport solution, it is also costly with a short shelf life and many of the ingredients, designed to inhibit tissue degradation, interfere with the intentionally catabolic activity of collagenase and neutral protease (Contractor et al. 1995). Other cold storage solutions have been proposed including histidine-tryptophan-ketoglutarate (HTK), Celsior, and the Kyoto solutions, but UW remains the most common for pancreas hypothermic preservation. In trimming solution, a modified UW reverses the Na^+/K^+ ratio to mimic the natural extracellular environment and exchanges lactobionate for the less expensive but equally effective gluconate. Since we accept cadaveric donors from a variety of institutions, we recommend allograft pancreata be stored in either UW or HTK solution for transport to our lab (Barlow et al. 2013).

Packaging

For transporting a pancreas, we use simple cold storage in trimming solution – the organ placed in a 1 L Nalgene, filled to the top with solution, and packed on ice inside a cooler. For allograft isolations, we request organs to be packaged using the two-layer method (TLM) of oxygenated perfluorocarbon underneath a layer of either UW or HTK solution (Hering et al. 2002). Although the efficacy of TLM, hypothesized to promote aerobic respiration and ATP production, has been vigorously debated, a large-scale meta-study recently concluded that TLM did increase pancreatic islet yield and viability for long cold ischemia times, which are common for allogeneic isolations (Qin et al. 2011).

The Baylor group has published a series of studies demonstrating improvements in isolation yield using a combination of cold ductal flush during procurement and TLM packaging, even for short distances (Naziruddin et al. 2012). However, we

prefer to minimize manipulation of the pancreas prior to the start of isolation. In our autotransplantation series, a small number of autologous isolation cases have also necessitated long transports due to the patient's inability to travel, in which the resected pancreas was flown by plane to our isolation center in Minnesota (Rabkin et al. 1997, 1999b) or in a similar case to another center (Jindal et al. 2010; Khan et al. 2012). The islet mass was then flown back to the patient and transplanted at the original site, in several instances resulting in a complete return to normoglycemia (Rabkin et al. 1999b; Jindal et al. 2010). Despite the long travel time, the pancreatic tissue did not appear to suffer any ill effects from the use of simple storage in cold UW (Rabkin et al. 1997; Khan et al. 2012), offering hope that this kind of procedure could make IAT available at hospitals and surgical sites where a full isolation facility is not feasible.

Pancreas Trimming and Cannulation

After receiving a pancreas at our isolation facility, the temperature of the transport preservation solution is recorded and a 3 mL sample volume taken to assay for microbial contamination (aerobic/fungal, anaerobic, and gram stain). It is critical that the pancreas be kept cold from the time of organ removal to the initiation of enzymatic digestion (Lakey et al. 2002). In conjunction with the preservation solution, a low-temperature environment slows cellular respiration and tissue degradation ('tHart et al. 2002). Initial procedures should be performed in a cold room with prechilled solutions, but if this is not available, an ice bath can be prepared for working with the pancreas. At least 1 L of sterile saline should be frozen solid ahead of time. During setup, wrap the saline bag in a towel or cover to protect the integrity of the outer plastic while breaking up the inner ice block with a mallet or hammer. The bag should be opened using sterile technique and the saline ice fragments poured into a large tray, which is then covered with a sterile drape. A smaller dissection tray is placed inside, resting on top of the drape and underlying slush.

Trimming

The first isolation step inside the biosafety hood is to immerse the pancreas in ~500 mL of trimming solution inside a cold trimming pan. After a visual inspection, the organ is photographed for formal documentation. The gross morphology of every pancreas is different and we assess each as mild, moderate, or severe to determine enzyme dose and digestion conditions (Fig. 3). A trained team member then performs an initial brief trim, removing excess fat and connective tissue from around the pancreas but keeping the pancreatic capsule intact to reduce the likelihood of enzyme leakage during ductal perfusion. After trimming, the pancreas is submerged for 5 min in Antibiotic solution (Fungizone + Gentamicin + Cefazolin) that has been checked against potential patient allergies before treating the pancreas. If the solution

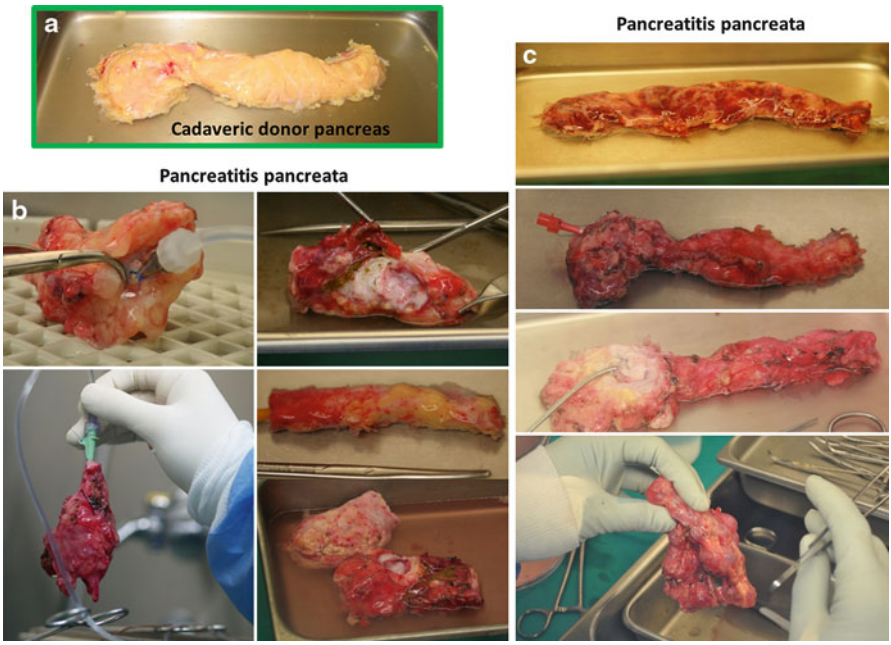

Fig. 3 Pancreas gross morphological differences between (**a**) normal cadaveric donor and (**b**), (**c**), chronic pancreatitis pancreata. For enzyme dosing, our lab categorizes the pancreas into mild, moderate, and severe categories

beaker is resting on a pre-tared scale, the organ can also be weighed during this time in order to calculate the per gram enzyme concentration that will be needed for perfusion/distension. The pancreas is then rinsed in two consecutive beakers, each containing 500 mL of sterile Hanks balanced salt solution (HBSS), and placed in a pan with trimming solution in preparation for cannulation.

Cannulation

Up to this point, we have referred to the pancreas as a single intact organ. However, depending on the severity of a patient's pancreatitis and the degree of near or total pancreatectomy, the organ may arrive whole, partial, or in multiple pieces. In the case of a whole pancreas, we have achieved better enzyme distention by dividing the pancreatic lobes and cannulating the head and the body-tail portions individually. The selection of a catheter should be based on the size of the pancreatic duct, which may vary between lobes. Typically, sizes range from 14 to 24 G but severe fibrosis or a dilated duct may require either a Christmas or metal catheter. The selected catheter should be sutured firmly in place to avoid enzyme backflow during perfusion. With the catheters secured, the pancreatic lobes are moved to the perfusion tray and the tray's basin filled with the prepared enzyme solution.

Enzyme Selection

Enzymatic tissue dissociation has been used to separate the exocrine and endocrine components of the pancreas since 1967, when crude collagenase enzyme, derived from the bacteria *Clostridium histolyticum*, was first used to isolate guinea pig and rat islets (Moskalewski 1965; Lacy and Kostianovsky 1967). Since then, researchers have observed the best tissue dissociation resulting after the ductal perfusion of a blend of collagenase and protease enzymes into the main pancreatic duct (Kin 2010; Caballero-Corbalan et al. 2009, 2010). We tailor our enzyme mixture according to pancreas weight, with the final enzyme solution prepared by diluting the reconstituted enzymes into approximately 350 mL HBSS + 10U/mL heparin solution (or up to 450 mL for larger pancreases) (Balamurugan et al. 2012).

Enzyme Combination

In the past, the universal adoption of Liberase HI for enzyme digestion provided a convenient formula in which one complete vial could be dissolved into the final desired solution volume (Hering et al. 2005). When Liberase was made clinically unavailable, we switched to SERVA collagenase and neutral protease (Anazawa et al. 2009). However, we have recently identified a new enzyme mixture (NEM) that has improved both the yield and viability of our islet products. We currently use VitaCyte collagenase HA, which contains a high proportion of intact C1 collagenase, to obtain greater islet numbers compared to SERVA's analogous product (Balamurugan et al. 2010). On the other hand, we observed SERVA neutral protease NB (GMP or premium grade) to produce better-quality islets with a more solid and intact structural morphology compared to VitaCyte's equivalent, thermolysin (Balamurugan et al. 2012). This novel combination (VitaCyte Collagenase + SERVA neutral protease) resulted in total islet yields of >200,000 IEQ in 90 % of our attempted autologous isolations and doubled the number of allograft isolations that reached transplantation threshold in our recently published study (Balamurugan et al. 2012).

Enzyme Dose

Initially, our laboratory used only a 1:1 ratio of whole enzyme vials, even with the NEM. We have since observed that customizing the enzyme dose based on pancreas weight and other donor/organ characteristics results in more consistent islet release. Pancreas weight is estimated based on the actual pre-distension weight minus the anticipated weight of non-pancreatic trimmed tissue, evaluated by an experienced team member based on visual and tactile observation. Following distension and final trimming, the actual pancreas weight is recorded and the real enzyme dose can be calculated. Improper enzyme concentration can then be compensated for by adjusting the temperature and timing of the digestion phase or by adding additional enzyme directly into the digest circuit. To produce our

NEM results, we used a dose formula of 22 W unit/g pancreas of collagenase and 1.5 DMC unit/g pancreas of neutral protease (Balamurugan et al. 2012). In practice, we vary the collagenase dose from 22 to 30 W unit/g and the neutral protease dose from 1.5 to 3.0 DMC unit/g pancreas depending on pancreatic characteristics.

Enzymatic Perfusion of Pancreas

During perfusion, enzyme solution is administered to the pancreatic tissue through a pressurized injection, manual or automated, into the cannulated pancreatic duct of either the whole or segmented pancreas (Lakey et al. 1999, 2003). Enzyme distension is the critical step in the islet isolation process (Fig. 4). Complete delivery of enzyme to the entire pancreatic parenchyma will reduce the remaining undigested tissue in the digestion chamber and maximize the final islet yield recovered and ultimately delivered to the patient.

Perfusion Method

Historically, enzyme solution was loaded directly into the ductal cannula with a handheld syringe, relying on retrograde perfusion to distend the pancreas. This approach diminishes the ability to address leaks as they occur and does not offer quantified values about pressure and flow rate. However, the manual method is still in common practice as either a primary or alternative perfusion technique at many clinical islet transplant (CIT) centers. The first use of a fully automated recirculating pump to perfuse a human pancreas through the pancreatic duct was published in 1999 and shown to improve islet yields over traditional syringe loading (Lakey et al. 1999). The superior yield produced by this method was confirmed using refined rather than crude collagenase (Linetsky et al. 1997a, b). In addition to improved distension and yield, automated pump perfusion provides precise control over injection pressure and enzyme solution temperature.

The modern automated perfusion system is equipped with peristaltic pumps, two pressure sensors, a heater, a touch screen, and data acquisition software (Bio-Rep) that combines the convenience of hands-free automation with the flexibility to make manual adjustments to a variety of programmable parameters. This is especially important to achieve even enzymatic distribution of cadaveric donor organs (Lakey et al. 1999) or to control pressure in a diseased pancreas with severe fibrosis or ductal alterations. This semiautomated system is used by several prominent CIT centers and is the primary perfusion method used in our laboratory.

Temperature, Pressure, and Flow Rate

Distension pressure, pump speed, flow rate, and temperature can all be monitored and controlled using a semiautomated perfusion system. Throughout the enzyme

perfusion process, the temperature is kept between 6 °C and 16 °C while the desired injection pressure is maintained between 60 and 80 mmHg for the first 4 min and gradually increased to a perfusion pressure of 160–180 mmHg until completion (approximately 10–12 min total distention time). However, perfusion pressure can vary significantly depending on the condition of the organ. Distention pressures could be low for a severely damaged, leaking, pancreas or high for an organ with abnormal ductal anatomy (stricture or blockage) or severe fibrosis.

While it is important to maintain adequate perfusion pressure, vigilance is also required to maintain the pump speed and flow rate above 30 mL/min. A ductal occlusion or misplaced cannula can obstruct the flow of liquid, increasing the pressure and slowing the automated pump. However, enzyme injected at a low flow rate, even with a normal pressure reading, would result in an ineffective distension. In this case, the cannula may need to be repositioned or the team may decide that higher pressure or an interstitial perfusion method is required. Similarly an enzyme leak may cause the pump speed and flow rate to suddenly increase as the perfusion pressure drops and a stream of solution may be visible ejecting from the pancreas. Since leaks can significantly detriment the quality of the perfusion (Goto et al. 2004; Johnson 2010), they should be clamped or sutured immediately to ensure a complete and efficient distension.

Interstitial Perfusion

In some cases the extent of parenchymal fibrosis is so high that ductal enzyme perfusion is ineffective at delivering enzyme to the entire body of the pancreas (Fig. 4). In these cases, interstitial perfusion can be performed by repeated manual injections of cold enzyme solution into the tissue with a needle and syringe (Al-Abdullah et al. 1994) (Fig. 4d). On the other hand, the distal half of a pancreas, particularly if perfused intact, may fail to effectively distend with solution. This may be due to duct alterations caused by inflammation and fibrosis or intraductal calcification deposits, which have obstructed the flow of fluid through the duct. In these cases, it is possible to make a complete transverse cut before the distal section after the proximal end has finished distending and re-cannulate the distal end to attempt further distention in this area.

Post-distension Trimming

After adequate distension has been achieved, the pancreas is transferred to a trimming pan with fresh heparinized phase 1 solution. Final trimming of the pancreas should be performed as quickly as possible to reduce overall cold ischemia time. This includes removal of the pancreatic capsule, surface fat, vasculature tissue, and any sutures of staples used during the surgical procedure. Surface fat is a particular concern as it can clog the Ricordi chamber screen during digestion and obstruct the free flow of islets and solution. For normal pancreata, the trimmed

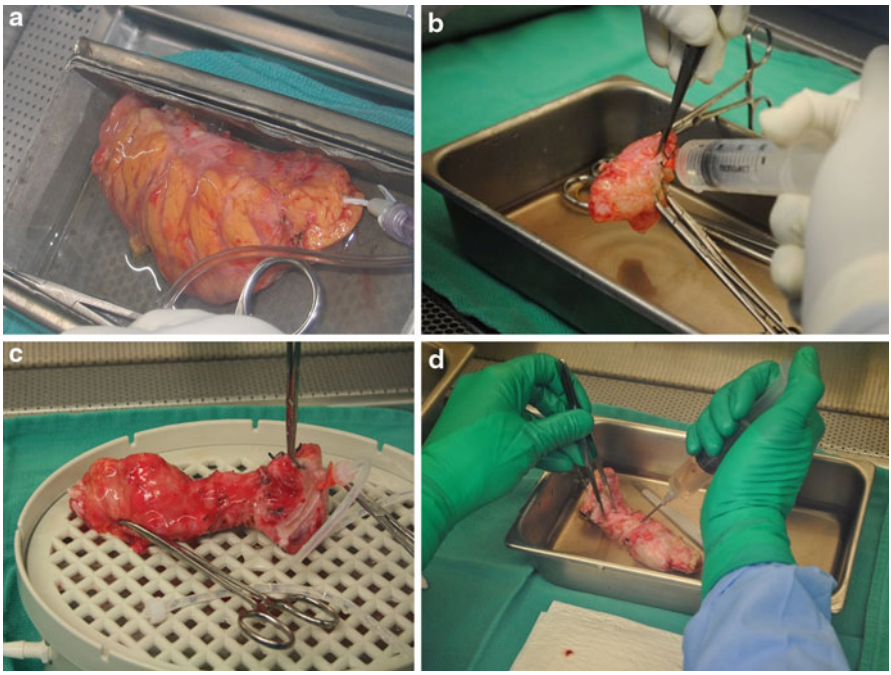

Fig. 4 Based on the nature of pancreas, the enzymatic distention will be performed using (**a**) a simple perfusion machine or (**b**) a hand syringe injection through the pancreatic duct or (**c**) an automated perfusion apparatus injection through the pancreatic duct or (**d**) in case of severely fibrosis or ductal collapsed pancreas, interstitial enzymatic distention (by using needle and syringe) should be performed

organ should be cut into large (3–5 cm diameter) pieces prior to digestion. For an inadequately distended or severely fibrotic pancreas, the tissue can be cut into many small pieces (up to 30 pieces, 2 cm in diameter) to enhance the exposed surface area available for mechanical and enzymatic digestion.

Tissue Digestion

The perfused enzyme begins to bind to the matrix tissue of the pancreas as soon as it is injected so we should not delay between the end of distension and the beginning of digestion. Currently, the semiautomated method for tissue digestion is employed by all centers isolating human islet preparations intended for clinical transplant programs (Ricordi 2003; Ricordi et al. 1988). Developed in 1988 by Dr. Camillo Ricordi, the digestion method utilizes a specialized "Ricordi" chamber to contain and collect the pancreatic digest as islets are released (Ricordi et al. 1988). Along with the pieces of pancreatic tissue, multiple stainless steel marbles are sealed inside the chamber to mechanically assist enzymatic dissociation as the chamber is

gently shaken. This method has proven superior to manual methods in isolating high quantities of viable human islets for successful transplantation (Ricordi 2003; Ricordi et al. 1988; Paget et al. 2007).

Digest Sampling

To monitor the progress of tissue dissociation, it is critical to collect samples of the digest tissue and solution throughout the digestion process. We withdraw a 2 mL volume from a sterile syringe connected to an outlet port in the tubing circuit, which circulates solution through the Ricordi chamber lid. The sample is stained with dithizone inside a small petri dish and evaluated under a light microscope for quantity of acinar tissue, acinar diameter, number of islets, percent free islets, percent fragmented (over-digested) islets, and average islet score. These are recorded, along with the circuit temperature, at regular intervals. During phase I, sampling begins after 8 min of recirculation digestion and occurs every 90–120 s. After the switch to phase II collection, samples can be taken every 5–10 min.

Digestion: Phase 1, Recirculation

The distended and trimmed pancreas is aseptically transferred to a Ricordi chamber (usually 600 mL) connected to a digestion apparatus, along with all enzyme solution left in the perfusion tray or trimming pan. The digestion apparatus consists of a peristaltic pump that moves fluid through a closed circuit of tubing with a reservoir, a heat transfer coil, an inlet for diluent, and outlets for tissue collection or sampling. Once the tissue and bathing solution is inside the chamber, a 400–600 μm mesh screen (typically ~500 um) is placed across the opening before sealing the lid with a rubber gasket and screws or a large clamp. H phase 1 solution (+10 U/mL heparin) is added to fill the digestion circuit and purge air from the system. The start time of digestion is recorded when sufficient liquid has filled the reservoir to allow for sampling.

At the start of digestion, the pump speed is set to deliver a flow rate of 200 mL/min and the tubing clamps are adjusted to recirculate solution through the system. Over the first 5 min, the temperature is gradually increased to 34–37 °C while the chamber is gently rocked. When the target temperature has been reached (34–35 °C standard), the heating coil is removed from the heated water bath, the pump slowed to 100 mL/min, and more forceful shaking of the chamber begins. After 8 min (or when liberated tissue appears in the tubing), 2 mL samples of digest tissue are taken by syringe from the sampling port and viewed under a microscope after staining with dithizone. Successive samples with large quantities of free islets indicate the completion of phase 1 digestion and suggest a transition to phase 2 (switch time).

As mentioned above (see section "Enzyme Dose"), the actual enzyme dose, calculated by subtracting the real trimmed tissue weight from the original pancreas

weight, can be a useful indicator to adjust the digestion settings. The target temperature set point can be raised or lowered to compensate for off-target enzyme concentrations, resulting from inaccurate estimation of the final trim or for a sluggish phase 1 progression. Additionally, in cases where phase 1 is proceeding exceedingly slow, extra collagenase or neutral protease can be added to the digest circuit directly to rapidly increase digestion rates. It is important to keep phase 1 as short as possible because prolonged exposure to active enzyme, and an increasingly basic chamber environment, can be harmful to islet yield and integrity (Gray and Morris 1987; Balamurugan et al. 2003; Tsukada et al. 2012). Temperature rate and set point, enzyme dose, and switch time should be determined by qualified and experienced personnel based on multiple factors such as the % dissociation of tissue in the Ricordi chamber and the progressive condition of the samples including amount of tissue, % of free islets, size/condition of acinar tissue, and morphology of the islets.

Digestion: Phase 2, Collection

Phase 2 of digestion starts by increasing the pump speed to a flow rate of 200 mL/min and switching the tubing clamps from recirculation of the digest material to collection. Cold phase 2 solution (RPMI 1640) is added to progressively weaken the enzyme concentration inside the digest circuit. The first 2 L of digest tissue is collected into four 1 L flasks in a series of increasing dilutions: first 25 % (250 mL of digest + 750 mL of cold RPMI 1640/2.5 % human serum albumin (HSA)) then 50 %, 50 %, and 75 % v/v. The prechilled collection media and the HSA both work to inhibit collagenase and neutral protease activity, which would otherwise continue to break down islet integrity during recombination. The decreasing dilution factors provide a greater inhibitory buffer during the initial collection fractions with the highest concentration of enzyme. Following the fourth flask, digest fractions are collected into 250 mL conical tubes, prefilled with 6.25 mL of chilled 25 % HSA. The stop point for phase II tissue collection is determined by the minimal presence or complete absence of islets and/or tissue in the dithizone-stained samples that have been under regular evaluation throughout digestion. As digestion comes to an end, the remaining RPMI is collected from the chamber and air introduced into the tubing to flush out the final solution. During this last wash, the chamber should be gently rotated to ensure that all fine tissue has been moved through the circuit. The weight of undigested tissue in the chamber can be used to ascertain the efficacy of the enzyme dose and the efficiency of the tissue dissociation.

Digestion of the Severely Fibrotic Pancreas

A common side effect of CP inflammation is the progressive development of fibrosis as a result of increased deposition and reduced degradation of ECM

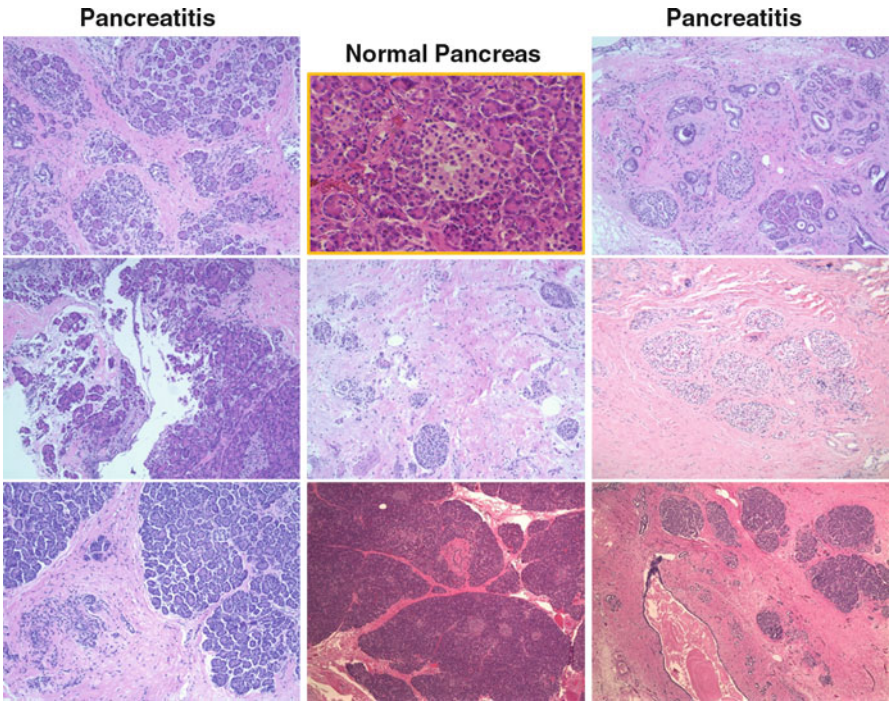

Fig. 5 Hematoxylin and eosin-stained histological images of normal (*middle*) and chronic pancreatitis pancreata. Cellular architecture differs in every case with the amount of fibrous tissue

materials (Fig. 5). This excess and hardened tissue is more resistant to enzyme digestion and can greatly lower islet yield if appropriate adjustments are not made to the procedure. Maintaining the solution temperature at the high end of the acceptable range (36.5–37) can intensify enzyme activity. If unusually little digestion is observed after 25 min, an enzyme recirculation protocol may be helpful (Balamurugan et al. 2003). This procedural variation involves the collection of free islets from the recirculating system early after release. The free islets are pelleted by a quick centrifugation step and transferred into fresh, cold media in the recombination container. The supernatant, containing active enzyme, is recycled back into the digestion system, which increases the effective enzyme dose for the undigested tissue remaining in the Ricordi chamber (Balamurugan et al. 2003). This recirculation can be repeated until nearly all tissue mass has dispersed or healthy islets are no longer apparent in the digestion samples.

Other Common Complications

In addition to the extent of fibrosis, other donor characteristics – especially age, pancreas weight, and fat infiltration – can lead to extreme discrepancies in islet

release as the digestion phase progresses (Miki et al. 2009; Eckhard et al. 2004; Loganathan et al. 2013; Lake et al. 1989). A common observation in pancreas digestion from young donors is the release of mantle islets, which are embedded in a corona of acinar cells (Ricordi 2003; Balamurugan et al. 2006). These islets are difficult to recover after purification as the extra cells increase the islet density, settling them into a heavier and less differentiable density layer during COBE processing (Miki et al. 2009). Prolonged enzyme exposure or an extra chamber screen, to restrict the passage of larger particles, may be effective in reducing mantle islets collected during isolation of a young pancreas (Balamurugan et al. 2006). With very small pancreases, common with pediatric donors or near-total pancreatectomy, a smaller digest chamber (250 mL) is typically more effective by concentrating the enzyme activity and shortening the duration of the digestion phase. Excess liquid volume inside the chamber should be avoided since it can act as a cushion, making it difficult to mechanically agitate the tissue. As with the distention phase, experienced islet researchers and lab technologists can greatly enhance the success of the isolation by making slight modifications to the standard procedure.

Temperature, circulation speed, enzyme dose, apparatus setup, and/or the level of mechanical shaking can all be used to accommodate the inherent variation in pancreatic tissue condition caused by different disease pathologies. An understanding of how these digestion parameters affect the rate and quality of tissue dissociation is essential to minimize the amount of undigested tissue left in the Ricordi chamber and to maximize the number and quality of liberated islets obtained. This is particularly critical for autograft patients, who have invested their entire hope for insulin independence on the success of a single isolation, as opposed to allograft recipients, who may have the opportunity to receive islets from multiple donors and transplantations. Since the first autologous islet transplantation was performed at the University of Minnesota in 1977, international islet transplant registry data indicate that transplant recipients are achieving longer durations of insulin independence. As pancreas procurement, preservation, and islet isolation techniques have advanced, average islet yields and positive patient outcomes have increased, spurring support for the further expansion of islet autotransplantation.

Tissue Recombination

Fraction Collection

The recombination phase is closely associated with the digestion phase, beginning immediately after the digestion switch point. All digest solution collected in phase 2 contains a suspension of free islets that must be preserved from further enzyme degradation. The first four digest fractions, collected successively into four 1 L flasks of RPMI/HSA (see section "Digestion: Phase 2, Collection"), are each transferred to a separate biological safety cabinet hood and divided into 250 mL conicals then centrifuged at 140–170 × g, 2–10 °C, for 3 min. The resulting cell

pellets are separated by decanting, discarding the supernatant, and aspirating the remaining pellet into a 1 L flask prefilled with 750 mL of cold recombination solution (wash media). This cold storage/purification stock solution (Mediatech, Inc., Manassas, VA, USA) is supplemented with 10 U/mL of heparin and 2 % v/v penta starch. This latter ingredient prevents exocrine cells from swelling and becoming less dense, which would reduce the efficacy of the density gradient-dependent purification process later in the isolation (Eckhard et al. 2004). Subsequent digest fractions are drawn off directly into 250 mL conicals and can be centrifuged immediately after collection.

In some instances of CP, calcification deposits, up to 3 mm in diameter, may be observed in the collection conicals and should be removed before centrifugation. These deposits are much denser and larger than the observable digest tissue and can be mechanically separated by pipet aspiration. The calcifications should be gathered into a separate 250 mL conical where they can be rinsed with recombination solution to rescue any islets aspirated with the deposits.

Recombination

When phase 2 digestion has been completed and all tissue mass spun out and collected into the final recombination flask, 5 mL of tissue suspension is removed for density determination (see section "Analytical Test Gradient System"). The recombination flask is divided evenly into 4–5 conicals, rinsed thoroughly with wash media to scavenge all islets, and spun again. After decanting off the supernatant, these pellets are combined into a single conical and resuspended in fresh media up to 200 mL total volume. At this point, two samples of well-dispersed tissue suspension are taken to provide a "post-digest" islet count. This is done by gently rocking the capped conical, to evenly disrupt and distribute tissue aggregates, and then using a pipet to aspirate 100 μL of tissue suspension into 35 mm petri dishes containing 1 mL of wash media each. Packed pellet volume is estimated by bringing the recombination conical solution volume up to 250 mL before centrifuging at a higher speed, 220 × g, and reading the conical gradations. The count samples and total packed tissue volume are critical determinants for deciding whether a purification step is necessary. In all allograft cases and for autograft tissue volumes >20 mL, isopycnic gradient purification is advisable. Autograft pellet sizes smaller than 15 mL are usually not purified, to maximize the recovered islet mass, but tissue volume can be diminished by repeated washing: resuspending the pellet in fresh media, spinning at 140 × g, and decanting off the supernatant.

If islets are not purified, the final combined pellet is washed repeatedly with room-temperature transplant media (unsupplemented CMRL with 2.5 % HSA and 25 mM HEPES) until the supernatant is translucent and free of cell debris. The tissue pellet is then quantitatively transferred from the conical into a T-75 flask and brought up to a 100 mL volume with fresh transplant media. The islets are allowed to settle for 5 min so that samples of the supernatant may be aspirated for

sterility testing and archival storage. The 100 μL sample counts from earlier are used to calculate the volume of islet-suspended solution that should be collected for islet viability/potency assays, minimally fluorescein diacetate (FDA)/propidium iodide (PI) (Loganathan et al. 2013), and others depending on total yield and patient consent. Density gradient purification necessitates additional recombination and sampling steps, which are described in the next section.

Purification Process

The goal of purification is to reduce tissue volume, particularly exocrine cell contamination, while minimizing islet loss. Several purification techniques have been reported but isopycnic density gradient centrifugation on the COBE 2991 cell processor is the only method that has been consistently successful and used clinically for large-scale human islet purification (Lake et al. 1989; Anazawa et al. 2011). This purification technique is accomplished by employing centrifugation through a density gradient to separate the less dense islets from the more dense exocrine tissue (Fig. 6).

The expediency of islet purification is debatable (Gores and Sutherland 1993) and should take into consideration the particular patient and potential for transplant complications. The decision to purify or not should be made by qualified and experienced personnel, often in consultation with the attending physician/surgeon. Avoiding density gradient purification is generally beneficial for islet viability as it avoids exposing the islets to harsh gradient solutions and additional mechanical stress (Mita et al. 2009). Some researchers also argue that exocrine-islet signaling plays an important role in islet survival and posttransplant function and that removing these communication pathways is detrimental to overall graft survival (Webb et al. 2012). On the other hand, significant evidence from transplant case reports describes the risks associated with transplanting large tissue volumes into the portal venous network, including portal vein thrombosis and portal hypertension (Bucher et al. 2004). Liver embolism, thrombosis, damage, and even death have been documented in clinical settings immediately after intraportal infusion of large amounts of tissue (Walsh et al. 1982; Kawahara et al. 2012). In addition, recent evidence from our laboratory indicates that activated proteolytic enzymes released by dying acinar cells and co-transplanted with islets may reduce in vivo islet function by degrading insulin (Loganathan et al. 2011).

Purification is also a risk when pellet size as small as 10–40 % of the total IEQ recovered from digest may be lost (Kin 2010) and it is well known that the total IEQ/kg of patient body weight is the current best predictor of a successful clinical outcome (Bellin et al. 2012b). As a general rule, we allow up to 0.25 cm^3 of tissue volume per kg of patient weight (in press, American Journal of Transplantation) for safe intraportal infusion, although we have safely infused a higher tissue content on several occasions when purification was anticipated to result in a particularly low yield (unpublished data).

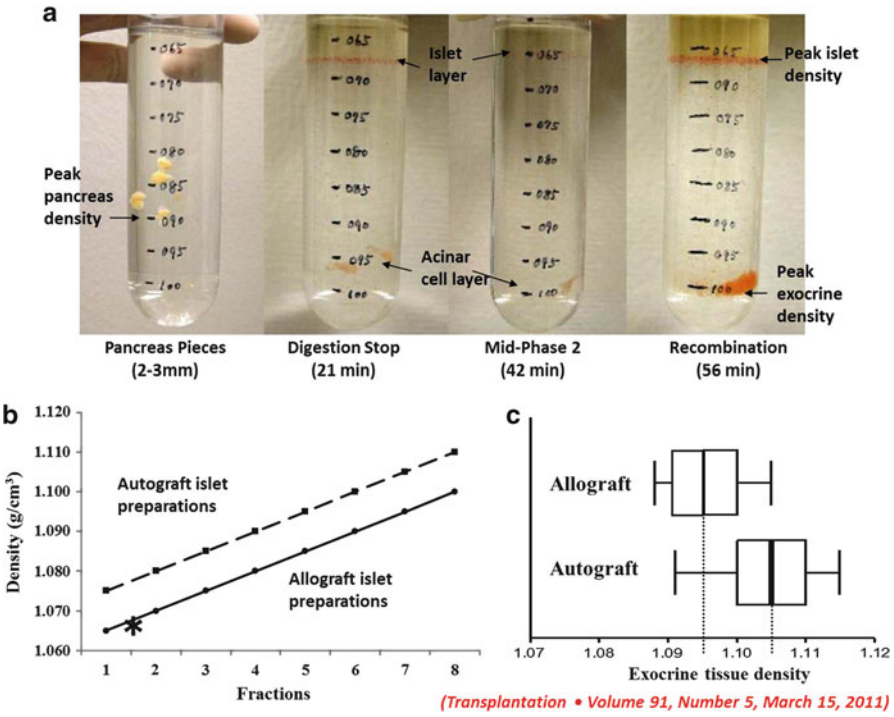

Fig. 6 (**a**) Examination of the density profile by prelabeled ATGS. The density at which most of the tissue floated is defined as the tissue density. Sequential photographs of digest tissue display a change in tissue density over time. Peak islet density is 1.068 g/cm^3, and exocrine tissue density is 1.101 g/cm^3. (**b**) The linearity of the analytical test gradient system (*ATGS*). For allograft purification (*solid line*), the ATGS is constructed of eight iodixanol-based density gradient levels, each increasing by 0.005 g/cm^3 at 5 mL intervals, running from heavy (1.105 g/cm^3) to light (1.065 g/cm^3) density. For autograft preparations (*dashed line*), the ATGS is constructed with a density gradient between 1.115 g/cm^3 (heavy) and 1.075 g/cm^3 (light). (**c**) Actual exocrine tissue density from all isolations indicated that autograft preparations were heavier than allograft preparations. The box-and-whiskers plot presents the median (*dark line*), the interquartile range (*box*), and the range between 10th percentile and 90th percentile (whiskers), $*P < 0.001$

Standard Density Gradients

When islets are purified with a COBE 2991 cell processor, the purification gradient used are often fixed between 1.060 and 1.100 g/cm^3, values currently utilized by the Clinical Islet Transplantation Consortium (Anazawa et al. 2011). A predefined, standard density gradient is less successful when exocrine density is unusually light (<1.100 g/cm^3), lowering the post-purification purity, or when islet density is unusually heavy (>1.100 g/cm^3, common for mantle islets), causing the islets to sediment into the COBE bag and reducing total recovery (Anazawa et al. 2011).

Traditionally, however, the exact density of pancreatic tissue components is unknown prior to purification, which enhances the difficulty of predicting or controlling the outcome of purification.

Analytical Test Gradient System

Predicting exocrine tissue and islet density is important for the selection of an optimal density gradient range for the COBE process, to maximize islet yield and purity (Fig. 6). Consequently, our center has developed an analytical test gradient system (ATGS) to determine the true density distribution of human pancreatic tissue components before purification (Anazawa et al. 2011). This method mimics the actual purification process but uses only a minute fraction of recombination tissue in a single tube. ATGS results can be quickly interpreted in order to customize the gradient for a full COBE purification.

The ATGS is prepared by using a small gradient maker (Hoefer SG30, GE Healthcare Bio-Sciences Corp, CA, USA) filled with heavy-density (1.115 g/cm^3) and light-density (1.065 g/cm^3) solutions in either of two chambers to create a continuous test gradient inside a 50 mL conical tube. Specific density solutions are made by changing the volumetric ratio of iodixanol (1.320 g/cm^3, OptiPrep, Axis-Shield, Oslo, Norway) and cold storage/purification stock solution (1.025 g/cm^3). The ATGS conical is first filled with 5 mL of pure heavy solution to create the bottom layer. A peristaltic pump (flow rate 2 mL/min) is then used to progressively mix the contents of the two machine chambers and force a gradually less dense solution into the conical. This procedure creates a 40 mL continuous density gradient from 1.115 to 1.065 g/cm^3 with 0.005 g/cm^3 increments every 5 mL (Fig. 6).

It is advisable to create the density test gradient during digestion so that it will be available as soon as the recombination product can be sampled. From the recombination flask, 5 mL of pancreatic digest (~0.1 mL of packed tissue) is carefully added to the top of the gradient. The ATGS conical is centrifuged at 400 × g for 3 min at 4 ºC with no brake, similar to the actual COBE purification process. After centrifugation, the layers of acinar cells and islets are separated and settle at their respective tissue densities. Using the graduations on the side of the 50 mL conical, it is easy to determine the peak islet and acinar tissue density (Fig. 6a).

High-Density Gradients

Interpancreatic variations in exocrine and islet tissue density is influenced by the donor characteristics, the secretory status of exocrine cells, the pancreas procurement, the preservation protocols that affect cellular swelling and tissue edema, and the islet isolation procedure, which determines the extent of tissue dissociation and the size of aggregates formed (Anazawa et al. 2011; Chadwick et al. 1993;

London et al. 1998; Hering 2005). In particular, when isolating islets from CP pancreata for autograft transplant, many centers have observed frequent settling of islets in the COBE bag. Our data from use of the ATGS substantiates this observation, revealing a pattern of exocrine density discrepancy between living, chronic pancreatitis, donors (mean 1.105, range 1.085–1.115 g/cm^3), and brain-dead donors (mean 1.095, range 1.080–1.105 g/cm^3) (Anazawa et al. 2011) (Fig. 6b, c). To compensate for this effect, our center prefers to use a more dense gradient range for some autologous islet isolations, pushing the islets out of the COBE bag and into the collection fractions. As demonstrated, a more dense heavy solution effectively reduced tissue volume 63 % (from 30 ± 10.5 to 11 ± 9.2 ml) but also maintained a high post-purification islet recovery (84 ± 29.2 %). Following purification, autograft islets were found in all 12 collection fractions while allograft islets were primarily settled into the first 8, less dense layers.

COBE Purification Process

The COBE purification process begins after recombination has finished and the total tissue pellet has been consolidated into a single 250 mL conical. If the packed tissue volume is >25 mL, it may be split in half as a single COBE is likely to be less effective at segregating out exocrine tissue, which could result in significant islet sedimentation into the COBE bag. All purification steps should be done inside a cold room or custom-built refrigerator to ensure that the COBE apparatus and collection solutions are refrigerated throughout the process. The COBE 2991 cell processor is loaded with a larger gradient maker but in a similar manner as described earlier (see section "Analytical Test Gradient System"). We adjust our standard gradient range, 1.065–1.115 g/cm^3, according to the results of the analytical test gradient obtained during the recombination phase.

The centrifuged pellet is prepared for purification by decanting off the last of the wash media and resuspending the tissue in 20 mL of 25 % HSA. This suspension is transferred to a sterile 250 mL beaker; the conical is rinsed with cold storage solution and brought to a final volume of 120 mL by weight. The tissue is top loaded into the COBE bag by peristaltic pump (flow rate 20 mL/min), gently swirling the beaker for an even distribution of tissue, followed by an additional 30 mL of cold storage solution to rinse the beaker and tubing. After loading, the inlet tube is clamped and the COBE bag vented by carefully opening the outlet clamp and allowing the machinery to spin at $400 \times g$ for 3 min. Subsequently, the first contents of the COBE bag are collected into an empty "waste" conical. When the tissue starts to appear in the tubing, the purification product is then distributed by 25 mL volumes into a consecutive series of 12 conicals, each prefilled with 225 mL of cold supplemented CMRL media.

The efficacy of the purification can be evaluated by taking a 1 mL sample from each well-mixed collection fraction, as well as a 200 μL sample of residual tissue remaining in the COBE bag. If a significant quantity of free islets is observed in the COBE bag, re-purification may be necessary. Assuming a successful purification,

each collection conical is centrifuged at 220 × g, 2–10 °C, for 3 min. We use an estimation of each tube's pellet volume in conjunction with the sample counts to decide which fractions will be combined into a transplantable product. Ideally, the final pellet size will be <15 mL (<20 mL is acceptable) and contain the maximum number of islets with the minimum amount of contaminating tissue. After combining the selected fractions into a single 250 mL conical, the amassed pellet is washed in transplant media, under the same centrifugation parameters. The final pellet is transferred to a T-75 flask and resuspended in 100 mL of room-temperature transplant media, from which 100 μL samples are obtained for a "post-COBE" islet count and additional samples taken for potency and product sterility testing (see section "Recombination").

There have been some interesting innovations proposed in the last few years to improve and standardize this purification process. Friberg et al. have developed a computer-controlled closed system for loading the COBE 2991 that successfully reproduces a continuous density gradient but minimizes the potential for manual variation and contamination (Friberg et al. 2008). The Baylor research group found evidence that passage through the narrow neck of the COBE bag produces fluid shearing forces that encourage islet fragmentation (Shimoda et al. 2012b). They have shown some improvements in post-purification islet yield and size by using a wide plastic bottle with a lower-viscosity density gradient to reduce the necessary centrifugation speed (Shimoda et al. 2012c).

Transplant Preparation

Although quality control and sterility test results will not be available until after the product is released, the islet preparation should be immediately packaged for transplantation to avoid excess delay and operating time for the patient. Any subsequent failure to meet post-release criteria should be reported to the patient physician when assessed (especially sterility positives). Procedural deviations that have occurred during tissue processing should be reported to the physician before product release.

Once the tissue pellet, purified or unpurified, has been washed in transplant media and settled into 100 mL of fresh media for 5 min, the last sterility and retention samples can be taken from the supernatant to ensure the status of the final islet product immediately before packaging. From this point, extreme care should be taken to ensure the continued sterility of the product. All items in the biological safety cabinet where loading will take place should be sterile (if in contact with the product) or thoroughly disinfected and the operator should wear sterile gloves and sleeves during the loading process. If the packed tissue volume is greater than 10 mL, the pellet must be divided evenly and loaded into two 200 mL transplant bags, each labeled with the correct patient identification and FDA-required labeling. Each tissue load is suspended in 100 mL of transplant media and will require an additional 100 mL of media as a rinse solution. If the patient has no known allergy to ciprofloxacin, add 0.4 mL of Cipro® (1 % = 10,000 μg/mL) to each volume of rinse media.

After affixing a 60 mL syringe to the transplant bag, place the syringe upright in a clamp stand and transfer the 100 mL tissue suspension into the bag through the syringe. Rinse the tissue conical twice with 50 mL volumes of rinse solution to transfer any residual islets. Aseptically recap and clamp the bag's inlet tubing to ensure a thorough seal for transport. The sealed bag should be gently rocked to evenly suspend the islets. Repeat these steps for additional transplant bags if needed. Once the transplant physician at the operation room has been alerted, the islet preparation can be readied for transport in a room-temperature cooler equipped with temperature stabilizers (Kaddis et al. 2013).

Conclusion

Autologous islet isolation and transplantation has repeatedly demonstrated the ability to improve clinical outcomes by diminishing the impact of iatrogenic diabetes on patients undergoing pancreatectomy to alleviate CP or other disabling conditions. As practical experience has accumulated at an increasing number of qualified isolation centers, islet yield and viability, critical factors for achieving postoperative insulin independence, have progressively improved. Since performing the first IAT after CP pancreatectomy in 1977, our team at the University of Minnesota has been dedicated to understanding and improving the technical aspects of the isolation procedure that directly impact its immediate and posttransplant success. This includes the introduction of the simplified ATGS to improve purification yield (Anazawa et al. 2011) and the identification of post-isolation factors that detriment graft function (Loganathan et al. 2011). In the last few years, we have focused our research on the mechanics of enzyme digestion, proposing a new enzyme mixture and variable dose classes that have increased the flexibility of the procedure to respond to different donor and tissue characteristics (Balamurugan et al. 2010, 2012). Despite these and other advances, the best islet yields still average significantly less than the available store, indicating the need for more research.

There remains a clear need for better understanding enzyme mechanisms: the best role and timing for collagenase versus neutral protease, their functional ingredients, and how each interacts with different ECM components to define the length and efficiency of digestion. While good work has been done to identify factors that influence islet yield and viability, including donor and tissue characteristics, we need more specific techniques to be hypothesized and tested for overcoming these inevitable obstacles. Furthermore, there is currently a heavy cost burden to establish facilities and perform these procedures, which severely limits their availability, especially in developing countries. Ultimately, islet isolation should be considered a flexible and optimizable set of techniques, rather than a one-size-fits-all procedure. With well-trained teams and a deeper understanding of technical principles, it is our belief that IAT will continue to expand its reach all over the world, to prolong life and alleviate suffering among an increasingly diverse pool of potential recipients.

Acknowledgments The authors would like to thank Josh Wilhelm, Muhamad Abdulla, Mukesh Tiwari, Tom Gilmore, and Jeff Ansite.

Cross-References

- ▶ Advances in Clinical Islet Isolation
- ▶ Basement Membrane in Pancreatic Islet Function
- ▶ Human Islet Autotransplantation
- ▶ Successes and Disappointments with Clinical Islet Transplantation

References

Al-Abdullah IH, Kumar MS, Abouna GM (1994) Combined intraductal and interstitial distension of human and porcine pancreas with collagenase facilitates digestion of the pancreas and may improve islet yield. Transplant Proc 26:3384

Alsaif F, Molinari M, Al-Masloom A, Lakey JR, Kin T, Shapiro AM (2006) Pancreatic islet autotransplantation with completion pancreatectomy in the management of uncontrolled pancreatic fistula after whipple resection for ampullary adenocarcinoma. Pancreas 32:430–431

Anazawa T, Balamurugan AN, Bellin M, Zhang HJ, Matsumoto S, Yonekawa Y, Tanaka T, Loganathan G, Papas KK, Beilman GJ, Hering BJ, Sutherland DE (2009) Human islet isolation for autologous transplantation: comparison of yield and function using SERVA/Nordmark versus Roche enzymes. Am J Transplant 9:2383–2391

Anazawa T, Matsumoto S, Yonekawa Y, Loganathan G, Wilhelm JJ, Soltani SM, Papas KK, Sutherland DE, Hering BJ, Balamurugan AN (2011) Prediction of pancreatic tissue densities by an analytical test gradient system before purification maximizes human islet recovery for islet autotransplantation/allotransplantation. Transplantation 91:508–514

Balamurugan AN, Chang Y, Fung JJ, Trucco M, Bottino R (2003) Flexible management of enzymatic digestion improves human islet isolation outcome from sub-optimal donor pancreata. Am J Transplant 3:1135–1142

Balamurugan AN, Chang Y, Bertera S, Sands A, Shankar V, Trucco M, Bottino R (2006) Suitability of human juvenile pancreatic islets for clinical use. Diabetologia 49:1845–1854

Balamurugan AN, Breite AG, Anazawa T, Loganathan G, Wilhelm JJ, Papas KK, Dwulet FE, McCarthy RC, Hering BJ (2010) Successful human islet isolation and transplantation indicating the importance of class 1 collagenase and collagen degradation activity assay. Transplantation 89:954–961

Balamurugan AN, Loganathan G, Bellin MD, Wilhelm JJ, Harmon J, Anazawa T, Soltani SM, Radosevich DM, Yuasa T, Tiwari M, Papas KK, McCarthy R, Sutherland DE, Hering BJ (2012) A new enzyme mixture to increase the yield and transplant rate of autologous and allogeneic human islet products. Transplantation 93:693–702

Barlow AD, Hosgood SA, Nicholson ML (2013) Current state of pancreas preservation and implications for DCD pancreas transplantation. Transplantation 95:1419–1424

Bellin MD, Carlson AM, Kobayashi T, Gruessner AC, Hering BJ, Moran A, Sutherland DE (2008) Outcome after pancreatectomy and islet autotransplantation in a pediatric population. J Pediatr Gastroenterol Nutr 47:37–44

Bellin MD, Sutherland DE, Beilman GJ, Hong-McAtee I, Balamurugan AN, Hering BJ, Moran A (2011) Similar islet function in islet allotransplant and autotransplant recipients, despite lower islet mass in autotransplants. Transplantation 91:367–372

Bellin MD, Beilman GJ, Dunn TB, Pruett TL, Chinnakotla S, Wilhelm JJ, Ngo A, Radosevich DM, Freeman ML, Schwarzenberg SJ, Balamurugan AN, Hering BJ, Sutherland DE (2012a)

Islet autotransplantation to preserve β cell mass in selected patients with chronic pancreatitis and diabetes mellitus undergoing total pancreatectomy. Pancreas 42:317–321

Bellin MD, Balamurugan AN, Pruett TL, Sutherland DE (2012b) No islets left behind: islet autotransplantation for surgery-induced diabetes. Curr Diab Rep 12:580–586

Berney T, Mathe Z, Bucher P, Demuylder-Mischler S, Andres A, Bosco D, Oberholzer J, Majno P, Philippe J, Buhler L, Morel P (2004) Islet autotransplantation for the prevention of surgical diabetes after extended pancreatectomy for the resection of benign tumors of the pancreas. Transplant Proc 36:1123–1124

Blondet JJ, Carlson AM, Kobayashi T, Jie T, Bellin M, Hering BJ, Freeman ML, Beilman GJ, Sutherland DE (2007) The role of total pancreatectomy and islet autotransplantation for chronic pancreatitis. Surg Clin North Am 87:1477–1501, x

Braganza JM, Lee SH, McCloy RF, McMahon MJ (2011) Chronic pancreatitis. Lancet 377:1184–1197

Bucher P, Mathe Z, Bosco D, Becker C, Kessler L, Greget M, Benhamou PY, Andres A, Oberholzer J, Buhler L, Morel P, Berney T (2004) Morbidity associated with intraportal islet transplantation. Transplant Proc 36:1119–1120

Caballero-Corbalan J, Friberg AS, Brandhorst H, Nilsson B, Andersson HH, Felldin M, Foss A, Salmela K, Tibell A, Tufveson G, Korsgren O, Brandhorst D (2009) Vitacyte collagenase HA: a novel enzyme blend for efficient human islet isolation. Transplantation 88:1400–1402

Caballero-Corbalan J, Brandhorst H, Asif S, Korsgren O, Engelse M, de Koning E, Pattou F, Kerr-Conte J, Brandhorst D (2010) Mammalian tissue-free liberase: a new GMP-graded enzyme blend for human islet isolation. Transplantation 90:332–333

Chadwick DR, Robertson GS, Rose S, Contractor H, James RF, Bell PR, London NJ (1993) Storage of porcine pancreatic digest prior to islet purification. The benefits of UW solution and the roles of its individual components. Transplantation 56:288–293

Contractor HH, Johnson PR, Chadwick DR, Robertson GS, London NJ (1995) The effect of UW solution and its components on the collagenase digestion of human and porcine pancreas. Cell Transplant 4:615–619

D'Haese JG, Ceyhan GO, Demir IE, Tieftrunk E, Friess H (2013) Treatment options in painful chronic pancreatitis: a systematic review. HPB, Oxford

DiMagno MJ, DiMagno EP (2013) Chronic pancreatitis. Curr Opin Gastroenterol 29:531–536

Eckhard M, Brandhorst D, Brandhorst H, Brendel MD, Bretzel RG (2004) Optimization in osmolality and range of density of a continuous ficoll-sodium-diatrizoate gradient for isopycnic purification of isolated human islets. Transplant Proc 36:2849–2854

Forster S, Liu X, Adam U, Schareck WD, Hopt UT (2004) Islet autotransplantation combined with pancreatectomy for treatment of pancreatic adenocarcinoma: a case report. Transplant Proc 36:1125–1126

Friberg AS, Stahle M, Brandhorst H, Korsgren O, Brandhorst D (2008) Human islet separation utilizing a closed automated purification system. Cell Transplant 17:1305–1313

Gachago C, Draganov PV (2008) Pain management in chronic pancreatitis. World J Gastroenterol 14:3137–3148

Garraway NR, Dean S, Buczkowski A, Brown DR, Scudamore CH, Meloche M, Warnock G, Simons R (2009) Islet autotransplantation after distal pancreatectomy for pancreatic trauma. J Trauma 67:E187–E189

Gores PF, Sutherland DE (1993) Pancreatic islet transplantation: is purification necessary? Am J Surg 166:538–542

Goto M, Eich TM, Felldin M, Foss A, Kallen R, Salmela K, Tibell A, Tufveson G, Fujimori K, Engkvist M, Korsgren O (2004) Refinement of the automated method for human islet isolation and presentation of a closed system for in vitro islet culture. Transplantation 78:1367–1375

Gray DW, Morris PJ (1987) Developments in isolated pancreatic islet transplantation. Transplantation 43:321–331

Gruessner RW, Sutherland DE, Dunn DL, Najarian JS, Jie T, Hering BJ, Gruessner AC (2004) Transplant options for patients undergoing total pancreatectomy for chronic pancreatitis. J Am Coll Surg 198:559–567; discussion 568–559

Hering BJ (2005) Repurification: rescue rather than routine remedy. Am J Transplant 5:1–2
Hering BJ, Matsumoto I, Sawada T, Nakano M, Sakai T, Kandaswamy R, Sutherland DE (2002) Impact of two-layer pancreas preservation on islet isolation and transplantation. Transplantation 74:1813–1816
Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, Matsumoto I, Ihm SH, Zhang HJ, Parkey J, Hunter DW, Sutherland DE (2005) Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. JAMA 293:830–835
Jindal RM, Ricordi C, Shriver CD (2010) Autologous pancreatic islet transplantation for severe trauma. New Engl J Med 362:1550
Johnson PR (2010) Suitable donor selection and techniques of islet isolation and purification for human islet-allograft transplantation. In: Hakim NS, Stratta RJ, Gray DW, Friend P, Colman A (eds) Pancreas, islet and stem cell transplantation for diabetes, 2nd edn. Oxford University Press, New York, pp 387–396
Kaddis JS, Hanson MS, Cravens J, Qian D, Olack B, Antler M, Papas KK, Iglesias I, Barbaro B, Fernandez L, Powers AC, Niland JC (2013) Standardized transportation of human islets: an islet cell resource center study of more than 2,000 shipments. Cell Transplant 22:1101–1111
Kawahara T, Kin T, Shapiro AM (2012) A comparison of islet autotransplantation with allotransplantation and factors elevating acute portal pressure in clinical islet transplantation. J Hepatobiliary Pancreat Sci 19:281–288
Khan A, Jindal RM, Shriver C, Guy SR, Vertrees AE, Wang X, Xu X, Szust J, Ricordi C (2012) Remote processing of pancreas can restore normal glucose homeostasis in autologous islet transplantation after traumatic whipple pancreatectomy: technical considerations. Cell Transplant 21:1261–1267
Kin T (2010) Islet isolation for clinical transplantation. Adv Exp Med Biol 654:683–710
Kin T, Shapiro AM (2010) Surgical aspects of human islet isolation. Islets 2:265–273
Kobayashi T, Manivel JC, Carlson AM, Bellin MD, Moran A, Freeman ML, Bielman GJ, Hering BJ, Dunn T, Sutherland DE (2011) Correlation of histopathology, islet yield, and islet graft function after islet autotransplantation in chronic pancreatitis. Pancreas 40:193–199
Lacy PE, Kostianovsky M (1967) Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 16:35–39
Lake SP, Bassett PD, Larkins A, Revell J, Walczak K, Chamberlain J, Rumford GM, London NJ, Veitch PS, Bell PR et al (1989) Large-scale purification of human islets utilizing discontinuous albumin gradient on IBM 2991 cell separator. Diabetes 38(Suppl 1):143–145
Lakey JR, Warnock GL, Shapiro AM, Korbutt GS, Ao Z, Kneteman NM, Rajotte RV (1999) Intraductal collagenase delivery into the human pancreas using syringe loading or controlled perfusion. Cell Transplant 8:285–292
Lakey JR, Kneteman NM, Rajotte RV, Wu DC, Bigam D, Shapiro AM (2002) Effect of core pancreas temperature during cadaveric procurement on human islet isolation and functional viability. Transplantation 73:1106–1110
Lakey JR, Burridge PW, Shapiro AM (2003) Technical aspects of islet preparation and transplantation. Transpl Int 16:613–632
Linetsky E, Bottino R, Lehmann R, Alejandro R, Inverardi L, Ricordi C (1997a) Improved human islet isolation using a new enzyme blend, liberase. Diabetes 46:1120–1123
Linetsky E, Lehmann R, Li H, Fernandez L, Bottino R, Selvaggi G, Alejandro R, Ricordi C (1997b) Human islet isolation using a new enzyme blend. Transplant Proc 29:1957–1958
Loganathan G, Dawra RK, Pugazhenthi S, Guo Z, Soltani SM, Wiseman A, Sanders MA, Papas KK, Velayutham K, Saluja AK, Sutherland DE, Hering BJ, Balamurugan AN (2011) Insulin degradation by acinar cell proteases creates a dysfunctional environment for human islets before/after transplantation: benefits of α-1 antitrypsin treatment. Transplantation 92:1222–1230
Loganathan G, Graham ML, Radosevich DM, Soltani SM, Tiwari M, Anazawa T, Papas KK, Sutherland DE, Hering BJ, Balamurugan AN (2013) Factors affecting transplant outcomes in diabetic nude mice receiving human, porcine, and nonhuman primate islets: analysis of 335 transplantations. Transplantation 95:1439–1447

London NJ, Swift SM, Clayton HA (1998) Isolation, culture and functional evaluation of islets of Langerhans. Diabetes Metab 24:200–207

Miki A, Ricordi C, Messinger S, Yamamoto T, Mita A, Barker S, Haetter R, Khan A, Alejandro R, Ichii H (2009) Toward improving human islet isolation from younger donors: rescue purification is efficient for trapped islets. Cell Transplant 18:13–22

Mita A, Ricordi C, Miki A, Barker S, Khan A, Alvarez A, Hashikura Y, Miyagawa S, Ichii H (2009) Purification method using iodixanol (OptiPrep)-based density gradient significantly reduces cytokine chemokine production from human islet preparations, leading to prolonged β-cell survival during pretransplantation culture. Transplant Proc 41:314–315

Morrison CP, Wemyss-Holden SA, Dennison AR, Maddern GJ (2002) Islet yield remains a problem in islet autotransplantation. Arch Surg 137:80–83

Moskalewski S (1965) Isolation and culture of the islets of Langerhans of the Guinea pig. Gen Comp Endocrinol 44:342–353

Najarian JS, Sutherland DE, Matas AJ, Goetz FC (1979) Human islet autotransplantation following pancreatectomy. Transplant Proc 11:336–340

Nakanishi W, Imura T, Inagaki A, Nakamura Y, Sekiguchi S, Fujimori K, Satomi S, Goto M (2012) Ductal injection does not increase the islet yield or function after cold storage in a vascular perfusion model. PLoS One 7:e42319

Naziruddin B, Matsumoto S, Noguchi H, Takita M, Shimoda M, Fujita Y, Chujo D, Tate C, Onaca N, Lamont J, Kobayashi N, Levy MF (2012) Improved pancreatic islet isolation outcome in autologous transplantation for chronic pancreatitis. Cell Transplant 21:553–558

Paget M, Murray H, Bailey CJ, Downing R (2007) Human islet isolation: semi-automated and manual methods. Diab Vasc Dis Res 4:7–12

Qin H, Matsumoto S, Klintmalm GB, De vol EB (2011) A meta-analysis for comparison of the two-layer and university of Wisconsin pancreas preservation methods in islet transplantation. Cell Transplant 20:1127–1137

Rabkin JM, Leone JP, Sutherland DE, Ahman A, Reed M, Papalois BE, Wahoff DC (1997) Transcontinental shipping of pancreatic islets for autotransplantation after total pancreatectomy. Pancreas 15:416–419

Rabkin JM, Olyaei AJ, Orloff SL, Geisler SM, Wahoff DC, Hering BJ, Sutherland DE (1999a) Distant processing of pancreas islets for autotransplantation following total pancreatectomy. Am J Surg 177:423–427

Rabkin JM, Olyaei AJ, Orloff SL, Geisler SM, Wahoff DC, Hering BJ, Sutherland DE (1999b) Distant processing of pancreas islets for autotransplantation following total pancreatectomy. Am J Surg 177:423–427

Ricordi C (2003) Islet transplantation: a brave new world. Diabetes 52:1595–1603

Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW (1988) Automated method for isolation of human pancreatic islets. Diabetes 37:413–420

Sah RP, Dawra RK, Saluja AK (2013) New insights into the pathogenesis of pancreatitis. Curr Opin Gastroenterol 29:523–530

Shimoda M, Itoh T, Sugimoto K, Iwahashi S, Takita M, Chujo D, Sorelle JA, Naziruddin B, Levy MF, Grayburn PA, Matsumoto S (2012a) Improvement of collagenase distribution with the ductal preservation for human islet isolation. Islets 4:130–137

Shimoda M, Itoh T, Iwahashi S, Takita M, Sugimoto K, Kanak MA, Chujo D, Naziruddin B, Levy MF, Grayburn PA, Matsumoto S (2012b) An effective purification method using large bottles for human pancreatic islet isolation. Islets 4:398–404

Shimoda M, Itoh T, Iwahashi S, Takita M, Sugimoto K, Kanak MA, Chujo D, Naziruddin B, Levy MF, Grayburn PA, Matsumoto S (2012c) An effective purification method using large bottles for human pancreatic islet isolation. Islets 4:398–404

Sutherland DE, Gruessner AC, Carlson AM, Blondet JJ, Balamurugan AN, Reigstad KF, Beilman GJ, Bellin MD, Hering BJ (2008) Islet autotransplant outcomes after total pancreatectomy: a contrast to islet allograft outcomes. Transplantation 86:1799–1802

Sutherland DE, Radosevich DM, Bellin MD, Hering BJ, Beilman GJ, Dunn TB, Chinnakotla S, Vickers SM, Bland B, Balamurugan AN, Freeman ML, Pruett TL (2012) Total pancreatectomy and islet autotransplantation for chronic pancreatitis. J Am Coll Surg 214:409–424; discussion 424–406

'tHart NA, Leuvenink HG, Ploeg RJ (2002) New solutions in organ preservation. Transplant Rev 16:131–141

Tsukada M, Saito T, Ise K, Kenjo A, Kimura T, Satoh Y, Saito T, Anazawa T, Oshibe I, Suzuki S, Hashimoto Y, Gotoh M (2012) A model to evaluate toxic factors influencing islets during collagenase digestion: the role of serine protease inhibitor in the protection of islets. Cell Transplant 21:473–482

Wahlberg JA, Southard JH, Belzer FO (1986) Development of a cold storage solution for pancreas preservation. Cryobiology 23:477–482

Walsh TJ, Eggleston JC, Cameron JL (1982) Portal hypertension, hepatic infarction, and liver failure complicating pancreatic islet autotransplantation. Surgery 91:485–487

Watkins JG, Krebs A, Rossi RL (2003) Pancreatic autotransplantation in chronic pancreatitis. World J Surg 27:1235–1240

Webb MA, Dennison AR, James RF (2012) The potential benefit of non-purified islets preparations for islet transplantation. Biotechnol Genet Eng Rev 28:101–114

Human Islet Autotransplantation

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Martin Hermann, Raimund Margreiter, and Paul Hengster

Contents

Abstract

Total pancreatectomy is a last option for patients in whom all other efforts for managing intractable pain caused by chronic pancreatitis have failed. When performed with a simultaneous islet autotransplantation, endogenous insulin production can be preserved to some extent. Although it does not necessarily prevent any future need for exogenous insulin, the diabetic state of the patient is less severe compared to pancreatectomy alone.

In contrast to islet allotransplantation, the patients do not require immunosuppressive drugs and are not at risk for autoimmune destruction or alloimmune rejection. Consequently, insulin independence is more likely to be preserved in

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_21,

autoislet-transplanted patients than in those patients who received alloislet transplants. Therefore, the setting of islet autotransplantation allows the study of the transplanted islets without interference from medication or immunological variables.

Keywords
Human islet autotransplantation • Islet shipment • Real-time live confocal microscopy

Introduction

Chronic pancreatitis (CP) is a progressive inflammatory disease causing irreversible structural damage to the pancreatic parenchyma. Besides affecting the pancreatic exocrine function, in severe cases, the endocrine function may also be impaired leading to the onset of diabetes mellitus (Steer et al. 1995). As in many patients CP is clinically silent, its prevalence can only be estimated and ranges from 0.4 % to 5 % before the onset of clinically apparent disease. Besides heavy consumption of alcohol (150–170 g/day), pancreatic obstructions such as posttraumatic ductal strictures, pseudocysts, mechanical or structural changes of the pancreatic-duct sphincter, and periampullary tumors may result in chronic pancreatitis. Of high importance is the recent recognition of a set of genetic mutations such as the loss-of-function mutations of pancreatic secretory trypsin inhibitor (SPINK1), which were shown to be present in CP cases that previously had been considered idiopathic (for review see Naruse et al. 2007). Also, sphincter of Oddi dysfunction (SOD) has increasingly been recognized as being present in CP (McLoughlin and Mitchell 2007).

Due to the progress in imaging techniques such as endoscopic retrograde cholangiopancreatography, magnetic resonance imaging, and cross-sectional imaging, we now have a better understanding of the pathophysiology and origin of inflammation and pain in CP. Nevertheless, chronic pancreatitis still remains an inscrutable process of uncertain pathogenesis, unpredictable clinical course, and difficult treatment (Steer et al. 1995; Riediger et al. 2007). Chronic pancreatitis is associated with a mortality rate that approaches 50 % within 20–25 years. Approximately 15–20 % of patients die of complications associated with acute attacks of pancreatitis (Steer et al. 1995).

Complications such as biliary or duodenal stenosis, as well as intractable pain, are the current indications for surgery in patients with CP. Surgical drainage of the duct in CP has largely been replaced by endoscopic duct drainage procedures of sphincterotomy and stent placement in the duct. Patients with CP whose pain persists after endoscopic pancreatic-duct drainage are candidates for total pancreatectomy and islet autotransplantation (IAT) (Evans et al. 2008). Islet autotransplantation was also shown to be a successful option for patients suffering severe trauma requiring a complete removal of the pancreas (Khan et al. 2012; Jindal et al. 2010).

In the Cincinnati series of total pancreatectomy in combination with simultaneous IAT, unremitting abdominal pain refractory to high-dose narcotics was the indication for surgery (Ahmad et al. 2005; Rodriguez Rilo et al. 2003). Narcotic independence due to pain relief after total pancreatectomy and islet autotransplantation was achieved in 58–81 % of the patients (Ahmad et al. 2005; Blondet et al. 2007). Notably, in a retrospective survey, more than 95 % of the patients stated they would recommend total pancreatectomy in combination with islet autotransplantation (Blondet et al. 2007).

Mortality as well as morbidity associated with pancreatic resections in patients suffering from chronic pancreatitis was shown to be very low and normally leads to adequate pain control in the majority of CP patients. One drawback of surgical resection is the development of exo- and endocrine insufficiencies. Therefore, surgical resection of the pancreas is considered as a final option in the treatment of CP. Nevertheless, the addition of an islet autotransplant offers the possibility of a postoperative glucose control and should therefore always be a considerable option.

Besides being applicable to prevent surgical diabetes after extensive pancreatic resection for chronic pancreatitis, islet autotransplantation is additionally pertinent in benign tumors located at the neck of the pancreas. Even without pancreatic inflammation, extensive pancreatic resection of more than 70 % of the pancreas may cause diabetes (Slezak and Andersen 2001).

Islet autotransplantation, after extended pancreatectomy performed for the resection of benign tumors of the mid-segment of the pancreas, was shown to be a feasible option with excellent metabolic results and low morbidity. Due to the non-inflammatory nature of the pancreata, higher islet yields and, consequently, higher transplanted islet masses were achieved compared to those from organs resected for chronic pancreatitis. At a median follow-up of 5 years (range, 1–8 years), all patients ($n = 7$) had β;-cell function as assessed by a positive C-peptide level. Six out of the seven patients were insulin independent (Berney et al. 2004). Pivotal for such an approach is the unequivocal diagnosis of the benign nature of the tumor, before making the decision to perform the isolation and transplantation procedure.

The first total pancreatectomy in combination with islet autotransplantation to treat chronic pancreatitis (CP) in humans was performed 30 years ago at the University of Minnesota (Sutherland et al. 1978). Besides aiming to relieve the pain of the CP patient in whom other measures had failed, the additional goal was to preserve β-cell mass and insulin secretion in order to avoid the otherwise inevitable surgical diabetes. Since then, more than 300 islet autotransplantations have been performed and reported worldwide, most of them at the University of Minnesota. With a few exceptions, the intraportal site has been predominantly applied as an implantation site for the transplanted islets (Blondet et al. 2007; White et al. 2000). Since 1990 the results of autologous islet transplantation have been reported to the International Islet Transplant Registry (ITR) in Giessen, Germany (Bretzel et al. 2007).

Combined pancreatectomy and islet autotransplantation can be performed in adults, as well as in pediatric patients. For both patient populations, the procedures

are identical and described in detail elsewhere (Bellin et al. 2008; Wahoff et al. 1995; White et al. 2000). Performing islet autotransplantations provides the possibility to compare the metabolic outcomes between islet autografts and islet allografts, the latter still being subject to declining function with time (Shapiro et al. 2006). Besides, and prior to, the outstanding results from the Edmonton study fuelling the whole field of islet transplantation with new energy, the "Minnesota islet autotransplantation" provided the pivotal biological "proof of principle" for the feasibility of a long-lasting successful glucose control after islet transplantation.

Islet allotransplantation shows a 5-year post-islet transplantation graft survival of approximately 80 %, and insulin independence around 10 % at 5 years (Ryan et al. 2005). Differences in the success of allogenic islet transplantation among different centers illustrate the complexity of the procedure (Shapiro et al. 2006). Therefore, the ultimate goal, defined by insulin independence in the long term being achieved on a regular basis, has still not been achieved.

Notably, the results from islet autotransplantation obtained so far clearly show that long-term insulin independence after islet transplantation is a goal which can be realized, although also here not on a regular basis (Blondet et al. 2007; Robertson et al. 1999, 2001). In a recently published study, the outcomes of islet function over time were compared between intraportal islet autotransplant recipients at the University of Minnesota and diabetic islet allograft recipients as reported by the Collaborative Islet Transplant Registry (CITR). With regard to insulin independence, 74 % of islet autotransplant recipients retained insulin independence at 2-year posttransplant versus only 45 % of the CITR allograft recipients who initially became insulin independent. Notably, 46 % of the islet autotransplant patients were still insulin independent at 5 years and 28 % at 10 years posttransplant (Sutherland et al. 2008).

Lessons from Islet Autotransplantations

Three metabolic states were described in patients after islet autotransplantations: one third of the patients after islet autotransplantation in the University of Minnesota series were long-term insulin independent, another third of the recipients became fully diabetic, and the last third achieved near normoglycemia and are therefore partially insulin independent requiring only one daily injection of insulin (Fig. 1) (Sutherland et al. 2012; Blondet et al. 2007).

A remarkable result when comparing islet allo- with islet autotransplantation is the generally higher long-term success rate of the latter (Robertson et al. 2001; Ryan et al. 2005). There are at least three known causes (Fig. 2) for organ/cell stress which are present in islet allotransplantation but not in autotransplantation, thereby possibly explaining the better long-term success rates of the latter:

1. Brain death: In islet allotransplantation, the organ is obtained from brain dead patients. In animal models, brain death was shown to negatively affect islet yield as well as function due to the activation of pro-inflammatory cytokines (Contreras et al. 2003).

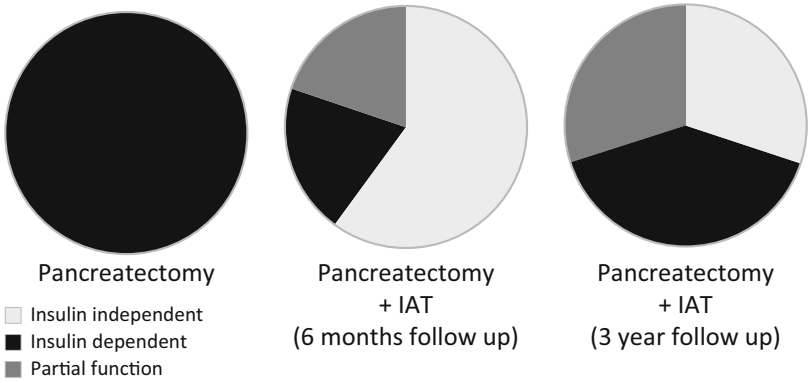

Fig. 1 A schematic representation of the three metabolic states described in patients after pancreatectomy with or without islet autotransplantation. Total pancreatectomy without a simultaneous islet autotransplantation unequivocally leads to insulin dependence of the patient. In contrast, approximately one third of autoislet transplant recipients become insulin independent and an additional one third require minimal insulin replacement (Bellin et al. 2011; Blondet et al. 2007)

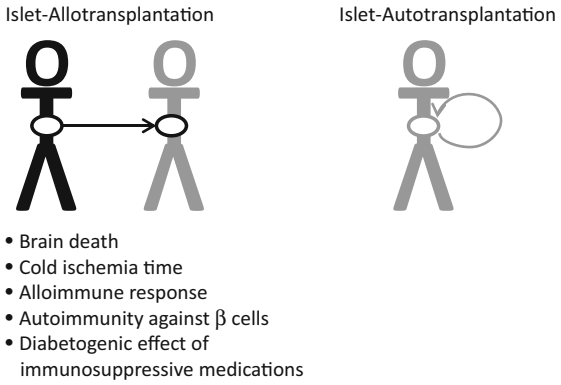

Fig. 2 In contrast to islet autografts, islet allografts are subject to several additional cell stress conditions. Brain death (Contreras et al. 2003), longer cold ischemia times before islet isolation from the donor pancreas (Hering et al. 2002), the patients' alloimmune response to the donor tissue, the autoimmunity against β cells (Huurman et al. 2008; Monti et al. 2008), and the diabetogenic effect of the immunosuppressive medications (Egidi et al. 2008) are the main reasons limiting long-term success of islet allotransplantation

2. Ischemia: In islet autotransplantation, the organ is not subjected to prolonged cold ischemia times which are normally present in islet allotransplantation due to the transport of the organ to the islet procurement center. Such cold ischemia times are known to damage the organ and impair cell viability, as well as function (Emamaullee and Shapiro 2007).

3. Immunosuppression: Besides ischemia-associated organ damage, the need for immunosuppression in islet allotransplantation is the third major limiting cause in the long-term success of islet allotransplantation (Emamaullee and Shapiro 2007). In human islet allotransplantation, immunosuppressive regimens are implemented in order to cope with both auto- and alloimmunity after transplantation. However, many of the immunosuppressive drugs are known to be directly β-cell toxic. Using a transgenic mouse model for conditional ablation of pancreatic β cells in vivo, Nir and coworkers elegantly demonstrated that β cells have a significant regenerative capacity which is prevented by the addition of the immunosuppressant drugs sirolimus and tracrolimus (Nir et al. 2007). As shown in humans, up to 15 % of nondiabetic patients who received solid organ transplantation were shown to develop posttransplant diabetes as a result of calcineurin inhibitor therapy (i.e., tacrolimus) (Emamaullee and Shapiro 2007). Therefore, the declining function of β cells after human allotransplantation may also be explained by the inhibition of β-cell turnover due to the administration of immunosuppressive drugs (Ruggenenti et al. 2008).

Allograft rejection and recurrent autoimmunity, both conditions not present in islet autotransplant recipients, may additionally contribute to the decreasing insulin independence over time observed in the allogeneic setting (Huurman et al. 2008; Monti et al. 2008). Recently it was shown that immunosuppression with FK506 and rapamycin after islet transplantation in patients with autoimmune diabetes induced homeostatic cytokines that expand autoreactive memory T cells. It was therefore proposed that such an increased production of cytokines might contribute to recurrent autoimmunity in transplanted patients with autoimmune disease and that a therapy that prevents the expansion of autoreactive T cells will improve the outcome of islet allotransplantation (Monti et al. 2008).

Another recently published study reports that cellular islet autoimmunity associates with the clinical outcome of islet allotransplantation. In this study, 21 type 1 diabetic patients received islet grafts prepared from multiple donors, while immunosuppression was maintained by means of antithymocyte globulin (ATG) induction and tacrolimus and mycophenolate treatment. Immunity against auto- and alloantigens was measured before and during 1 year after transplantation. Interestingly, cellular autoimmunity before and after transplantation was shown to be associated with delayed insulin independence and lower circulating C-peptide levels during the first year after islet allotransplantation. While seven out of eight patients without preexistent T-cell autoreactivity became insulin independent, none of the four patients reactive to both islet autoantigens GAD and IA-2 achieved insulin independence. Consequently, tailored immunotherapy regimens targeting cellular islet autoreactivity may be required (Huurman et al. 2008).

An additional explanation for the lack of long-term insulin independence after islet transplantation was suggested to be the detrimental effect of hyperglycemia on β-cell physiology. As shown in mice, increased apoptosis and reduced β-cell mass were found in islets exposed to chronic hyperglycemia (Biarnes et al. 2002). Consequently, both (auto- and allo-) human islet recipients usually receive insulin early on to maintain euglycemia as much as possible. However, no study in humans

has been performed so far comparing islet engraftment with and without this measure.

Still Open Issues in Islet Autotransplantation

Islet Mass

The timing of the pancreatectomy and simultaneous islet allotransplantation has a direct impact on islet yield. The highest islet yields and insulin independence can be achieved when the islet autotransplantation is performed earlier in the disease course of CP (Ahmed et al. 2006; Rodriguez Rilo et al. 2003). The search for the optimal enzyme blend that maximizes human islet yield for transplantation is still ongoing (Balamurugan et al. 2012).

Although the islet yield is an important predictor of insulin independence (Gruessner et al. 2004), there are exceptions: one patient who received only 954 IEQ/kg remained insulin-free even 4 years after transplantation (Webb et al. 2006, 2008). Considering the scarcity of available organs, such results are a crucial proof of principle showing that even very low amounts of transplanted islets may be sufficient to provide long-term insulin independence. Interestingly islet autografts show durable function and, once established, are associated with a persisting high rate of insulin independence, although the β-cell mass transplanted is lesser than that used for islet allografts (Sutherland et al. 2008).

Evaluating and comparing the different outcomes after islet allo- versus autotransplantations may help clarify the extent to which different stress parameters account for islet damage resulting in limited success rates of islet allotransplantation. There are several causes for cellular stress in islet autotransplantation.

Islet Shipment

Exposure of islets to a series of damaging physicochemical stresses already during explantation of the pancreas may amplify the damage caused during cold storage as well as the following islet isolation procedure. There is consensus among the major islet transplantation centers that islet yields and quality can be improved with better pancreas procurement techniques such as in situ regional organ cooling which protects the pancreas from warm ischemic injury (for review see Iwanaga et al. 2008). In addition, the development of more sophisticated pancreas preservation protocols promises to translate into an improved islet yield as well as quality.

While pancreatectomy can be performed at most hospitals, only a few centers are able to perform islet isolations. Therefore, human islet autotransplantation is often limited due to the absence of an on-site islet-processing facility. The setup of an islet isolation facility, designed according to the rules of good manufacturing practice, is a technically challenging and cost- and time-intensive process (Hengster

et al. 2005; Guignard et al. 2004). Consequently, several institutions have decided to perform transplantation of islets isolated at another center with already established expertise. Such an "outsourcing solution" was not only shown to be applicable in human islet allotransplantation (Guignard et al. 2004; Ichii et al. 2007; Yang et al. 2004) but also in human islet autotransplantation (Rabkin et al. 1997, 1999). In the latter, the resected pancreata were transferred to an islet-processing laboratory, which then sent back the freshly isolated islets that were transplanted into the same patient. All five patients experienced complete relief from pancreatic pain, and three of the five patients had minimal or no insulin requirement, thereby demonstrating the feasibility of islet shipment for autotransplantation (median follow-up of 23 months) (Rabkin et al. 1999). A recent case reports a successful emergency autologous islet transplantation after a traumatic Whipple operation with islets processed in a remote center. Although the number of islets was suboptimal, near-normal glucose tolerance was achieved, thereby showing that islets processed at a remote site are also suitable for transplantation (Khan et al. 2012; Jindal et al. 2010).

Although practicability as well as feasibility of islet transportation has already been proven, many questions such as the one addressing the optimal transport conditions for islets remain to be answered. While there is a worldwide consensus of how to isolate islets under GMP conditions, this is not the case for the transport of the freshly isolated islets. Many different media and transport devices have been used, ranging from 50 ml flasks, syringes, and gas permeable bags (Ichii et al. 2007). Other solutions such as rotary devices avoiding detrimental cell compaction (Merani et al. 2006) may be an alternative, especially when vitality parameters such as temperature, pH, or oxygen concentration are actively controlled (Wurm et al. 2007). Determining the optimal conditions for the transport of islets promises to yield better islet quality after the transport of islets and consequently an improved transplantation outcome. In addition, a gain of knowledge concerning the issues addressing the regeneration potential of freshly isolated islets may help not only to avoid unnecessary additional cellular stress but also counterbalance it in a preemptive way.

In this context, the topic of islet quality assessment has to be mentioned: similar to the transport conditions of human islets, this issue remains a matter of debate. Predicting the outcome of islet transplantation is still not possible due to the lack of reliable markers of islet potency, which might potentially be used to screen human islet preparations prior to transplantation. According to these pretransplant criteria, islet preparations that failed to reverse diabetes were indistinguishable from those that exhibited excellent function (Ichii et al. 2007).

Therefore, one of the primary challenges also in islet autotransplantation is to identify and understand the changes taking place in islets after the isolation, culture, and transport. Description of such changes in living islet cells offers insights not achievable by the use of fixed cell techniques. Combining real-time live confocal microscopy with three fluorescent dyes, dichlorodihydrofluorescein (DCF) diacetate, tetramethylrhodamine methyl ester (TMRM) perchlorate, and fluorescent wheat germ agglutinin (WGA), offers the possibility to assess overall oxidative

stress, time-dependent mitochondrial membrane potentials, and cell morphology (Hermann et al. 2005, 2007). The advantage of such a method resides in the fast and accurate imaging at a cellular and even subcellular level. Taking into account the use of other fluorescent dyes which can be used to visualize additional cell viability parameters such as calcium concentrations (measured with rhod-2) or apoptosis (measured with annexin-V), such an approach promises to be of great value for a better future islet assessment, post-isolation, culture, and/or transport. For detecting islets at transplantation in a clinical setting, intraoperative ultrasound examination was shown to be useful (Sakata et al. 2012).

Cell Death

A significant proportion of the transplanted islet mass fails to engraft due to apoptotic cell death. Several strategies have been implemented to inhibit this process by blocking the extrinsic apoptosis-inducing signals (cFLIP or A20), although only with limited impact. More recently, investigations of downstream apoptosis inhibitors that block the final common pathway (i.e., X-linked inhibitor of apoptosis protein [XIAP]) have shown promising results, in human (Emamaullee et al. 2005a, b; Hui et al. 2005) as well as in rodent (Plesner et al. 2005) models of islet engraftment. XIAP-transduced human islets were significantly less apoptotic in an in vitro system that mimics hypoxia-induced injury. In addition, transplanting a series of marginal mass islet graft transplants in streptozotocin-induced diabetic NOD-RAG$^{-/-}$ mice resulted in 89 % of the animals becoming normoglycemic, with only 600 XIAP-transduced human islets (Emamaullee et al. 2005b). Moreover, XIAP overexpression has been shown to prevent the diabetogenicity of the immunosuppressive drugs tacrolimus and sirolimus in vitro (Hui et al. 2005).

Which Are the Best Islets? Does Size Matter?

In islet allo- as well as autotransplantation, it is still a matter of debate to define the features of an ideal islet able to ensure proper long-lasting glucose homeostasis after transplantation into the liver. The central question is whether bigger islets are better suited than smaller islets.

In the early phase after transplantation, the islets are supplied with oxygen and nutrients only by diffusion. In addition, data obtained from rat islet transplantations have shown that being in the portal vein, islets encounter a hypoxic state with an oxygen tension of 5 mmHg compared to 40 mmHg in the pancreas (Carlsson et al. 2001). In a study determining whether the size of the islets could influence the success rates of islet transplantations in rats, the small islets ($<$125 μm) were shown to be superior compared to their larger counterparts ($>$150 μm). The superiority of small islets was shown in vitro, via functional assays, as well as in vivo after transplanting them under the kidney capsule of diabetic rats. Using only marginal islet equivalencies for the renal subcapsular transplantation, large

islets failed to produce euglycemia in any recipient rat, whereas small islets were successful in 80 % of the cases (MacGregor et al. 2006). A recent study analyzed the influence of islet size on insulin production in human islet transplantation. The results convincingly showed that small islets are superior to large islets with regard to in vitro insulin secretion and higher survival rates (Lehmann et al. 2007). Therefore, islet size seems to be of importance for the success of human islet transplantation, and at least regarding islets, it might be stated: "Small is beautiful!"

The question that remains to be answered is how to improve the transplantation outcome, when using large islets. Besides applying measures that promote islet engraftment, such as the addition of the iron chelator deferoxamine which increases vascular endothelial growth factor expression (Langlois et al. 2008), an alternative would be to customize large islets into small "pseudoislets" using the hanging drop technique (Cavallari et al. 2007).

The Role of the Surrounding Tissue: Site Matters

To what extent is the surrounding tissue necessary or beneficial for islet function?

Besides the long-lasting functionality of autologous transplanted islets, there are at least two additional findings in islet autotransplantation that merit attention: the relatively low amounts of islets needed to achieve normoglycemia and the impurity of transplanted islets.

In islet allotransplantation, about 850,000 islets, normally obtained from two to four pancreases, are needed to achieve insulin independence in a single type 1 diabetes patient. As a consequence, the available pool of pancreata for islet allotransplantation is limited and is therefore one of the foremost problems in islet transplantation. Interestingly islet autotransplantation has shown us that even low amounts of islets may result in long-lasting insulin independence (Robertson et al. 2001; Pyzdrowski et al. 1992).

Due to extensive fibrosis, which is often present in pancreata of pancreatitis patients, the digestion process is incomplete. Theoretically, such an incomplete digestion might result in lower success rates after islet transplantation. Surprisingly, in a recent study, 8 of 12 patients who showed insulin independence after islet autotransplantation had less than 40 % islet cleavage (Webb et al. 2008). Therefore, a protective role of the tissue surrounding the islets might be postulated. Besides postulating such a protective role of the surrounding tissue, one could speculate that the digestion process may also lead to the loss of the basement membrane surrounding the islets (Rosenberg et al. 1999) which might be detrimental as it is a well-recognized fact that the extracellular matrix provides the islets with biotrophic support (Ilieva et al. 1999; Pinkse et al. 2006; Rosenberg et al. 1999).

Besides the innate surrounding tissue of the islets, the ectopic site into which the islets are implanted also seems to exert an influence on their biology: while autoislet β-cell biology can be normal (as shown by fasting glucose and hemoglobin A1c levels and intravenous glucose disappearance rates) for up to 13 years

(Robertson et al. 2001), there seem to be abnormalities in α-cell responsiveness to insulin-induced hypoglycemia.

Although responses from intrahepatically autotransplanted islets to intravenous arginine were shown to be present, their responsiveness to insulin-induced hypoglycemia was absent (Kendall et al. 1997). Similar observations were also made in islet allotransplantation: two normoglycemic type 1 diabetic patients who had been successfully transplanted with alloislets into the liver also failed to secrete glucagon during hypoglycemia (Kendall et al. 1997). These findings led to a study comparing the α-cell function between autoislets transplanted either in the liver or in the peritoneal cavity of dogs. As expected from the situation in humans, the animals that received their islets transplanted into the liver did not have a glucagon response during hypoglycemic clamps. Interestingly, in the animals that received their autoislets transplanted into the peritoneal cavity, the glucagon response was present. Both groups showed similar responses to intravenous arginine (Gupta et al. 1997).

In a recent study, autologous pancreatic islets were successfully transplanted into a human bone marrow. In all four patients islet function was sustained up to the maximum follow-up of 944 days (Maffi et al. 2013).

Taking everything together it could be said, "site matters," and there is certainly not just one site which deserves a closer "sight-seeing."

Conclusion

The technical feasibility of islet autotransplantation has been demonstrated by several centers (Clayton et al. 2003; Gruessner et al. 2004; Rodriguez Rilo et al. 2003). In spite of the problems that autologous transplanted islets encounter in their new surrounding, pancreatic islet autotransplantation has prevented the onset of diabetes in pancreatectomized patients for more than two decades (Robertson 2004). Therefore, the biological proof of principle, for a long-lasting stable glucose control by islets transplanted into the liver, has already been established. This success is equally surprising as well as inspiring for the more difficult task of islet allotransplantation. Understanding how autotransplanted islets can sustain their homeostasis and function in the liver, even for decades, might help us to find answers for still open questions regarding the molecular and cellular basis necessary for a successful islet allotransplantation.

Islet autotransplantation can abrogate the onset of diabetes and may therefore be considered as a valuable addition to surgical resection of the pancreas. The results obtained after islet autotransplantation have definitively provided a significant proof of principle: islets are able to regulate glucose homeostasis over decades when transplanted into the liver.

In times like these, when the enthusiasm regarding clinical islet allotransplantation has been dampened by the inadequate long-term results, such a proof of principle is a vital beacon reminding us of the ultimate goal and prospects of islet transplantation.

Cross-References

- ▶ Advances in Clinical Islet Isolation
- ▶ Approaches for Imaging Islets: Recent Advances and Future Prospects
- ▶ Islet Isolation from Pancreatitis Pancreas for Islet Autotransplantation

References

Ahmad SA, Lowy AM, Wray CJ, D'Alessio D, Choe KA, James LE, Gelrud A, Matthews JB, Rilo HL (2005) Factors associated with insulin and narcotic independence after islet autotransplantation in patients with severe chronic pancreatitis. J Am Coll Surg 201:680–687

Ahmed SA, Wray C, Rilo HL, Choe KA, Gelrud A, Howington JA, Lowy AM, Matthews JB (2006) Chronic pancreatitis: recent advances and ongoing challenges. Curr Probl Surg 43:127–238

Balamurugan AN, Loganathan G, Bellin MD, Wilhelm JJ, Harmon J, Anazawa T, Soltani SM, Radosevich DM, Yuasa T, Tiwari M, Papas KK, McCarthy R, Sutherland DE, Hering BJ (2012) A new enzyme mixture to increase the yield and transplant rate of autologous and allogeneic human islet products 2. Transplantation 93:693–702

Bellin MD, Carlson AM, Kobayashi T, Gruessner AC, Hering BJ, Moran A, Sutherland DE (2008) Outcome after pancreatectomy and islet autotransplantation in a pediatric population. J Pediatr Gastroenterol Nutr 47:37–44

Bellin MD, Sutherland DE, Beilman GJ, Hong-McAtee I, Balamurugan AN, Hering BJ, Moran A (2011) Similar islet function in islet allotransplant and autotransplant recipients, despite lower islet mass in autotransplants 5. Transplantation 91:367–372

Berney T, Mathe Z, Bucher P, Demuylder-Mischler S, Andres A, Bosco D, Oberholzer J, Majno P, Philippe J, Buhler L, Morel P (2004) Islet autotransplantation for the prevention of surgical diabetes after extended pancreatectomy for the resection of benign tumors of the pancreas. Transplant Proc 36:1123–1124

Biarnes M, Montolio M, Nacher V, Raurell M, Soler J, Montanya E (2002) β-cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. Diabetes 51:66–72

Blondet JJ, Carlson AM, Kobayashi T, Jie T, Bellin M, Hering BJ, Freeman ML, Beilman GJ, Sutherland DE (2007) The role of total pancreatectomy and islet autotransplantation for chronic pancreatitis. Surg Clin North Am 87:1477–1501, x

Bretzel RG, Jahr H, Eckhard M, Martin I, Winter D, Brendel MD (2007) Islet cell transplantation today. Langenbecks Arch Surg 392:239–253

Carlsson PO, Palm F, Andersson A, Liss P (2001) Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. Diabetes 50:489–495

Cavallari G, Zuellig RA, Lehmann R, Weber M, Moritz W (2007) Rat pancreatic islet size standardization by the "hanging drop" technique. Transplant Proc 39:2018–2020

Clayton HA, Davies JE, Pollard CA, White SA, Musto PP, Dennison AR (2003) Pancreatectomy with islet autotransplantation for the treatment of severe chronic pancreatitis: the first 40 patients at the leicester general hospital. Transplantation 76:92–98

Contreras JL, Eckstein C, Smyth CA, Sellers MT, Vilatoba M, Bilbao G, Rahemtulla FG, Young CJ, Thompson JA, Chaudry IH, Eckhoff DE (2003) Brain death significantly reduces isolated pancreatic islet yields and functionality in vitro and in vivo after transplantation in rats. Diabetes 52:2935–2942

Egidi MF, Lin A, Bratton CF, Baliga PK (2008) Prevention and management of hyperglycemia after pancreas transplantation. Curr Opin Organ Transplant 13:72–78

Emamaullee JA, Shapiro AM (2007) Factors influencing the loss of β-cell mass in islet transplantation. Cell Transplant 16:1–8

Emamaullee J, Liston P, Korneluk RG, Shapiro AM, Elliott JF (2005a) XIAP overexpression in islet β-cells enhances engraftment and minimizes hypoxia-reperfusion injury. Am J Transplant 5:1297–1305

Emamaullee JA, Rajotte RV, Liston P, Korneluk RG, Lakey JR, Shapiro AM, Elliott JF (2005b) XIAP overexpression in human islets prevents early posttransplant apoptosis and reduces the islet mass needed to treat diabetes. Diabetes 54:2541–2548

Evans KA, Clark CW, Vogel SB, Behrns KE (2008) Surgical management of failed endoscopic treatment of pancreatic disease. J Gastrointest Surg 12:1924–1929

Gruessner RW, Sutherland DE, Dunn DL, Najarian JS, Jie T, Hering BJ, Gruessner AC (2004) Transplant options for patients undergoing total pancreatectomy for chronic pancreatitis. J Am Coll Surg 198:559–567

Guignard AP, Oberholzer J, Benhamou PY, Touzet S, Bucher P, Penfornis A, Bayle F, Kessler L, Thivolet C, Badet L, Morel P, Colin C (2004) Cost analysis of human islet transplantation for the treatment of type 1 diabetes in the Swiss-French Consortium GRAGIL. Diabetes Care 27:895–900

Gupta V, Wahoff DC, Rooney DP, Poitout V, Sutherland DE, Kendall DM, Robertson RP (1997) The defective glucagon response from transplanted intrahepatic pancreatic islets during hypoglycemia is transplantation site-determined. Diabetes 46:28–33

Hengster P, Hermann M, Pirkebner D, Draxl A, Margreiter R (2005) Islet isolation and GMP, ISO 9001:2000: what do we need – a 3-year experience. Transplant Proc 37:3407–3408

Hering BJ, Matsumoto I, Sawada T, Nakano M, Sakai T, Kandaswamy R, Sutherland DE (2002) Impact of two-layer pancreas preservation on islet isolation and transplantation. Transplantation 74:1813–1816

Hermann M, Pirkebner D, Draxl A, Margreiter R, Hengster P (2005) "Real-time" assessment of human islet preparations with confocal live cell imaging. Transplant Proc 37:3409–3411

Hermann M, Margreiter R, Hengster P (2007) Molecular and cellular key players in human islet transplantation. J Cell Mol Med 11:398–415

Hui H, Khoury N, Zhao X, Balkir L, D'Amico E, Bullotta A, Nguyen ED, Gambotto A, Perfetti R (2005) Adenovirus-mediated XIAP gene transfer reverses the negative effects of immunosuppressive drugs on insulin secretion and cell viability of isolated human islets. Diabetes 54:424–433

Huurman VA, Hilbrands R, Pinkse GG, Gillard P, Duinkerken G, van de Linde P, van der Meer-Prins PM, Versteeg-van der Voort Maarschalk MF, Verbeeck K, Alizadeh BZ, Mathieu C, Gorus FK, Roelen DL, Claas FH, Keymeulen B, Pipeleers DG, Roep BO (2008) Cellular islet autoimmunity associates with clinical outcome of islet cell transplantation. PLoS ONE 3:e2435

Ichii H, Sakuma Y, Pileggi A, Fraker C, Alvarez A, Montelongo J, Szust J, Khan A, Inverardi L, Naziruddin B, Levy MF, Klintmalm GB, Goss JA, Alejandro R, Ricordi C (2007) Shipment of human islets for transplantation. Am J Transplant 7:1010–1020

Ilieva A, Yuan S, Wang RN, Agapitos D, Hill DJ, Rosenberg L (1999) Pancreatic islet cell survival following islet isolation: the role of cellular interactions in the pancreas. J Endocrinol 161:357–364

Iwanaga Y, Sutherland DE, Harmon JV, Papas KK (2008) Pancreas preservation for pancreas and islet transplantation. Curr Opin Organ Transplant 13:445–451

Jindal RM, Ricordi C, Shriver CD (2010) Autologous pancreatic islet transplantation for severe trauma. N Engl J Med 362:1550

Kendall DM, Teuscher AU, Robertson RP (1997) Defective glucagon secretion during sustained hypoglycemia following successful islet allo- and autotransplantation in humans. Diabetes 46:23–27

Khan A, Jindal RM, Shriver C, Guy SR, Vertrees AE, Wang X, Xu X, Szust J, Ricordi C (2012) Remote processing of pancreas can restore normal glucose homeostasis in autologous islet transplantation after traumatic whipple pancreatectomy: technical considerations. Cell Transplant 21:1261–1267

Langlois A, Bietiger W, Mandes K, Maillard E, Belcourt A, Pinget M, Kessler L, Sigrist S (2008) Overexpression of vascular endothelial growth factor in vitro using deferoxamine: a new drug to increase islet vascularization during transplantation. Transplant Proc 40:473–476

Lehmann R, Zuellig RA, Kugelmeier P, Baenninger PB, Moritz W, Perren A, Clavien PA, Weber M, Spinas GA (2007) Superiority of small islets in human islet transplantation. Diabetes 56:594–603

MacGregor RR, Williams SJ, Tong PY, Kover K, Moore WV, Stehno-Bittel L (2006) Small rat islets are superior to large islets in in vitro function and in transplantation outcomes. Am J Physiol Endocrinol Metab 290:E771–E779

Maffi P, Balzano G, Ponzoni M, Nano R, Sordi V, Melzi R, Mercalli A, Scavini M, Esposito A, Peccatori J, Cantarelli E, Messina C, Bernardi M, Del Maschio A, Staudacher C, Doglioni C, Ciceri F, Secchi A, Piemonti L (2013) Autologous pancreatic islet transplantation in human bone marrow 1. Diabetes 62(10):3523–3531

McLoughlin MT, Mitchell RM (2007) Sphincter of Oddi dysfunction and pancreatitis. World J Gastroenterol 13:6333–6343

Merani S, Schur C, Truong W, Knutzen VK, Lakey JR, Anderson CC, Ricordi C, Shapiro AM (2006) Compaction of islets is detrimental to transplant outcome in mice. Transplantation 82:1472–1476

Monti P, Scirpoli M, Maffi P, Ghidoli N, De TF, Bertuzzi F, Piemonti L, Falcone M, Secchi A, Bonifacio E (2008) Islet transplantation in patients with autoimmune diabetes induces homeostatic cytokines that expand autoreactive memory T cells. J Clin Invest 118:1806–1814

Naruse S, Fujiki K, Ishiguro H (2007) Is genetic analysis helpful for diagnosing chronic pancreatitis in its early stage? J Gastroenterol 42(Suppl 17):60–65

Nir T, Melton DA, Dor Y (2007) Recovery from diabetes in mice by β cell regeneration. J Clin Invest 117:2553–2561

Pinkse GG, Bouwman WP, Jiawan-Lalai R, Terpstra OT, Bruijn JA, de Heer E (2006) Integrin signaling via RGD peptides and anti-β1 antibodies confers resistance to apoptosis in islets of Langerhans. Diabetes 55:312–317

Plesner A, Liston P, Tan R, Korneluk RG, Verchere CB (2005) The X-linked inhibitor of apoptosis protein enhances survival of murine islet allografts. Diabetes 54:2533–2540

Pyzdrowski KL, Kendall DM, Halter JB, Nakhleh RE, Sutherland DE, Robertson RP (1992) Preserved insulin secretion and insulin independence in recipients of islet autografts. N Engl J Med 327:220–226

Rabkin JM, Leone JP, Sutherland DE, Ahman A, Reed M, Papalois BE, Wahoff DC (1997) Transcontinental shipping of pancreatic islets for autotransplantation after total pancreatectomy. Pancreas 15:416–419

Rabkin JM, Olyaei AJ, Orloff SL, Geisler SM, Wahoff DC, Hering BJ, Sutherland DE (1999) Distant processing of pancreas islets for autotransplantation following total pancreatectomy. Am J Surg 177:423–427

Riediger H, Adam U, Fischer E, Keck T, Pfeffer F, Hopt UT, Makowiec F (2007) Long-term outcome after resection for chronic pancreatitis in 224 patients. J Gastrointest Surg 11:949–959

Robertson RP (2004) Islet transplantation as a treatment for diabetes – a work in progress. N Engl J Med 350:694–705

Robertson RP, Sutherland DE, Lanz KJ (1999) Normoglycemia and preserved insulin secretory reserve in diabetic patients 10–18 years after pancreas transplantation. Diabetes 48:1737–1740

Robertson RP, Lanz KJ, Sutherland DE, Kendall DM (2001) Prevention of diabetes for up to 13 years by autoislet transplantation after pancreatectomy for chronic pancreatitis. Diabetes 50:47–50

Rodriguez Rilo HL, Ahmad SA, D'Alessio D, Iwanaga Y, Kim J, Choe KA, Moulton JS, Martin J, Pennington LJ, Soldano DA, Biliter J, Martin SP, Ulrich CD, Somogyi L, Welge J, Matthews JB, Lowy AM (2003) Total pancreatectomy and autologous islet cell transplantation as a means to treat severe chronic pancreatitis. J Gastrointest Surg 7:978–989

Rosenberg L, Wang R, Paraskevas S, Maysinger D (1999) Structural and functional changes resulting from islet isolation lead to islet cell death. Surgery 126:393–398

Ruggenenti P, Remuzzi A, Remuzzi G (2008) Decision time for pancreatic islet-cell transplantation. Lancet 371:883–884

Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, Shapiro AM (2005) Five-year follow-up after clinical islet transplantation. Diabetes 54:2060–2069

Sakata N, Goto M, Gumpei Y, Mizuma M, Motoi F, Satomi S, Unno M (2012) Intraoperative ultrasound examination is useful for monitoring transplanted islets: a case report 1. Islets 4:339–342

Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, Cagliero E, Alejandro R, Ryan EA, DiMercurio B, Morel P, Polonsky KS, Reems JA, Bretzel RG, Bertuzzi F, Froud T, Kandaswamy R, Sutherland DE, Eisenbarth G, Segal M, Preiksaitis J, Korbutt GS, Barton FB, Viviano L, Seyfert-Margolis V, Bluestone J, Lakey JR (2006) International trial of the Edmonton protocol for islet transplantation. N Engl J Med 355:1318–1330

Slezak LA, Andersen DK (2001) Pancreatic resection: effects on glucose metabolism. World J Surg 25:452–460

Steer ML, Waxman I, Freedman S (1995) Chronic pancreatitis. N Engl J Med 332:1482–1490

Sutherland DE, Matas AJ, Najarian JS (1978) Pancreatic islet cell transplantation. Surg Clin North Am 58:365–382

Sutherland DE, Gruessner AC, Carlson AM, Blondet JJ, Balamurugan AN, Reigstad KF, Beilman GJ, Bellin MD, Hering BJ (2008) Islet autotransplant outcomes after total pancreatectomy: a contrast to islet allograft outcomes. Transplantation 86:1799–1802

Sutherland DE, Radosevich DM, Bellin MD, Hering BJ, Beilman GJ, Dunn TB, Chinnakotla S, Vickers SM, Bland B, Balamurugan AN, Freeman ML, Pruett TL (2012) Total pancreatectomy and islet autotransplantation for chronic pancreatitis 1. J Am Coll Surg 214:409–424

Wahoff DC, Papalois BE, Najarian JS, Kendall DM, Farney AC, Leone JP, Jessurun J, Dunn DL, Robertson RP, Sutherland DE (1995) Autologous islet transplantation to prevent diabetes after pancreatic resection. Ann Surg 222:562–575

Webb MA, Illouz SC, Pollard CA, Musto PP, Berry D, Dennison AR (2006) Long-term maintenance of graft function after islet autotransplantation of less than 1000 IEQ/kg. Pancreas 33:433–434

Webb MA, Illouz SC, Pollard CA, Gregory R, Mayberry JF, Tordoff SG, Bone M, Cordle CJ, Berry DP, Nicholson ML, Musto PP, Dennison AR (2008) Islet auto transplantation following total pancreatectomy: a long-term assessment of graft function. Pancreas 37:282–287

White SA, Robertson GS, London NJ, Dennison AR (2000) Human islet autotransplantation to prevent diabetes after pancreas resection. Dig Surg 17:439–450

Wurm M, Lubei V, Caronna M, Hermann M, Margreiter R, Hengster P (2007) Development of a novel perfused rotary cell culture system. Tissue Eng 13:2761–2768

Yang Z, Chen M, Deng S, Ellett JD, Wu R, Langman L, Carter JD, Fialkow LB, Markmann J, Nadler JL, Brayman K (2004) Assessment of human pancreatic islets after long distance transportation. Transplant Proc 36:1532–1533

Successes and Disappointments with Clinical Islet Transplantation

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_23,

Abstract

Islet transplantation is considered a therapeutic option for patients with type 1 diabetes who have life-threatening hypoglycemic episodes. After the procedure, the frequency and severity of hypoglycemic episodes generally decrease and the majority of patients have sustained graft function as indicated by detectable levels of C-peptide. However, true insulin independence is seldom achieved and generally not long-lasting. Apart from the low insulin-independence rates, reasons for concern regarding this procedure are the side effects of the immunosuppressive therapy, alloimmunization, and the high costs. Moreover, whether islet transplantation prevents the progression of diabetic micro- and macrovascular complications more effectively than standard insulin therapy is largely unknown. Areas of current research include the development of less toxic immunosuppressive regimens, the control of the inflammatory reaction immediately after transplantation, the identification of the optimal anatomical site for islet infusion, and the possibility to encapsulate transplanted islets to protect them from the alloimmune response. Nowadays, islet transplantation is still an experimental procedure, which is only indicated for a highly selected group of type 1 diabetic patients with life-threatening hypoglycemic episodes.

Keywords

Islet transplantation • Type 1 diabetes • Immunosuppression • Diabetic complications

Introduction

Type 1 diabetes is the clinical consequence of immune-mediated destruction of insulin-producing pancreatic β cells. Since from its first description, the main goal of treatment has been the identification of strategies to replace insulin deficiency by exogenous insulin and, later on, pancreas transplantation. More recently, islet transplantation has been proposed as an alternative, supposedly safer way to replace β-cell function compared to whole pancreas transplantation. However, despite early excitement and optimism, results have been unsatisfactory for many years.

In 2000, research in the field of pancreatic islet cell transplantation was boosted by a key paper reporting insulin independence in seven out of seven patients with type 1 diabetes mellitus over a median follow-up of 12 months (Shapiro et al. 2000). The two major novelties of this protocol were the administration of pancreatic islet doses higher than those previously used and a steroid-free immunosuppression. Until then, clinical outcomes had been disappointing. Of the 267 islet preparations transplanted since 1990, less than 10 % had resulted in insulin independence for more than 1 year (Brendel et al. 1999). With the new protocol, success rates have increased in parallel with significant improvements in the technical procedure and medical management of islet transplantation. However, true insulin-independence rates for a prolonged period of time are still very low, and patients are required to take immunosuppressive medication as long as there is evidence of residual graft

function. Moreover, islet transplantation remains a highly complex procedure that typically requires the use of at least two donor pancreases and may compete with the number of organs available for whole organ transplantation. Thus, islet transplantation is still far from representing an effective and widely available cure for type 1 diabetes. This review describes the successes and disappointments of clinical islet transplantation programs.

The Burden of Type 1 Diabetes Mellitus

Type 1 diabetes is the most common metabolic disease in childhood with incidence rates ranging from 8 to >50 per 100,000 population per year in Western countries (Daneman 2009). For children aged 0–14 years, the prevalence of type 1 diabetes is estimated to be at least 1 million worldwide by the year 2025 (Green 2008). Onkamo et al. reported that the global incidence of type 1 diabetes is increasing by 3 % per year (Onkamo et al. 1999) and the Project Group estimated a global annual increase in incidence of 2.8 %, from 1990 to 1999 (DIAMOND Project Group 2006).

One of the most accredited theories to explain the increase in type 1 diabetes incidence is the hygiene hypothesis (Strachan 1989), suggesting that exposure to a variety of infectious agents during early childhood might protect against autoimmune diseases, including type 1 diabetes (Kolb and Elliott 1994). Consistently, the constant increase in the incidence of type 1 diabetes reported by a variety of epidemiological studies (Gale 2005; Soltesz et al. 2007) has been paralleled by a gradual decrease in infectious diseases, such as tuberculosis, mumps, measles, hepatitis A, and enteroviruses (Coppieters et al. 2012). Other factors, however, are probably involved in the increased type 1 diabetes incidence (reviewed in Egro (2013))

Children with type 1 diabetes usually present with a several day history of symptoms such as frequent urination, excessive thirst, and weight loss, which appear when about 80 % of the pancreatic β cells have already been lost. If those symptoms are misinterpreted, progressive insulin deficiency leads to a potentially life-threatening condition in the form of diabetic ketoacidosis. Patients with type 1 diabetes require daily subcutaneous injections of insulin in an effort to mimic the physiological release of insulin during meals and during fasting periods. Optimal glycemic control is crucial to reduce the incidence and slow the progression of microvascular and macrovascular complications (The Diabetes Control and Complications Trial Research Group 1993; Nathan et al. 2005).

Pathophysiology of Type 1 Diabetes Mellitus

Most people with type 1 diabetes do not have a family history of the disease; nonetheless, there is clearly a genetic predisposition for developing β-cell autoimmunity (Harjutsalo et al. 2006; Rich 1990; Tuomilehto et al. 1995) with first-degree relatives having a lifetime risk of developing type 1 diabetes of 6 % versus 0.4 % in

the general population. In addition, there is a strong, approximately 50 %, concordance in identical twins for the development of type 1 diabetes (Kyvik et al. 1995; Redondo et al. 1999). Human leukocyte antigen (HLA)-related immunogenotype accounts for approximately 60 % of the genetic influence in type 1 diabetes. In individuals who are genetically at risk, an environmental trigger is thought to initiate an immune response targeting the insulin-secreting pancreatic islet β cells (Bluestone et al. 2010; Pugliese 2013). The initial immune response may initiate secondary and tertiary immune responses that contribute to further impair β-cell function and to their destruction (Peakman 2013).

The initial manifestation of β-cell injury is the appearance of diabetes-related autoantibodies. Whether they exert a direct pathogenic role or they are just markers of such injury is still debated. Regardless, with progressive impairment of β-cell function, metabolic abnormalities become measurable, initially either as loss of early insulin response to intravenous glucose (Chase et al. 2001) or as reduced β-cell sensitivity to glucose resulting in decreased insulin secretion (Ferrannini et al. 2010) and eventually as impaired glucose tolerance and hyperglycemia (Sosenko et al. 2009).

At the point of development of overt diabetes, there is still evidence of persistent β-cell function shown through measurement of C-peptide. After diagnosis of type 1 diabetes, however, there is a progressive decline in C-peptide as β-cell function decays (Greenbaum et al. 2012). Nonetheless, even many years after diagnosis, some patients with type 1 diabetes may have low detectable levels of C-peptide (Keenan et al. 2010).

In a prospective study, Ziegler et al. (2013) showed that among 585 children who developed two or more diabetes-related autoantibodies, nearly 70 % (280 of 401 available for follow-up) had developed type 1 diabetes within 10 years, and 84 % (299 of 355 available for follow-up) had developed type 1 diabetes within 15 years. Because the participants were recruited from both the general population and offspring of parents with type 1 diabetes, the similar findings take on added significance, suggesting that the same sequence of events occurs in individuals with so-called sporadic type 1 diabetes and in relatives of individuals with type 1 diabetes.

These data offer new opportunities for type 1 diabetes prevention in patients with autoantibodies. Importantly, evidence has been generated that β cells can regenerate, suggesting that even secondary prevention of type 1 diabetes could be possible. Importantly, evidence has been provided that mature β cells can proliferate not only in the presence of euglycemia (Dor et al. 2004) but also in the diabetic "milieu." Using genetically modified mice made diabetic by inducing 80 % β-cell ablation via endogenous diphtheria toxin production, Nir et al. (2007) showed that β-cell mass and glucose homeostasis can be fully restored in a few weeks after exhaustion of toxin production. Finding that this was accompanied by a dramatically increased proliferation rate even in face of severe hyperglycemia challenged the common belief that glucotoxicity is a major impediment to β-cell survival. Thus, provided the autoimmune process is inhibited, the possibility to induce surviving cells to proliferate and replenish the β-cell compartment might allow

restoring normal glucose homeostasis and achieving freedom from exogenous insulin dependency for millions of type 1 diabetics worldwide.

Standard Management of Patients with Type 1 Diabetes

Glycemic control is the keystone of treatment for type 1 diabetes, which requires a meticulous balance of insulin replacement with diet and exercise. In 1993, the Diabetes Control and Complications Trial (DCCT) showed that a system of intensive diabetes management aimed at near-normal glycemic control dramatically reduces the risk of microvascular complications and favorably affects the risk of macrovascular complications compared to a less strict control approach (The Diabetes Control and Complications Trial Research Group 1993; Nathan et al. 2005; Lachin et al. 2008). Over a median follow-up period of 22 years, extension studies of the DCCT trial showed that intensive diabetes therapy significantly prevented reduction in glomerular filtration rate versus conventional diabetes therapy (de Boer et al. 2011). Unfortunately, the treatment regimens used by subjects randomized to the intensive treatment arm of the DCCT also significantly increased their risk of severe hypoglycemia and led to more weight gain (The Diabetes Control and Complications Trial Research Group 1993). Since the publication of the DCCT results, a variety of insulin analogues, better and more sophisticated insulin pumps, and faster and more accurate glucose meters have become widely used in the treatment of type 1 diabetes, making the prospects for patients with type 1 diabetes far better than they were in the past (Nordwall et al. 2004; Nathan et al. 2009).

In most type 1 diabetes patients, however, the goal of near normalization of glycated hemoglobin (HbA1c, a parameter of glucose control over the last 3 months) remains elusive. Several large, multicenter studies demonstrated a persistent gap between attained and target HbA1c levels. Successful implementation of intensive diabetes management in routine clinical practice continues to be a major challenge. The unremitting daily task of controlling blood glucose while avoiding hypoglycemia is arduous and often frustrating. A meta-analysis show that the use of insulin analogues and pump therapy, when compared with conventional insulins and injection-based regimens, respectively, has had only a modest impact on glycemic control and rates of adverse events (Yeh et al. 2012).

Glycemic control is particularly challenging in adolescent patients. In the DCCT the mean HbA1c for adolescents as compared to adults was 1–2 % higher in both the intensive and conventionally treated arms. Despite this, rates of hypoglycemia were higher in adolescents than in adults (Pescovitz et al. 2009). Studies published after DCCT have shown that mean levels of HbA1c have remained higher than current glycemic goals (Danne et al. 2001). Management of type 1 diabetes requires many life-long daily tasks that the child and/or family must perform to maintain a relatively healthy metabolism and glycemic control. Although in younger children these tasks are performed primarily by the care givers, in the teenage years the burden of diabetes management falls on the adolescents themselves. These patients

more than others also require considerable psychosocial support, ongoing education, and guidance from cohesive diabetes team working with each patient to set and achieve individualized treatment goals.

Who May Benefit from Islet Transplantation?

Frequent and severe hypoglycemic events are most common indications for islet transplantation alone. Patients with so-called "brittle" diabetes may have an improvement in quality of life or may even be saved from fatal hypoglycemia when provided with functionally active β cells (Ryan et al. 2006). In addition, islet transplantation may be considered in patients with severe clinical and emotional problems with exogenous insulin therapy (Robertson et al. 2006).

The Edmonton group has proposed two scores to quantify the severity of labile diabetes. The HYPO score quantifies the extent of the problem of hypoglycemia by assigning scores to capillary glucose readings from a four-week observation period in combination with a score for self-reported hypoglycemic episodes in the previous year. The lability index (LI) quantifies the extent of glucose excursions over time and is calculated using the formula as described by this group (Ryan et al. 2004a).

The American Diabetes Association acknowledges the potential advantages of islet transplantation over whole pancreas transplantation in terms of morbidity and mortality associated with the operative procedure. However, they clearly state that islet transplantation is still an experimental procedure, only to be performed in the setting of controlled research studies. As for patients who will also be receiving a kidney transplantation, simultaneous pancreas transplantation is the treatment of choice, because it may improve kidney survival and will provide insulin independence in the majority of patients (Robertson et al. 2006). Islet after kidney transplantation is restricted to selected patients with end-stage renal disease affected by type 1 diabetes who underwent kidney transplantation alone or who rejected the pancreas after simultaneous pancreas-kidney transplantation.

Islet Transplantation: A Historical Perspective

The first evidence that islet transplantation might be considered a cure for type 1 diabetes emerged in 1972, when experiments in rodents showed that artificially induced diabetes mellitus could be reversed by transplanted pancreatic islets (Ballinger and Lacy 1972). In 1977, Paul Lacy discussed the feasibility of islet transplantation to treat type 1 diabetes in humans (Lacy 1978), which was subsequently attempted in patients. Success rates, however, were generally low, with less than 10 % of patients being insulin independent at 1 year after transplantation (Sutherland 1981). More encouraging results were obtained in patients who had already had a kidney transplant, with higher rates of insulin independency and graft function as defined by C-peptide secretion (Secchi et al. 1997; Benhamou

et al. 2001). In 2000, a report was published describing seven type 1 diabetes patients with a history of severe hypoglycemia and poor metabolic control who underwent islet transplantation alone using a modified, steroid-free immunosuppressive protocol. In addition, each patient received at least two different islet transplantations; thus, the total transplanted islet mass per patient was remarkably higher than in previous series. Over a median follow-up of 11.9 months (range 4.4–14.9), all patients were insulin-independent (Shapiro et al. 2000). The so-called Edmonton protocol was subsequently adopted and modified by many centers. Results of a large multicenter trial using the Edmonton protocol were published in 2006 (Shapiro et al. 2006). Seventy percent of patients had an improved glycemic control after 2 years, but the insulin-independence rate was disappointingly low (14 %).

Clinical Outcomes of Islet Transplantation

Insulin Independence and Improved Glycemic Control

Many centers have published results obtained in their islet transplant programs (Hirshberg et al. 2003; Frank et al. 2004; Goss et al. 2004; Hering et al. 2004, 2005; Froud et al. 2005; Hafiz et al. 2005; Warnock et al. 2005; Keymeulen et al. 2006; O'Connell et al. 2006; Maffi et al. 2007; Gangemi et al. 2008; Gillard et al. 2008). Here, we present some of the largest reports from diverse geographic regions.

In 2005, single-center outcomes of 65 islet transplant recipients treated according to the Edmonton protocol were reported, showing that 44 (68 %) patients had become insulin independent, with a median duration of insulin independency of 15 months (IQR 6.2–25.5). Five of these subjects received only a single islet infusion, 33 received two infusions, and six received three infusions. Insulin independency after 5 years was 10 %. Nonetheless, after 5 years, some residual graft function could be demonstrated in about 80 % of patients on the basis of detectable serum C-peptide levels. Diabetic lability and the occurrence of severe hypoglycemia were effectively diminished (Ryan et al. 2005).

Following the initial Edmonton results in 2000, a large international trial in nine centers in the United States and Europe was initiated by the Immune Tolerance Network to examine the feasibility and reproducibility of islet transplantation using the Edmonton protocol. The primary end point, defined as insulin independency with adequate glycemic control 1 year after the final transplantation, was met by 16 out of 36 subjects (44 %). Only five of these patients were still insulin independent after 2 years (14 %). Of note, the considerable differences in results obtained by the various participating sites emphasize the need for concentration of this procedure in highly experienced centers. Again, graft function as defined by detectable C-peptide levels and associated improvements in diabetic control was preserved in a higher percentage of patients (70 % after 2 years) (Shapiro et al. 2006).

The Groupe de Recherche Rhin Rhone Alpes Geneve pour la transplantation d'Ilots de Langerhans (GRAGIL) reported results obtained in 10 patients who received one or two islet infusions. Only three out of ten patients had prolonged insulin independence after 1 year of follow-up. However, five more transplantations were considered successful, since after 1 year recipients fulfilled the predefined criteria of success consisting of a basal C-peptide $\geq$ 0.5 ng/ml, HbA1c $\leq$ 6.5 %, disappearance of hypoglycemic events, and $\geq$ 30 % reduction of insulin needs (Badet et al. 2007).

A recent report from the Japanese Trial of Islet Transplantation showed that only 3 out of 18 recipients of islet transplantation achieved insulin independency and only for a period of 2 weeks to 6 months. Graft function was preserved in 63 % after 2 years. As in the other reports, HbA1c levels decreased and blood glucose levels stabilized, with disappearance of hypoglycemia unawareness. In this report, no information was provided about the amount of islet equivalents (IEQ; number of islets in a preparation adjusted for size of the islet, one IEQ equals a single islet of 150 μm in diameter) per kg body weight infused. Of note, in Japan all pancreata are obtained from non-heart-beating donors, since pancreata from brain-dead donors are usually allocated to whole pancreas or pancreas-kidney transplantation. In addition, the presence of brain death is frequently not examined because of cultural reasons, and invasive procedures are usually not allowed even in brain-dead donors before cardiac arrest occurs. This may lead to decreased viability of pancreatic tissue when compared with pancreata from brain-dead donors (Kenmochi et al. 2009).

The largest registry of islet transplant data is the Collaborative Islet Transplant Registry (CITR), which retrieves its data mainly from US and Canadian medical institutions and two European centers. In their 2008 update considering 279 recipients of an islet transplantation reported between 1999 and 2007, the registry reported 24 % insulin independence after 3 years. Graft function as defined by detectable C-peptide levels after 3 years was 23–26 %. The prevalence of hypoglycemic events decreased dramatically, and mean HbA1c levels substantially improved. Predictors of better islet graft function were higher number, size, and viability of infused islets; older age and lower HbA1c levels in the recipient, whether the processing center was affiliated with the transplantation center; and the use of daclizumab, etanercept, or calcineurin inhibitors in the immunosuppressive regimens. In-hospital administration of steroids was associated with a negative outcome (Alejandro et al. 2008; Collaborative Islet Transplantation 2010).

Recently, the CITR reported the outcome data of 627 islet transplants performed between 1999 and 2010. Insulin independence at 3 years after transplant improved from 27 % in patients transplanted between 1999 and 2002 to 44 % in patients who received a transplant between 2007 and 2010 (Fig. 1). This success, however, was probably more the result of a careful selection of recipients (lower serum creatinine and donor-specific antibody titer) than of a real improvement in the transplant procedure. Consistently, the number of islet transplant performed each year did decline during the last era (Barton et al. 2012).

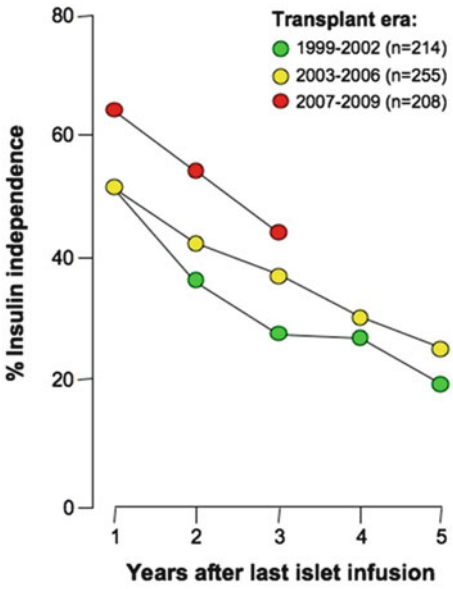

Fig. 1 Rate of insulin independence at different time points after islet infusion according to the transplant era (Data derived from Collaborative Islet Transplantation Registry (CITR) 2012, Barton et al. (2012))

Table 1 Clinical outcomes of whole pancreas transplantation versus islet transplantation

Insulin independence	Whole pancreas	Islets
At 1 year	77 %	47 %
Long term	58 % (5 years)	24 % (3 years)

Data were derived from the International Pancreas Transplant Registry (until June 2004, $n = 1{,}008$ pancreas transplantation alone) and from the Collaborative Islet Transplantation Registry (until January 2008, $n = 279$ islet transplantation alone) (Alejandro et al. 2008; Gruessner and Sutherland 2005)

Table 1 shows success rates for pancreatic islet transplantation compared with whole pancreas transplantation alone as reported by the Collaborative Islet Transplantation Registry and the International Pancreas Transplant Registry, respectively (Collaborative Islet Transplantation 2010; Gruessner and Sutherland 2005). Indications for pancreas transplantation alone are similar to those for islet transplantation. However, whole pancreas transplantation is more frequently performed simultaneously with kidney transplantation or after kidney transplantation in type 1 diabetic patients with end-stage renal disease. For simultaneous whole pancreas-kidney transplantation, favorable effects on micro- and possibly macrovascular diabetic complications have consistently been described (White et al. 2009). For pancreas-after-kidney and for pancreas transplantation alone, data are less consistent, and mild or no benefits or even worsening of patient survival after these procedure have been reported compared to insulin-treated patients (Venstrom et al. 2003; Gruessner et al. 2005).

Reduction of Hypoglycemic Episodes

Intrahepatic islet transplantation restores physiologic islet cell hormonal responses to insulin-induced hypoglycemia in type 1 diabetes patients whereby endogenous insulin secretion is appropriately suppressed and glucagon secretion is partially restored (Rickels et al. 2005a).

Studies using continuous glucose monitoring systems in islet transplant recipients have shown significant decreases to abolition of time spent in the hypoglycemic range (<60 mg/dL) (Paty et al. 2006). Indeed, in addition to normalizing the glycemic threshold for counter-regulatory glucagon secretion, islet transplant recipients have normalization of the glycemic thresholds for counter-regulatory epinephrine, autonomic symptom, and growth hormone (GH) responses (Fig. 2). A study using paired hyperinsulinemic, hypoglycemic, and euglycemic clamps with stable isotope tracers in islet transplant recipients has preliminarily shown that the recovery of intact islet cell and sympathoadrenal responses is associated with a restored endogenous (primarily hepatic) glucose production response that is ultimately required to protect patients from the development of low blood glucose (Rickels et al. 2011).

Even patients with graft failure after islet transplantation showed significantly fewer hypoglycemic episodes compared with pretransplant (Leitao et al. 2008). Whether this result is due to the reduction in exogenous insulin requirement or to the restoration of glucose counter-regulation is a matter of active investigation (Rickels et al. 2005b).

Long-Term Diabetic Complications

Until now, it has not been sufficiently established whether pancreatic islet transplantation can prevent diabetic complications or halt their progression (Lee et al. 2006; Fiorina et al. 2008).

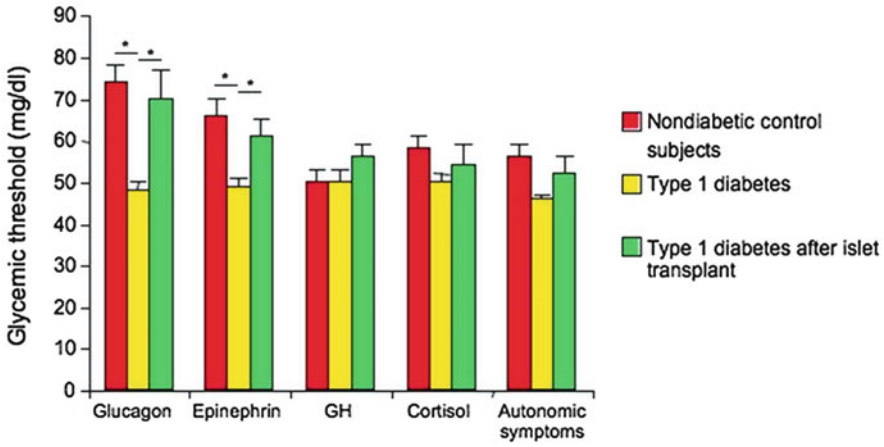

Fig. 2 Glycemic thresholds for counterregulatory responses in patients with type 1 diabetes, in islet transplant recipients, and in healthy controls. *$P < 0.05$ (Data from Rickels et al. 2007)

Cardiovascular Complications– In a retrospective study, cardiovascular function was compared between a group of 17 patients who received an islet-after-kidney transplantation and a group of 25 patients with previous kidney transplantation who were still on the waiting list for islet transplantation or who had experienced early islet graft failure. Baseline characteristics for both groups were similar. Islet transplantation was associated with an improvement in ejection fraction and left ventricular diastolic function compared to baseline. Moreover, arterial intima-media thickness was stable in the islet transplant group but worsened in the kidney-only group (Fiorina et al. 2005). Recently, data have been published showing that in a cohort of 15 consecutive islet transplant recipients who reached insulin independence carotid intima-media thickness did actually decrease after the procedure, suggesting that optimal glycemic control may lead to a regression of atherosclerotic lesions (Danielson et al. 2012).

Nephropathy– Increased kidney graft survival rates and stabilization of microalbuminuria have been reported in kidney transplant recipients with successful islet transplantation (fasting C-peptide levels of >0.5 ng/ml for >1 year) compared to kidney transplant patients with unsuccessful islet transplantation at 4 years after surgery (Fiorina et al. 2003). The effect of islet transplantation on kidney transplant survival compared with insulin therapy is still unknown.

An uncontrolled, observational study by the Edmonton group suggested an overall decline in estimated glomerular filtration rate during 4 years of follow-up after islet transplantation alone and an increase in albuminuria in a significant proportion of patients independently from insulin activity (Senior et al. 2007). Subsequently, Maffi et al. showed that even a mildly decreased renal function, before transplantations should be considered a contraindication for the currently used immunosuppressive regimen of sirolimus in combination with tacrolimus, since it was associated with progression to end-stage renal disease (Maffi et al. 2007). In patients with renal impairment, nephrotoxicity of immunosuppressive drugs like calcineurin and mTOR inhibitors might offset the benefits of improved metabolic control. Renal impairment progressively worsens even in those selected patients with type 1 diabetes who benefit of a 5-year normoglycemia period after a single pancreas transplantation, as a result of both immunosuppressive drug toxicity and, probably, the marginal effect of glycemia control on already damaged diabetic kidney (Fioretto et al. 1993, 1998). The same applies also to the rare subjects with prolonged normoglycemia after islet transplantation, which limits the indications for the procedure to those type 1 diabetes patients with normal renal function. In this cohort, aggressive treatment of risk factors for nephropathy, such as blood pressure and low-density lipoprotein cholesterol, together with careful tacrolimus level monitoring have been associated with preserved renal function after islet transplantation according to a retrospective series of 35 patients (Leitao et al. 2009).

Retinopathy – The Edmonton and the Miami series reported ocular problems posttransplantation in 8.5 % and 15 % of patients, respectively. Adverse events included retinal bleeds, tractional retinal detachment, and central retinal vein occlusion (Hafiz et al. 2005; Ryan et al. 2005). However, after 1–2 years, diabetic retinopathy seems to stabilize (Lee et al. 2005). Moreover, at 1 year after

transplantation, arterial and venous retinal blood flow velocities are significantly increased, possibly indicating improved retinal microcirculation (Venturini et al. 2006). The acute adverse effects on retinopathy may be due to the sudden improvement in glycemic control after islet transplantation. The DCCT also reported initial deterioration of diabetic retinopathy in patients with preexisting disease who were treated in the intensive insulin treatment arm as compared to those in the conventional treatment arm; however, after 1 year differences between treatment arms disappeared and after 36 months of follow-up, intensive treatment was consistently associated with significantly less progression of diabetic retinopathy (The Diabetes Control and Complications Trial Research Group 1993). Consistently, a study on 44 patients showed that at 36 months after transplant progression of retinopathy was slower in subjects who received islet transplantation compared to those on intensive insulin therapy (Thompson et al. 2008). More studies are needed to assess whether the overall effect of islet transplantation on diabetic retinopathy is beneficial in the long term.

Neuropathy – Reports on the effect of islet transplantation on diabetic neuropathy suggest that the procedure has only marginal effect on this microvascular complication. Lee et al. performed nerve conduction studies in eight patients with at least 1 year of follow-up after transplantation. They concluded that peripheral neuropathy stabilized or maybe even improved, although no formal statistical analysis was provided and conclusions were based on clinical observations by a single neurologist (Lee et al. 2005). Del Carro et al. compared nerve conduction studies in patients who had received an islet-after-kidney transplantation to patients having received kidney transplantation only. In their interpretation of the results, they suggested that worsening of diabetic neuropathy seemed to be halted by islet transplantation, but no statistically significant differences between the two groups could be demonstrated (Del Carro et al. 2007). A prospective cohort study compared the progression of microvascular complications between 31 patients who received an islet transplant and 11 who remained on the waiting list. Despite an association with lower HbA1c levels and slower progression of retinopathy, islet transplantation did not lead to any benefit in neuropathy (Warnock et al. 2008).

By and large, the potential benefits of the islet transplant procedure have been identified only in the minority of patients who reach prolonged insulin independence. Moreover, lack of comparisons with control groups on insulin therapy prevents any consideration on real superiority of islet transplantation. Therefore, large, multicenter, randomized trials are needed to assess the role of islet transplantation in slowing the progression of diabetic complications over conventional supportive therapy.

Adverse Events in Islet Transplantation

Adverse events related to islet transplantation are principally related to the procedure itself and to the consequences of the immunosuppressive regimen. During the procedure, a large mass of β cells is percutaneously and transhepatically injected

into the portal vein. This may lead to portal vein thrombosis or thrombosis of segmental branches. On the other hand, incidence rates of up to 14 % have been reported for intraperitoneal bleeding, which may require blood transfusion or even surgical intervention. This complication can be effectively prevented by sealing the catheter tract using thrombostatic coils and tissue fibrin glue (Villiger et al. 2005). Other relatively frequent procedure-related complications are abdominal pain from puncturation of the peritoneum or gallbladder and a transient rise of hepatic enzymes (Ryan et al. 2004b). Posttransplantation focal hepatic steatosis occurs in approximately 20 % of patients, possibly due to a local paracrine effect of insulin, but its significance with regard to graft function is not clear yet (Markmann et al. 2003; Bhargava et al. 2004).

Type 1 diabetes patients receiving pancreatic islet transplantation may need an additional kidney and/or whole pancreas transplantation later in life. Therefore, posttransplantation alloimmunization in roughly 10–30 % of patients using immunosuppression is a cause for concern (Campbell et al. 2007a; Cardani et al. 2007). Of note, up to 100 % of patients develop HLA alloreactivity, with 71 % having HLA panel-reactive antibodies (PRA) $\geq$50 %, after withdrawal of immunosuppression because of islet graft failure or side effects (Campbell et al. 2007a; Cardani et al. 2007). Pre- or posttransplantation alloreactivity against HLA class I and II may also be associated with reduced pancreatic islet graft survival itself (Lobo et al. 2005; Campbell et al. 2007b), although some authors suggested that increased PRA had no clinical significance under adequate immunosuppression (Cardani et al. 2007). As opposed to solid organ transplantation, pretransplantation testing of PRA is currently not performed in pancreatic islet transplantation. Thus, the impact of PRA positivity on clinical outcome after islet transplantation or on future whole organ transplantation has to be further investigated.

Immunosuppressive Regimens for Islet Transplantation

Following the publication by the Edmonton group in 2000 (Shapiro et al. 2000), the steroid-free immune-suppressive protocol this group used was adopted by many centers, although it was not the only change being introduced. Changes with regard to recipient and donor selection, the technical procedure, and the infusion of a large number of pancreatic islets from multiple donors will all have contributed to the favorable short-term results. The Edmonton immunosuppressive regimen consists of induction therapy with a monoclonal antibody against the interleukin-2 receptor (daclizumab) and maintenance therapy with a calcineurin inhibitor (tacrolimus) and a mammalian mTOR inhibitor (sirolimus). Sirolimus has been shown to display significant synergy with calcineurin inhibitors, control autoimmunity, induce apoptosis of T cells and other inflammatory cells, and induce generation of regulatory T cells. However, data have also emerged showing its potentially harmful effects on β-cell regeneration (Nir et al. 2007; Berney and Secchi 2009). The same applies for calcineurin inhibitors; although proven to be very effective in organ transplantation, they are toxic to β cells and cause insulin resistance and diabetes mellitus.

Moreover, sirolimus and tacrolimus exert direct nephrotoxic effects and they often induce the development of hyperlipidemia and hypertension, which may further increase the risk of micro- and macrovascular complications (Halloran 2004). Therefore, the combined use of sirolimus and tacrolimus to prevent acute rejection of transplanted pancreatic islets is certainly not ideal.

To increase islet transplantation success rates and diminish the often severe side effects associated with chronic use of immunosuppressive drugs (Hafiz et al. 2005), various centers are implementing new immunosuppressive regimens, both for the induction and for the maintenance phase (Hering et al. 2005; Gillard et al. 2008; Ghofaili et al. 2007; Froud et al. 2008a; Mineo et al. 2009). In an attempt to promote a pro-tolerogenic state, Froud et al. tested induction therapy with alemtuzumab in three islet transplant recipients (Froud et al. 2008a). Alemtuzumab is a humanized monoclonal antibody against CD-52, which is present on the surface of mature lymphocytes. Its administration leads to severe lymphocyte depletion and may favorably influence the regulatory T-cell versus effector T-cell ratio during T-cell repopulation (Weaver and Kirk 2007). Indeed, in these three patients, glucose metabolism seemed to be better than in historic controls, with no major infectious complications. However, other changes in the immunosuppressive regimen, such as the use of steroids on the day before islet infusion, the early switch from tacrolimus to mycophenolate mofetil (MMF) during the maintenance phase, and the use of etanercept (see below) may all have contributed to improved outcomes in this study.

Tumor necrosis factor (TNF)α is a regulator of the immune response, and its activity is inhibited by etanercept, a recombinant TNFα receptor protein. From the University of Minnesota came an interesting report of high success rates in eight patients using a protocol in which etanercept was administered as induction therapy, combined with prednisone, daclizumab, and rabbit antithymocyte globulin. Of the eight patients, five were still insulin independent after 1 year. Of note, patients received an islet graft from a single donor (Hering et al. 2005). More centers are now using etanercept as additional induction therapy, a strategy which is supported by the fact that the CITR found an association between etanercept use and graft survival (Gangemi et al. 2008; Alejandro et al. 2008; Faradji et al. 2008).

Some studies investigated the combination of etanercept induction with long-term use of subcutaneous exenatide, a glucagon-like peptide-1 (GLP-1) analogue. GLP-1 is a hormone derived from the gut, which stimulates insulin secretion, suppresses glucagon secretion, and inhibits gastric emptying (Gentilella et al. 2009). Combined treatment with etanercept and exenatide in addition to the Edmonton immunosuppressive protocol was shown to reduce the number of islets needed to achieve insulin independence (Gangemi et al. 2008). In addition, combined etanercept and exenatide use improved glucose control and graft survival in patients who needed a second transplantation because of progressive graft dysfunction (Faradji et al. 2008). In two studies with islet transplantation patients, exenatide reduced insulin requirements, although in one study they tended to rise again at the end of the 3-month study period, possibly due to exhaustion of β cells (Ghofaili et al. 2007; Froud et al. 2008a, b). However, these studies were very small and

nonrandomized. Of note, exenatide use involves the administration of twice-daily subcutaneous injections, causes severe nausea, and may lead to hypoglycemia. Therefore, randomized controlled trials are needed to define whether its use confers additional benefit over immunosuppressive therapy alone in islet transplantation recipients (Rickels and Naji 2009).

An isolated case with more than 11 years of insulin independency after islet transplantation was described in 2009 (Berney et al. 2009). The intriguing question is which factors have contributed to the outcome in this particular patient. The patient had previously received a kidney transplant and was on an immunosuppressive regimen comprising antithymocyte globulin as induction therapy followed by prednisone (which was rapidly tapered), cyclosporine, and azathioprine, which was later switched to MMF. Interestingly, the authors investigated the cellular immune response and found that the patient was hyporesponsive toward donor antigens, possibly as a result of the expanded regulatory T-cell (Treg) pool. This may have contributed to the excellent long-term survival of the graft. Huurman et al. examined cytokine profiles and found that allograft-specific cytokine profiles were skewed toward a Treg phenotype in patients who achieved insulin independence and that expression of the Treg cytokine interleukin-10 was associated with low alloreactivity and superior islet function (Huurman et al. 2009). The role of Tregs in allograft tolerance has long been recognized in solid organ and bone marrow transplantation, and much research is devoted to translating this knowledge into therapeutic options, which may also benefit islet transplantation (Schiopu and Wood 2008).

Despite immunosuppressive therapy aimed at preventing rejection (i.e., alloimmunity), outcomes of islet transplantation may also be adversely influenced by autoimmune injury. A recent study showed delayed graft function in patients with pretransplant cellular autoreactivity to β-cell autoantigens; in 4 out of 10 patients with recurrence of autoreactivity posttransplantation, insulin independence was never achieved. Moreover, in 5 out of 8 patients in whom cellular autoreactivity occurred de novo after transplantation, time to insulin independence was prolonged (Huurman et al. 2008). In the international trial of the Edmonton protocol, patients with one or two autoantibodies in the serum before the final infusion had a significantly lower insulin-independence rate than those without autoantibodies (Shapiro et al. 2006).

Autologous Islet Transplantation

The concept of autologous islet transplantation after pancreatectomy arose at the University of Minnesota in the late 1970s (Sutherland et al. 1978). Pancreatectomy had already been performed as a treatment for patients with chronic, unrelentingly painful pancreatitis (Braasch et al. 1978). However, it was seen as an undesirable method, in part because removal of the gland inevitably causes insulin-dependent diabetes. This drawback led to the concept of not disposing of the resected pancreas but using it as a source of islet tissue that could be used for autologous transplant.

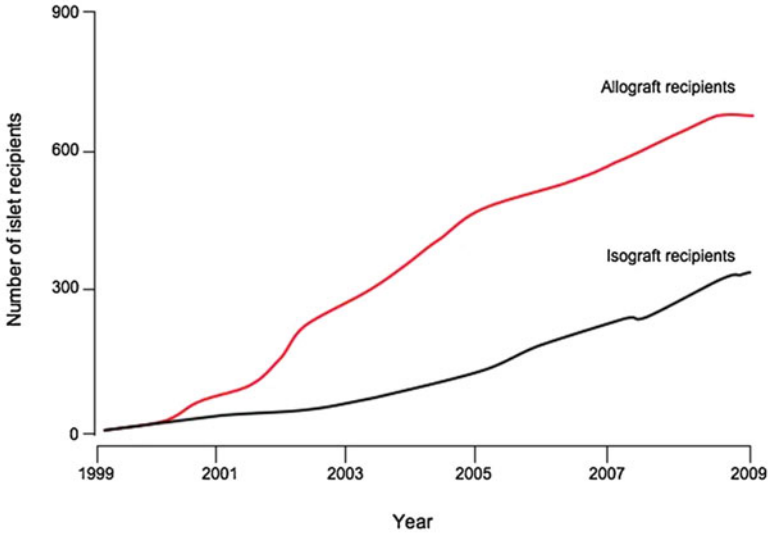

Fig. 3 Number of recipients of allograft and isograft islet transplantation, according to the Collaborative Islet Transplant Registry (CITR), 2011

In contrast to alloimmune islet transplantation in patients with type 1 diabetes mellitus, no immunosuppression is needed for patients receiving autologous islet infusion, due to the autologous source of the islets and lack of preexisting autoimmune reactivity. These considerations boosted the use of islet autotransplantation to prevent diabetes after pancreatectomy performed for chronic pancreatitis (Fig. 3). More recently, encouraging results have been published showing that islet autotransplantation can effectively prevent diabetes after 50–60 % distal partial pancreatectomy for benign pancreatic tumors (Jin et al. 2013).

A report of 85 total pancreatectomy patients from the United Kingdom showed that the group of 50 patients receiving concomitant autologous islet transplantation had a significantly lower median insulin requirement than those without concomitant transplantation, although only five patients remained insulin independent (Garcea et al. 2009). Of 173 recipients of autologous islet transplantations post-pancreatectomy at the University of Minnesota, 55 (32 %) were insulin independent, and 57 (33 %) had partial islet function as defined by the need of only once-daily long-acting insulin at some posttransplant point. Importantly, the rate of decline of insulin independence was remarkably limited, with 46 % insulin independence at 5 years follow-up and 28 % at 10 years (Sutherland et al. 2008). Despite the lower number of islets needed to provide insulin independence in autologous compared to allogeneic transplantation, islet cell mass is an important predictor of success in both transplant procedures (Shapiro et al. 2006; Wahoff et al. 1995). Therefore, improvements in islet yields from fibrotic and inflamed pancreata are expected to further improve outcomes of autologous islet transplantation (Naziruddin et al. 2012).

Cost-Effectiveness of Islet Transplantation

So far, no study has addressed the issue of the cost-effectiveness of islet transplantation in terms of the costs per quality-adjusted life year or per micro- or macrovascular diabetic complication prevented. The GRAGIL network has estimated the average cost of an islet transplantation in the year 2000 at €77,745.00. These costs even slightly exceed those for whole pancreas transplantation, mainly due to the high expenses of cell isolation (Guignard et al. 2004). A study by Frank et al. also found that pancreas processing-related costs led to higher total costs for isolated islet transplantation than for whole pancreas transplantation, even though the former was associated with less procedure-related morbidity and shorter hospital stays (Frank et al. 2004). These high costs may be justified in patients in whom islet transplantation is deemed to be lifesaving because of severe hypoglycemic episodes. However, in other settings, they will compare extremely unfavorably to the costs of current strategies to prevent diabetic complications, such as adequate glycemic control, blood pressure and lipid profile optimization, diet and weight loss, and angiotensin-converting enzyme inhibitor use.

According to the 2000 French National Cost Study (Guignard et al. 2004), the costs of hospitalization for pancreas transplantation (DRG 279) were €25,674. The processing of the 5.6 pancreata used for a single islet transplantation costs €23,755; with the hospitalization, the total costs are about €34,178. Since about 80 % of patients are still insulin independent at 3 years after a pancreas transplantation, compared to only 44 % of those who receive an islet transplantation (Barton et al. 2012; Boggi et al. 2012), it can be estimated that one patient achieving insulin independence at 3 year costs to health-care providers €29,175 or €77,677 if this target is achieved after pancreas or islet transplantation, respectively. Thus, achieving long-lasting insulin independency is almost threefold more expensive by islet than by pancreas transplantation. Based on these data, pancreas transplantation is likely to be remarkably more cost-effective compared to islet transplantation, even taking into account the higher risk of surgical complications related to whole organ grafting.

A recent cost-effectiveness exploratory analysis comparing islet transplantation with standard insulin therapy in type 1 diabetes adult patients affected by hypoglycemia unawareness showed that islet transplantation becomes cost saving at about 9–10 years after the procedure (Beckwith et al. 2012). However, since the vast majority of islet transplant recipients loose islet independence within 5 years after transplantation, this result in fact means that islet transplantation is never cost-effective compared to standard insulin therapy. Therefore, despite the absence of ad hoc studies formally addressing the issue of the cost-effectiveness of islet transplantation compared to other treatments for type 1 diabetes, available data suggest that such procedure is hardly superior to pancreas transplantation or insulin therapy.

A recent study showed that islet cell autotransplantation after total pancreatectomy for chronic pancreatitis resulted in improved survival over total pancreatectomy alone. Importantly, the cost of pancreatectomy plus islet cell autotransplantation with attendant admission and analgesia costs over the 16-year

survival period was £110,445 compared with £101,608 estimated 16-year costs if no pancreatectomy was undertaken (Garcea et al. 2013). This suggests that islet autotransplantation after pancreatectomy might be a cost-effective strategy to treat chronic pancreatitis.

Future Developments

New Sources of Islets

Islet cell harvest from whole pancreas remains a limiting step, as the efficiency of the procedure and cell viability postharvest is relatively poor. There are several steps in the whole procedure of islet transplantation which may be targeted in order to improve islet recovery and posttransplantation protection. Pretransplantation procedures related to pancreas preservation, enzymatic digestion, purification, culture, and shipment may be further refined (Ichii and Ricordi 2009). While many laboratories are developing methods to improve these processes, given the severe shortage of donor pancreases, other investigators are exploring alternative sources of β cells.

Other sources of pancreatic β cells are mesenchymal stem cells (MSCs) that according to some studies might possibly display the capability to transdifferentiate into insulin-producing cells (Porat and Dor 2007; Claiborn and Stoffers 2008). Of even more importance are the immunomodulatory and anti-inflammatory properties of MSCs, which might control the autoimmune response preventing immune injury of newly proliferating cells (English 2012). However, before clinical use, substantial issues should be addressed regarding the safety, function, mode of isolation, and experimental handling of MSCs (Bianco et al. 2013).

Alternative sources for β-cell replacement include human embryonic stem cells (hESCs). Recently, it has been shown that the small molecule (-)-indolactam V induces differentiation of hESCs into pancreatic progenitor cells in vitro (Chen et al. 2009). The more plentiful pancreatic ductal cells isolated from human donor pancreases can be transdifferentiated into β cells (Bonner-Weir et al. 2000). Similarly, mouse experiments have shown that bile duct epithelial cells (Nagaya et al. 2009), acinar cells (Zhou et al. 2008), and hepatic cells (Aviv et al. 2009) can also be transdifferentiated into β cells. The differentiation of human fibroblast-derived induced pluripotent stem cells (iPSCs) into β cells provides another alternative that is particularly appealing due to its potential avoidance of allogeneic rejection (Tateishi et al. 2008).

Another possible source of pancreatic islets is xenotransplantation, with which some experience has been gained in humans. Six groups have independently reported that pig islets transplanted into nonhuman primates can maintain normoglycemia for periods in excess of 6 months, and more positive results are expected by genetic manipulation of transplanted islets (van der Windt et al. 2012).

In 1994, a Swedish group reported xenotransplantation with fetal porcine pancreatic islets in 10 diabetic patients. Although insulin requirements did not

decrease, the procedure was well tolerated and there was no evidence of transmission of porcine endogenous retroviruses after 4–7 years of follow-up (Groth et al. 1994; Heneine et al. 1998). More recently, xenotransplantation has been performed in China, Russia, and Mexico (Valdes-Gonzalez et al. 2005; Wang 2007). In 2005, the group from Mexico reported a 4-year follow-up of 12 diabetic patients not taking immunosuppressive therapy who had received one to three subcutaneous implantations of a device containing porcine pancreatic islets and Sertoli cells. Sertoli cells, being immune-privileged, were added because they may confer immunoprotection to transplanted endocrine tissue. Follow-up showed a decreased insulin requirement in 50 % of patients, compared to pretransplant levels and non-transplanted controls. Porcine C-peptide was not detectable in the urine, and the significance of this study remains to be determined. Importantly, severe ethical issues have been raised with regard to xenotransplantation as it is currently being performed. The program in China was suspended, and the International Xenotransplantation Association has seriously objected to the Mexican and Russian studies, as they feel that the safety of the patient and of the general public (especially with regard to the spread of porcine endogenous retroviruses) is not sufficiently guaranteed (Sykes et al. 2006, 2007; Groth 2007). More experimental studies are needed before clinical trials in human can be initiated (Rajotte 2008).

In 2005, Matsumoto et al. performed the first islet transplantation from a living related donor in a patient who had brittle diabetes due to chronic pancreatitis. The procedure resulted in good glycemic control and no major complications in both the donor and the recipient (Matsumoto et al. 2005, 2006). However, results cannot be generalized to the type 1 diabetes population, as diabetic disease in the recipient did not result from an autoimmune process. Moreover, partial pancreatectomy in the donor implies major surgery with associated risks of morbidity and mortality. In the long term, donors may be at increased risk of developing diabetes mellitus themselves (Hirshberg 2006).

Improving the Transplant Procedure

Efficacy of islet transplant procedure is hampered by massive cell loss shortly after infusion because of an inflammatory reaction termed instant blood-mediated inflammatory reaction (IBMIR). This reaction involves activation of the complement and coagulation cascades, ultimately resulting in clot formation and infiltration of leukocytes into the islets, which leads to disruption of islet integrity and islet destruction (Fig. 4). Different strategies have been proposed to prevent this phenomenon. Since heparin can prevent clotting and decrease complement activation, peri-transplant heparinization of either the patient or, to prevent bleeding complications, the pancreatic islets themselves has been proposed as a strategy to improve outcomes (Johansson et al. 2005; Cabric et al. 2007; Koh et al. 2010). This may prevent the immediate and significant post-procedural islet loss. Moreover, it is now possible to visualize islets in the peri-transplantation phase using 18 -F-fluorodeoxyglucose positron-emission tomography combined with computed

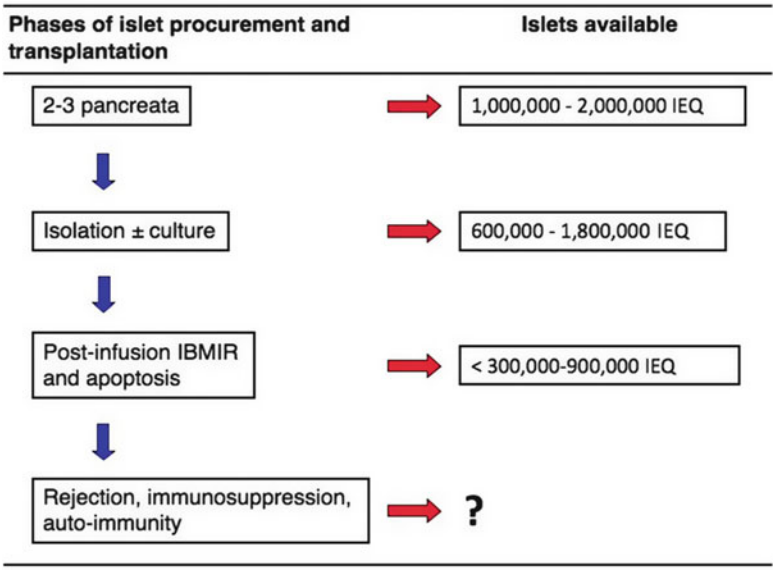

Fig. 4 Loss of pancreatic islet mass, from graft preparation to post-infusion degradation. *IEQ* islet equivalents, *IBMIR* instant blood-mediated inflammatory reaction. The IBMIR reduces islet mass by 50–70 % (Korsgren et al. 2005)

tomography in order to assess islet survival and distribution, which may also be used to evaluate alternative sites of implantation (Eich et al. 2007).

For the transplantation procedure itself, it has been recognized that the liver is not the ideal site for transplantation because of the procedure-related complications, the relatively low oxygen supply in the liver, the exposure to toxins absorbed from the gastrointestinal tract, and the IBMIR, which causes substantial islet loss shortly after infusion. Many alternative sites have been explored, including the omentum, pancreas, gastrointestinal tract, and muscular tissue. More recently, the bone marrow has been proposed as an alternative site of islet injection (Maffi et al. 2013). However, these alternative approaches have so far remained experimental, with none of them being convincingly superior to the currently used method (Merani et al. 2008; van der Windt et al. 2008).

Islet encapsulation as a strategy to improve graft survival is one of the main areas in experimental research. The use of semipermeable encapsulation material such as alginate gel or membrane devices should protect the islets against the alloimmune response while at the same time allowing them to sense glucose levels and secrete insulin (Figliuzzi et al. 2006; Beck et al. 2007). This technique must allow adequate diffusion of oxygen and nutrients to maintain islet viability and function but, at the same time, must be selective enough to prevent the permeation of host immune proteins. Islet encapsulation in alginate gel and in polysulfone hollow fibers allows adequate transport of nutrients to maintain islet function and viability, making the use of these immunoisolation strategies for transplantation a potentially important

field of investigation to transplant also xenogeneic islets (Cornolti et al. 2009; O'Sullivan et al. 2011).

Recently, four patients received an intraperitoneal infusion of encapsulated allogeneic islets. Despite no immunosuppression, all patients turned positive for serum C-peptide response, both in basal and after stimulation, and anti-MHC class I–II and GAD65 antibodies all tested negative at 3 years after transplant. Daily mean blood glucose, as well as HbA1c levels, significantly improved after transplant, with daily exogenous insulin consumption declining in all cases, but with full insulin independence reached, just transiently, in one single patient (Basta et al. 2011).

Induction of Immune Tolerance

Over the past several decades, the generation of a large array of immunosuppressive agents has increased the number of therapeutic tools available to prevent acute rejection. However, as detailed above, toxicity of immunosuppressive drugs may offset their benefits; hence, the final goal of transplant medicine is to achieve T- and β-cell tolerance that is antigen specific without the need for long-term generalized immunosuppression. Many strategies have been proposed to promote transplant tolerance in rodent model of transplantation, including infusion of regulatory T cells, mesenchymal stem cells, or immature dendritic cells. Most strategies, however, failed when transferred to nonhuman primate models. Recently, a treatment that selectively depletes activated cytopathic donor reactive T cells while sparing resting and immunoregulatory T cells has been tested in a model of islet transplantation. Short-term sirolimus and IL-2/Ig plus mutant antagonist-type IL-15/Ig cytolytic fusion proteins posttransplantation resulted in prolonged, drug-free engraftment (Koulmanda et al. 2012).

Novel Therapeutic Perspectives for Type 1 Diabetes Mellitus

Other therapeutic approaches for patients with type 1 diabetes are also underway. Indeed, refinement of insulin pumps in combination with continuous glucose monitoring systems may lead to better glycemic control (The Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group 2008). In the future, patients will ideally be able to use a closed-loop system consisting of a glucose sensor and an insulin pump, as well as software to automatically translate measured glucose levels into appropriate insulin doses. A recent trial showed that over a 3-month period, the use of sensor-augmented insulin-pump therapy with the threshold-suspend feature reduced nocturnal hypoglycemia without increasing glycated hemoglobin values compared to insulin pumps without the threshold-suspend feature (Bergenstal et al. 2013). Promising results from two random-order crossover studies show that "bionic" pancreas improves glycemic control and reduces hypoglycemic episodes compared to the insulin pumps (Russel et al. 2014).

Intriguingly, attempts have also been made to protect pancreatic islets from autoimmunity, allowing regeneration of β cells in the early phases of

type 1 diabetes. This appears to be a promising approach, since it is well documented that β cells can regenerate, as observed during pregnancy and in subjects with insulin resistance (Porat and Dor 2007; Claiborn and Stoffers 2008). Along this line, compelling evidence has accumulated suggesting that in addition to their immunosuppressive properties, CD3-specific antibodies can induce immune tolerance especially in the context of an ongoing immune response (Chatenoud and Bluestone 2007). Clinical studies have shown that this therapy may, at least partially, preserve β-cell mass in newly diagnosed type 1 diabetics (Chatenoud and Bluestone 2007; Keymeulen et al. 2005). A recent study showed that treatment with teplizumab, a nonactivating Fc-modified, anti-CD3 monoclonal antibody, significantly reduced C-peptide loss at 2 years after diagnosis, but its efficacy in delaying the need of insulin therapy was only partial (Hagopian et al. 2013). Modulation of T-cell costimulatory signals has been tested in a trial with 112 patients recently diagnosed with type 1 diabetes treated with CTLA4Ig fusion protein abatacept or vehicle control. Overall, abatacept slowed reduction in β-cell function (Orban et al. 2011). However, despite continued administration over 24 months, abatacept did not affect the decrease in β-cell function rate compared to placebo.

Starting from the rationale that interleukin-1 is involved in β-cell dysfunction and apoptosis, two recent randomized, controlled trials tested the effect of two interleukin-1 inhibitors, canakinumab and anakinra, on β-cell decline in recent-onset type 1 diabetes. Both drugs, however, failed to retard disease onset over placebo (Moran et al. 2013).

An alternative approach is targeting B lymphocytes, given the importance of the humoral response in the pathogenesis of type 1 diabetes and the fact that B lymphocytes also have a role as antigen-presenting cells. Promising results with a B lymphocyte depleting monoclonal antibody have been obtained in a mouse model of diabetes (Hu et al. 2007), and a clinical trial showed that a four-dose course of rituximab partially preserved β-cell function over a period of 12 months in patients with type 1 diabetes (Pescovitz et al. 2009).

A more drastic strategy to bypass autoimmunity is autologous non-myeloablative hematopoietic stem cell transplantation, which may reset autoreactive T cells and reverse the disease in new-onset type 1 diabetes (Couri et al. 2009). With this approach, persistent normoglycemia was achieved for a mean of 2.5 years in 60 % of patients. However, acute drug toxicity, risk of infections, and sterility may outweigh the benefits of this protocol.

Alternative approaches to the induction of tolerance include molecular biological strategies. In particular, evidence has been provided that immature dendritic cells (DCs) can promote tolerance. To this end, CD40, CD80, and CD86 cell surface molecules were specifically downregulated by ex vivo treating DCs from mice with a mixture of specific antisense oligonucleotides. This promoted the emergence of regulatory T cells that might possibly prevent the occurrence of diabetes (Machen et al. 2004). Intriguingly, to circumvent the technical issues of ex vivo DC manipulation, a recent study in mice showed that the same immature phenotype can be induced by using a microsphere-based vaccine injected subcutaneously (Phillips et al. 2008). This approach effectively prevented new-onset diabetes or even reversed it, providing the basis for testing this approach also in humans.

Conclusion

Islet transplantation is a dynamic field to which much time and resources are being devoted. If successful transplantation is defined as a transplant after which short-term quality of life and glycemic control improve, then success rates of this procedure are quite acceptable. However, if success is defined in terms of long-term insulin independence or prevention of diabetes-related complications, then outcomes are outright disappointing in the first, and largely unknown in the second.

At present, islet transplantation is far from representing a standard of care for the large majority of patients with type 1 diabetes (Cravedi et al. 2008; Ruggenenti et al. 2008). Only a highly selected group of patients with brittle diabetes may benefit from the procedure, which requires a high degree of expertise. In the absence of major advancements in islet transplantation, the 2006 recommendations by the American Diabetes Association (Robertson et al. 2006) that islet transplantation should be considered an experimental procedure appear still up to date.

Cross-References

- ▶ Advances in Clinical Islet Isolation
- ▶ Human Islet Autotransplantation
- ▶ Islet Isolation from Pancreatitis Pancreas for Islet Autotransplantation

References

Alejandro R, Barton FB, Hering BJ, Wease S (2008) 2008 update from the Collaborative Islet Transplant Registry. Transplantation 86:1783–1788

Aviv V, Meivar-Levy I, Rachmut IH, Rubinek T, Mor E, Ferber S (2009) Exendin-4 promotes liver cell proliferation and enhances the PDX-1-induced liver to pancreas transdifferentiation process. J Biol Chem 284:33509–33520

Badet L, Benhamou PY, Wojtusciszyn A et al (2007) Expectations and strategies regarding islet transplantation: metabolic data from the GRAGIL 2 trial. Transplantation 84:89–96

Ballinger WF, Lacy PE (1972) Transplantation of intact pancreatic islets in rats. Surgery 72:175–186

Barton FB, Rickels MR, Alejandro R et al (2012) Improvement in outcomes of clinical islet transplantation: 1999–2010. Diabetes Care 35:1436–1445

Basta G, Montanucci P, Luca G et al (2011) Long-term metabolic and immunological follow-up of nonimmunosuppressed patients with type 1 diabetes treated with microencapsulated islet allografts: four cases. Diabetes Care 34:2406–2409

Beck J, Angus R, Madsen B, Britt D, Vernon B, Nguyen KT (2007) Islet encapsulation: strategies to enhance islet cell functions. Tissue Eng 13:589–599

Beckwith J, Nyman JA, Flanagan B, Schrover R, Schuurman HJ (2012) A health economic analysis of clinical islet transplantation. Clin Transplant 26:23–33

Benhamou PY, Oberholzer J, Toso C et al (2001) Human islet transplantation network for the treatment of Type I diabetes: first data from the Swiss-French GRAGIL consortium (1999–2000). Groupe de Recherche Rhin Rhjne Alpes Geneve pour la transplantation d'Ilots de Langerhans. Diabetologia 44:859–864

Bergenstal RM, Klonoff DC, Garg SK et al (2013) Threshold-based insulin-pump interruption for reduction of hypoglycemia. N Engl J Med 369(3):224–232
Berney T, Secchi A (2009) Rapamycin in islet transplantation: friend or foe? Transpl Int 22:153–161
Berney T, Ferrari-Lacraz S, Buhler L et al (2009) Long-term insulin-independence after allogeneic islet transplantation for type 1 diabetes: over the 10-year mark. Am J Transplant 9:419–423
Bhargava R, Senior PA, Ackerman TE et al (2004) Prevalence of hepatic steatosis after islet transplantation and its relation to graft function. Diabetes 53:1311–1317
Bianco P, Cao X, Frenette PS et al (2013) The meaning, the sense and the significance: translating the science of mesenchymal stem cells into medicine. Nat Med 19:35–42
Bluestone JA, Herold K, Eisenbarth G (2010) Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature 464:1293–1300
Boggi U, Vistoli F, Egidi FM et al (2012) Transplantation of the pancreas. Curr Diab Rep 12:568–579
Bonner-Weir S, Taneja M, Weir GC et al (2000) In vitro cultivation of human islets from expanded ductal tissue. Proc Natl Acad Sci U S A 97:7999–8004
Braasch JW, Vito L, Nugent FW (1978) Total pancreatectomy of end-stage chronic pancreatitis. Ann Surg 188:317–322
Brendel M, Hering B, Schulz A, Bretzel R (1999) International islet transplant registry report. University of Giessen, Giessen, pp 1–20
Cabric S, Sanchez J, Lundgren T et al (2007) Islet surface heparinization prevents the instant blood-mediated inflammatory reaction in islet transplantation. Diabetes 56:2008–2015
Campbell PM, Senior PA, Salam A et al (2007a) High risk of sensitization after failed islet transplantation. Am J Transplant 7:2311–2317
Campbell PM, Salam A, Ryan EA et al (2007b) Pretransplant HLA antibodies are associated with reduced graft survival after clinical islet transplantation. Am J Transplant 7:1242–1248
Cardani R, Pileggi A, Ricordi C et al (2007) Allosensitization of islet allograft recipients. Transplantation 84:1413–1427
Chase HP, Cuthbertson DD, Dolan LM et al (2001) First-phase insulin release during the intravenous glucose tolerance test as a risk factor for type 1 diabetes. J Pediatr 138:244–249
Chatenoud L, Bluestone JA (2007) CD3-specific antibodies: a portal to the treatment of autoimmunity. Nat Rev Immunol 7:622–632
Chen S, Borowiak M, Fox JL et al (2009) A small molecule that directs differentiation of human ESCs into the pancreatic lineage. Nat Chem Biol 5:258–265
Claiborn KC, Stoffers DA (2008) Toward a cell-based cure for diabetes: advances in production and transplant of β cells. Mt Sinai J Med 75:362–371
Collaborative Islet Transplantation Registry (2010) Seventh annual report. http://citregistry.com Accessed on April 28, 2014
Coppieters KT, Wiberg A, Tracy SM, von Herrath MG (2012) Immunology in the clinic review series: focus on type 1 diabetes and viruses: the role of viruses in type 1 diabetes: a difficult dilemma. Clin Exp Immunol 168:5–11
Cornolti R, Figliuzzi M, Remuzzi A (2009) Effect of micro- and macroencapsulation on oxygen consumption by pancreatic islets. Cell Transplant 18:195–201
Couri CE, Oliveira MC, Stracieri AB et al (2009) C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA 301:1573–1579
Cravedi P, Mannon RB, Ruggenenti P, Remuzzi A, Remuzzi G (2008) Islet transplantation: need for a time-out? Nat Clin Pract Nephrol 4:660–661
Daneman D (2009) State of the world's children with diabetes. Pediatr Diabetes 10:120–126
Danielson KK, Hatipoglu B, Kinzer K et al (2012) Reduction in carotid intima-media thickness after pancreatic islet transplantation in patients with type 1 diabetes. Diabetes Care 19:2012 (Published ahead of print November)
Danne T, Mortensen HB, Hougaard P et al (2001) Persistent differences among centers over 3 years in glycemic control and hypoglycemia in a study of 3,805 children and adolescents with type 1 diabetes from the Hvidore Study Group. Diabetes Care 24:1342–1347

de Boer IH, Sun W, Cleary PA et al (2011) Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. N Engl J Med 365:2366–2376

Del Carro U, Fiorina P, Amadio S et al (2007) Evaluation of polyneuropathy markers in type 1 diabetic kidney transplant patients and effects of islet transplantation: neurophysiological and skin biopsy longitudinal analysis. Diabetes Care 30:3063–3069

DIAMOND Project Group (2006) Incidence and trends of childhood type 1 diabetes worldwide 1990–1999. Diabet Med 23:857–866

Dor Y, Brown J, Martinez OI, Melton DA (2004) Adult pancreatic β-cells are formed by self-duplication rather than stem-cell differentiation. Nature 429:41–46

Egro FM (2013) Why is type 1 diabetes increasing? J Mol Endocrinol 51(1):R1–R13

Eich T, Eriksson O, Lundgren T (2007) Visualization of early engraftment in clinical islet transplantation by positron-emission tomography. N Engl J Med 356:2754–2755

English K (2012) Mechanisms of mesenchymal stromal cell immunomodulation. Immunol Cell Biol 91(1):19–26

Faradji RN, Tharavanij T, Messinger S et al (2008) Long-term insulin independence and improvement in insulin secretion after supplemental islet infusion under exenatide and etanercept. Transplantation 86:1658–1665

Ferrannini E, Mari A, Nofrate V, Sosenko JM, Skyler JS (2010) Progression to diabetes in relatives of type 1 diabetic patients: mechanisms and mode of onset. Diabetes 59:679–685

Figliuzzi M, Plati T, Cornolti R et al (2006) Biocompatibility and function of microencapsulated pancreatic islets. Acta Biomater 2:221–227

Fioretto P, Mauer SM, Bilous RW, Goetz FC, Sutherland DE, Steffes MW (1993) Effects of pancreas transplantation on glomerular structure in insulin-dependent diabetic patients with their own kidneys. Lancet 342:1193–1196

Fioretto P, Steffes MW, Sutherland DE, Goetz FC, Mauer M (1998) Reversal of lesions of diabetic nephropathy after pancreas transplantation. N Engl J Med 339:69–75

Fiorina P, Folli F, Zerbini G et al (2003) Islet transplantation is associated with improvement of renal function among uremic patients with type I diabetes mellitus and kidney transplants. J Am Soc Nephrol 14:2150–2158

Fiorina P, Gremizzi C, Maffi P et al (2005) Islet transplantation is associated with an improvement of cardiovascular function in type 1 diabetic kidney transplant patients. Diabetes Care 28:1358–1365

Fiorina P, Shapiro AM, Ricordi C, Secchi A (2008) The clinical impact of islet transplantation. Am J Transplant 8:1990–1997

Frank A, Deng S, Huang X et al (2004) Transplantation for type I diabetes: comparison of vascularized whole-organ pancreas with isolated pancreatic islets. Ann Surg 240:631–643

Froud T, Ricordi C, Baidal DA et al (2005) Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. Am J Transplant 5:2037–2046

Froud T, Baidal DA, Faradji R et al (2008a) Islet transplantation with alemtuzumab induction and calcineurin-free maintenance immunosuppression results in improved short- and long-term outcomes. Transplantation 86:1695–1701

Froud T, Faradji RN, Pileggi A et al (2008b) The use of exenatide in islet transplant recipients with chronic allograft dysfunction: safety, efficacy, and metabolic effects. Transplantation 86:36–45

Gale EA (2005) Spring harvest? Reflections on the rise of type 1 diabetes. Diabetologia 48:2445–2450

Gangemi A, Salehi P, Hatipoglu B et al (2008) Islet transplantation for brittle type 1 diabetes: the UIC protocol. Am J Transplant 8:1250–1261

Garcea G, Weaver J, Phillips J et al (2009) Total pancreatectomy with and without islet cell transplantation for chronic pancreatitis: a series of 85 consecutive patients. Pancreas 38:1–7

Garcea G, Pollard CA, Illouz S, Webb M, Metcalfe MS, Dennison AR (2013) Patient satisfaction and cost-effectiveness following total pancreatectomy with islet cell transplantation for chronic pancreatitis. Pancreas 42:322–328

Gentilella R, Bianchi C, Rossi A, Rotella CM (2009) Exenatide: a review from pharmacology to clinical practice. Diabetes Obes Metab 11:544–556

Ghofaili KA, Fung M, Ao Z et al (2007) Effect of exenatide on β cell function after islet transplantation in type 1 diabetes. Transplantation 83:24–28
Gillard P, Ling Z, Mathieu C et al (2008) Comparison of sirolimus alone with sirolimus plus tacrolimus in type 1 diabetic recipients of cultured islet cell grafts. Transplantation 85:256–263
Goss JA, Goodpastor SE, Brunicardi FC et al (2004) Development of a human pancreatic islet-transplant program through a collaborative relationship with a remote islet-isolation center. Transplantation 77:462–466
Green A (2008) Descriptive epidemiology of type 1 diabetes in youth: incidence, mortality, prevalence, and secular trends. Endocr Res 33:1–15
Greenbaum CJ, Beam CA, Boulware D et al (2012) Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite type 1 diabetes trialNet data. Diabetes 61:2066–2073
Groth C (2007) Towards developing guidelines on Xenotransplantation in China. Xenotransplantation 14:358–359
Groth CG, Korsgren O, Tibell A et al (1994) Transplantation of porcine fetal pancreas to diabetic patients. Lancet 344:1402–1404
Gruessner AC, Sutherland DE (2005) Pancreas transplant outcomes for United States (US) and non-US cases as reported to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR) as of June 2004. Clin Transplant 19:433–455
Gruessner RW, Sutherland DE, Gruessner AC (2005) Survival after pancreas transplantation. JAMA 293:675
Guignard AP, Oberholzer J, Benhamou PY et al (2004) Cost analysis of human islet transplantation for the treatment of type 1 diabetes in the Swiss-French Consortium GRAGIL. Diabetes Care 27:895–900
Hafiz MM, Faradji RN, Froud T et al (2005) Immunosuppression and procedure-related complications in 26 patients with type 1 diabetes mellitus receiving allogeneic islet cell transplantation. Transplantation 80:1718–1728
Hagopian W, Ferry RJ Jr, Sherry N et al (2013) Teplizumab preserves C-peptide in recent-onset type 1 diabetes: 2-year results from the randomized, placebo-controlled Protege trial. Diabetes 62(11):3901–3908
Halloran PF (2004) Immunosuppressive drugs for kidney transplantation. N Engl J Med 351:2715–2729
Harjutsalo V, Reunanen A, Tuomilehto J (2006) Differential transmission of type 1 diabetes from diabetic fathers and mothers to their offspring. Diabetes 55:1517–1524
Heneine W, Tibell A, Switzer WM et al (1998) No evidence of infection with porcine endogenous retrovirus in recipients of porcine islet-cell xenografts. Lancet 352:695–699
Hering BJ, Kandaswamy R, Harmon JV et al (2004) Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. Am J Transplant 4:390–401
Hering BJ, Kandaswamy R, Ansite JD et al (2005) Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. JAMA 293:830–835
Hirshberg B (2006) Can we justify living donor islet transplantation? Curr Diab Rep 6:307–309
Hirshberg B, Rother KI, Digon BJ 3rd et al (2003) Benefits and risks of solitary islet transplantation for type 1 diabetes using steroid-sparing immunosuppression: the National Institutes of Health experience. Diabetes Care 26:3288–3295
Hu CY, Rodriguez-Pinto D, Du W et al (2007) Treatment with CD20-specific antibody prevents and reverses autoimmune diabetes in mice. J Clin Invest 117:3857–3867
Huurman VA, Hilbrands R, Pinkse GG et al (2008) Cellular islet autoimmunity associates with clinical outcome of islet cell transplantation. PLoS One 3:e2435
Huurman VA, Velthuis JH, Hilbrands R et al (2009) Allograft-specific cytokine profiles associate with clinical outcome after islet cell transplantation. Am J Transplant 9:382–388
Ichii H, Ricordi C (2009) Current status of islet cell transplantation. J Hepatobiliary Pancreat Surg 16:101–112

Jin SM, Oh SH, Kim SK et al (2013) Diabetes-free survival in patients who underwent islet autotransplantation after 50 % to 60 % distal partial pancreatectomy for benign pancreatic tumors. Transplantation 95:1396–1403

Johansson H, Lukinius A, Moberg L et al (2005) Tissue factor produced by the endocrine cells of the islets of Langerhans is associated with a negative outcome of clinical islet transplantation. Diabetes 54:1755–1762

Keenan HA, Sun JK, Levine J et al (2010) Residual insulin production and pancreatic β-cell turnover after 50 years of diabetes: Joslin Medalist Study. Diabetes 59:2846–2853

Kenmochi T, Asano T, Maruyama M et al (2009) Clinical islet transplantation in Japan. J Hepatobiliary Pancreat Surg 16:124–130

Keymeulen B, Vandemeulebroucke E, Ziegler AG et al (2005) Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. N Engl J Med 352:2598–2608

Keymeulen B, Gillard P, Mathieu C et al (2006) Correlation between β cell mass and glycemic control in type 1 diabetic recipients of islet cell graft. Proc Natl Acad Sci U S A 103:17444–17449

Koh A, Senior P, Salam A et al (2010) Insulin-heparin infusions peritransplant substantially improve single-donor clinical islet transplant success. Transplantation 89:465–471

Kolb H, Elliott RB (1994) Increasing incidence of IDDM a consequence of improved hygiene? Diabetologia 37:729

Korsgren O, Nilsson B, Berne C et al (2005) Current status of clinical islet transplantation. Transplantation 79:1289–1293

Koulmanda M, Qipo A, Fan Z et al (2012) Prolonged survival of allogeneic islets in cynomolgus monkeys after short-term triple therapy. Am J Transplant 12:1296–1302

Kyvik KO, Green A, Beck-Nielsen H (1995) Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins. BMJ 311:913–917

Lachin JM, Genuth S, Nathan DM, Zinman B, Rutledge BN (2008) Effect of glycemic exposure on the risk of microvascular complications in the diabetes control and complications trial – revisited. Diabetes 57:995–1001

Lacy PE (1978) Workshop on Pancreatic Islet Cell Transplantation in Diabetes sponsored by the National Institute of Arthritis, Metabolism, and Digestive Diseases and held at the National Institutes of Health in Bethesda, Maryland, on November 29 and 30, 1977. Diabetes 27:427–429

Lee TC, Barshes NR, O'Mahony CA et al (2005) The effect of pancreatic islet transplantation on progression of diabetic retinopathy and neuropathy. Transplant Proc 37:2263–2265

Lee TC, Barshes NR, Agee EE, O'Mahoney CA, Brunicardi FC, Goss JA (2006) The effect of whole organ pancreas transplantation and PIT on diabetic complications. Curr Diab Rep 6:323–327

Leitao CB, Tharavanij T, Cure P et al (2008) Restoration of hypoglycemia awareness after islet transplantation. Diabetes Care 31:2113–2115

Leitao CB, Cure P, Messinger S et al (2009) Stable renal function after islet transplantation: importance of patient selection and aggressive clinical management. Transplantation 87:681–688

Lobo PI, Spencer C, Simmons WD et al (2005) Development of anti-human leukocyte antigen class 1 antibodies following allogeneic islet cell transplantation. Transplant Proc 37:3438–3440

Machen J, Harnaha J, Lakomy R, Styche A, Trucco M, Giannoukakis N (2004) Antisense oligonucleotides down-regulating costimulation confer diabetes-preventive properties to nonobese diabetic mouse dendritic cells. J Immunol 173:4331–4341

Maffi P, Bertuzzi F, De Taddeo F et al (2007) Kidney function after islet transplant alone in type 1 diabetes: impact of immunosuppressive therapy on progression of diabetic nephropathy. Diabetes Care 30:1150–1155

Maffi P, Balzano G, Ponzoni M et al (2013) Autologous pancreatic islet transplantation in human bone marrow. Diabetes 62(10):3523–3531

Markmann JF, Rosen M, Siegelman ES et al (2003) Magnetic resonance-defined periportal steatosis following intraportal islet transplantation: a functional footprint of islet graft survival? Diabetes 52:1591–1594

Matsumoto S, Okitsu T, Iwanaga Y et al (2005) Insulin independence after living-donor distal pancreatectomy and islet allotransplantation. Lancet 365:1642–1644
Matsumoto S, Okitsu T, Iwanaga Y et al (2006) Follow-up study of the first successful living donor islet transplantation. Transplantation 82:1629–1633
Merani S, Toso C, Emamaullee J, Shapiro AM (2008) Optimal implantation site for pancreatic islet transplantation. Br J Surg 95:1449–1461
Mineo D, Sageshima J, Burke GW, Ricordi C (2009) Minimization and withdrawal of steroids in pancreas and islet transplantation. Transpl Int 22:20–37
Moran A, Bundy B, Becker DJ et al (2013) Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. Lancet 381:1905–1915
Nagaya M, Katsuta H, Kaneto H, Bonner-Weir S, Weir GC (2009) Adult mouse intrahepatic biliary epithelial cells induced in vitro to become insulin-producing cells. J Endocrinol 201:37–47
Nathan DM, Cleary PA, Backlund JY et al (2005) Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med 353:2643–2653
Nathan DM, Zinman B, Cleary PA et al (2009) Modern-day clinical course of type 1 diabetes mellitus after 30 years' duration: the diabetes control and complications trial/epidemiology of diabetes interventions and complications and Pittsburgh epidemiology of diabetes complications experience (1983–2005). Arch Intern Med 169:1307–1316
Naziruddin B, Matsumoto S, Noguchi H et al (2012) Improved pancreatic islet isolation outcome in autologous transplantation for chronic pancreatitis. Cell Transplant 21:553–558
Nir T, Melton DA, Dor Y (2007) Recovery from diabetes in mice by β cell regeneration. J Clin Invest 117:2553–2561
Nordwall M, Bojestig M, Arnqvist HJ, Ludvigsson J (2004) Declining incidence of severe retinopathy and persisting decrease of nephropathy in an unselected population of type 1 diabetes-the Linkoping Diabetes Complications Study. Diabetologia 47:1266–1272
O'Connell PJ, Hawthorne WJ, Holmes-Walker DJ et al (2006) Clinical islet transplantation in type 1 diabetes mellitus: results of Australia's first trial. Med J Aust 184:221–225
Onkamo P, Vaananen S, Karvonen M, Tuomilehto J (1999) Worldwide increase in incidence of Type I diabetes – the analysis of the data on published incidence trends. Diabetologia 42:1395–1403
Orban T, Bundy B, Becker DJ et al (2011) Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. Lancet 378:412–419
O'Sullivan ES, Vegas A, Anderson DG, Weir GC (2011) Islets transplanted in immunoisolation devices: a review of the progress and the challenges that remain. Endocr Rev 32:827–844
Paty BW, Senior PA, Lakey JR, Shapiro AM, Ryan EA (2006) Assessment of glycemic control after islet transplantation using the continuous glucose monitor in insulin-independent versus insulin-requiring type 1 diabetes subjects. Diabetes Technol Ther 8:165–173
Peakman M (2013) Immunological pathways to β-cell damage in type 1 diabetes. Diabet Med 30:147–154
Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H et al (2009) Rituximab, B-lymphocyte depletion, and preservation of β-cell function. N Engl J Med 361:2143–2152
Phillips B, Nylander K, Harnaha J et al (2008) A microsphere-based vaccine prevents and reverses new-onset autoimmune diabetes. Diabetes 57:1544–1555
Porat S, Dor Y (2007) New sources of pancreatic β cells. Curr Diab Rep 7:304–308
Pugliese A (2013) The multiple origins of type 1 diabetes. Diabet Med 30:135–146
Rajotte RV (2008) Moving towards clinical application. Xenotransplantation 15:113–115
Redondo MJ, Rewers M, Yu L et al (1999) Genetic determination of islet cell autoimmunity in monozygotic twin, dizygotic twin, and non-twin siblings of patients with type 1 diabetes: prospective twin study. BMJ 318:698–702
Rich SS (1990) Mapping genes in diabetes. Genetic epidemiological perspective. Diabetes 39:1315–1319
Rickels MR, Naji A (2009) Exenatide use in islet transplantation: words of caution. Transplantation 87:153

Rickels MR, Schutta MH, Mueller R et al (2005a) Islet cell hormonal responses to hypoglycemia after human islet transplantation for type 1 diabetes. Diabetes 54:3205–3211

Rickels MR, Schutta MH, Markmann JF, Barker CF, Naji A, Teff KL (2005b) β-Cell function following human islet transplantation for type 1 diabetes. Diabetes 54:100–106

Rickels MR, Schutta MH, Mueller R et al (2007) Glycemic thresholds for activation of counterregulatory hormone and symptom responses in islet transplant recipients. J Clin Endocrinol Metab 92:873–879

Rickels M, Cullison K, Fuller C (2011) Improvement of glucose counter-regulation following human islet transplantation in long-standing type 1 diabetes: preliminary results [abstract]. Diabetes 60:293, OR

Robertson RP, Davis C, Larsen J, Stratta R, Sutherland DE (2006) Pancreas and islet transplantation in type 1 diabetes. Diabetes Care 29:935

Ruggenenti P, Remuzzi A, Remuzzi G (2008) Decision time for pancreatic islet-cell transplantation. Lancet 371:883–884

Russell SJ1, El-Khatib FH, Sinha M, Magyar KL, McKeon K, Goergen LG, Balliro C, Hillard MA, Nathan DM, Damiano ER (2014) Outpatient Glycemic Control with a Bionic Pancreas in Type 1 Diabetes. N Engl J Med. Jun 15. [Epub ahead of print]

Ryan EA, Shandro T, Green K et al (2004a) Assessment of the severity of hypoglycemia and glycemic lability in type 1 diabetic subjects undergoing islet transplantation. Diabetes 53:955–962

Ryan EA, Paty BW, Senior PA, Shapiro AM (2004b) Risks and side effects of islet transplantation. Curr Diab Rep 4:304–309

Ryan EA, Paty BW, Senior PA et al (2005) Five-year follow-up after clinical islet transplantation. Diabetes 54:2060–2069

Ryan EA, Bigam D, Shapiro AM (2006) Current indications for pancreas or islet transplant. Diabetes Obes Metab 8:1–7

Schiopu A, Wood KJ (2008) Regulatory T cells: hypes and limitations. Curr Opin Organ Transplant 13:333–338

Secchi A, Socci C, Maffi P et al (1997) Islet transplantation in IDDM patients. Diabetologia 40:225–231

Senior PA, Zeman M, Paty BW, Ryan EA, Shapiro AM (2007) Changes in renal function after clinical islet transplantation: four-year observational study. Am J Transplant 7:91–98

Shapiro AM, Lakey JR, Ryan EA et al (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 343:230–238

Shapiro AM, Ricordi C, Hering BJ et al (2006) International trial of the Edmonton protocol for islet transplantation. N Engl J Med 355:1318–1330

Soltesz G, Patterson CC, Dahlquist G (2007) Worldwide childhood type 1 diabetes incidence – what can we learn from epidemiology? Pediatr Diabetes 8(Suppl 6):6–14

Sosenko JM, Palmer JP, Rafkin-Mervis L et al (2009) Incident dysglycemia and progression to type 1 diabetes among participants in the Diabetes Prevention Trial-Type 1. Diabetes Care 32:1603–1607

Strachan DP (1989) Hay fever, hygiene, and household size. BMJ 299:1259–1260

Sutherland DE (1981) Pancreas and islet transplantation. II. Clinical trials. Diabetologia 20:435–450

Sutherland DE, Matas AJ, Najarian JS (1978) Pancreatic islet cell transplantation. Surg Clin North Am 58:365–382

Sutherland DE, Gruessner AC, Carlson AM et al (2008) Islet autotransplant outcomes after total pancreatectomy: a contrast to islet allograft outcomes. Transplantation 86:1799–1802

Sykes M, Cozzi E, d'Apice A et al (2006) Clinical trial of islet xenotransplantation in Mexico. Xenotransplantation 13:371–372

Sykes M, Pierson RN 3rd, O'Connell P et al (2007) Reply to "critics slam Russian trial to test pig pancreas for diabetes". Nat Med 13:662–663

Tateishi K, He J, Taranova O, Liang G, D'Alessio AC, Zhang Y (2008) Generation of insulin-secreting islet-like clusters from human skin fibroblasts. J Biol Chem 283:31601–31607

The Diabetes Control and Complications Trial Research Group (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 329:977–986

The Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group (2008) Continuous glucose monitoring and intensive treatment of type 1 diabetes. N Engl J Med 359:1464–1476

Thompson DM, Begg IS, Harris C et al (2008) Reduced progression of diabetic retinopathy after islet cell transplantation compared with intensive medical therapy. Transplantation 85:1400–1405

Tuomilehto J, Podar T, Tuomilehto-Wolf E, Virtala E (1995) Evidence for importance of gender and birth cohort for risk of IDDM in offspring of IDDM parents. Diabetologia 38:975–982

Valdes-Gonzalez RA, Dorantes LM, Garibay GN et al (2005) Xenotransplantation of porcine neonatal islets of Langerhans and Sertoli cells: a 4-year study. Eur J Endocrinol 153:419–427

van der Windt DJ, Echeverri GJ, Ijzermans JN, Coopers DK (2008) The choice of anatomical site for islet transplantation. Cell Transplant 17:1005–1014

van der Windt DJ, Bottino R, Kumar G et al (2012) Clinical islet xenotransplantation: how close are we? Diabetes 61:3046–3055

Venstrom JM, McBride MA, Rother KI, Hirshberg B, Orchard TJ, Harlan DM (2003) Survival after pancreas transplantation in patients with diabetes and preserved kidney function. JAMA 290:2817–2823

Venturini M, Fiorina P, Maffi P et al (2006) Early increase of retinal arterial and venous blood flow velocities at color Doppler imaging in brittle type 1 diabetes after islet transplant alone. Transplantation 81:1274–1277

Villiger P, Ryan EA, Owen R et al (2005) Prevention of bleeding after islet transplantation: lessons learned from a multivariate analysis of 132 cases at a single institution. Am J Transplant 5:2992–2998

Wahoff DC, Papalois BE, Najarian JS et al (1995) Autologous islet transplantation to prevent diabetes after pancreatic resection. Ann Surg 222:562–575 (discussion 575–569, 1995)

Wang W (2007) A pilot trial with pig-to-man islet transplantation at the 3rd Xiang-Ya Hospital of the Central South University in Changsha. Xenotransplantation 14:358

Warnock GL, Meloche RM, Thompson D et al (2005) Improved human pancreatic islet isolation for a prospective cohort study of islet transplantation vs best medical therapy in type 1 diabetes mellitus. Arch Surg 140:735–744

Warnock GL, Thompson DM, Meloche RM et al (2008) A multi-year analysis of islet transplantation compared with intensive medical therapy on progression of complications in type 1 diabetes. Transplantation 86:1762–1766

Weaver TA, Kirk AD (2007) Alemtuzumab. Transplantation 84:1545–1547

White SA, Shaw JA, Sutherland DE (2009) Pancreas transplantation. Lancet 373:1808–1817

Yeh HC1, Brown TT, Maruthur N, Ranasinghe P, Berger Z, Suh YD, Wilson LM, Haberl EB, Brick J, Bass EB, Golden SH (2012) Comparative effectiveness and safety of methods of insulin delivery and glucose monitoring for diabetes mellitus: a systematic review and meta-analysis. Ann Intern Med. Sep 4;157(5):336–47

Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA (2008) In vivo reprogramming of adult pancreatic exocrine cells to β-cells. Nature 455:627–632

Ziegler AG, Rewers M, Simell O et al (2013) Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA 309:2473–2479

Islet Xenotransplantation: An Update on Recent Advances and Future Prospects

45

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_28,

Abstract

Type 1 diabetes (T1D) is an autoimmune disorder characterized by pancreatic β cell destruction, leading to a gradual loss of endogenous insulin secretion and ensuing hyperglycemia. Current treatment methods for T1D include the administration of exogenous insulin or the replacement of β-cell mass through either pancreas or islet transplantation. Current β-cell replacement trials have reported a lower incidence of potentially fatal hypoglycemic episodes when compared to exogenous insulin therapy. Islet allotransplants have also shown success in establishing insulin independence during the first year post transplant; however, transplant recipients progressively become increasingly dependent on insulin, and this therapy has been limited to a selected pool of patients due to a shortage of islet donors. The outcomes of the procedure have also been inconsistent, with the most success coming from highly experienced transplant centers.

Due to these inherent limitations of islet allotransplantation, xenotransplantation has been investigated as a possible alternative, since there are several potential advantages. Xenotransplantation has the potential of offering a reliable and consistent supply of islets. Having a readily available source of islets provides a significant advantage of being able to schedule the transplant procedure in advance. This is of great importance since most immunosuppression induction strategies require pre-dosing patients before the procedure. In addition, the development of specific pathogen-free (SPF) and designated disease pathogen-free (DPF) facilities and the propagation of animals with limited or no transmissible zoonotic diseases make xenotransplantation the treatment of choice for T1D patients.

This chapter aims to provide a comprehensive review of islet xenotransplantation for the treatment of type 1 diabetes, including a historical review, problems faced by xenotransplantation, current research on xenotransplantation outcomes and clinical trials, and future developments.

Keywords

Xenotransplantation • Type 1 diabetes • Islet transplant • Porcine islet • Piglet islet

Introduction to Type 1 Diabetes and Treatments

Treatment of Type 1 Diabetes

Type 1 diabetes (T1D) patients lack adequate endogenous insulin secretion due to the immune-mediated destruction of pancreatic β cells (Cnop et al. 2005). Current treatment regimens for T1DM patients aim to maintain blood glucose levels within

the physiological range and can take the form of either exogenous insulin injections several times a day or replacement of β-cells by pancreas or islet transplantation. Long-term studies have suggested that such efforts to maintain blood glucose control may delay the development and progression of chronic complications of T1D (National Kidney Disease Education Program 2013; Dvornik 1978).

In contrast to exogenous insulin administration, replacement of β-cells has shown more reliability in achieving good glycemic control and preventing hypoglycemic episodes (Heller 2008). In 2000, the Edmonton islet transplant group at the University of Alberta, Canada, achieved insulin independence in seven consecutive patients, using islets isolated from cadaver organ donors under a specific steroid-free immunosuppression protocol (Shapiro et al. 2000). Although still in its early stages, islet transplantation results in fewer complications while maintaining comparable patient outcomes and graft survival (Frank et al. 2004), making it a suitable alternative to whole pancreas transplantation (Sutherland et al. 2001). In 2005 a study out of the University of Minnesota reported that out of the eight islet transplant recipients, all became insulin independent, with five out of eight remaining independent after 1 year. All eight patients also had improved HA1c levels and demonstrated sustained C-peptide production 5 years post transplant (Hering et al. 2005).

Limitations to Allotransplantation

The scarcity of suitable donors represents the largest hurdle for islet transplantation. Donor criteria for islet transplantation are similar to criteria established for whole pancreas transplant but may be more lenient on donor parameters (Ris et al. 2004).

Pancreases are first offered for whole pancreas transplantation because of its long-standing record of long-term function and patient survival (Ris et al. 2004). Pancreases are then offered to islet isolation centers. Current donor criteria associated with optimal whole pancreas transplants are young donors (<50 years old) with low body mass indexes (BMI <30), while older donors (>50 years old) with high BMI (>30) are factors associated with improved islet yield, viability, and overall ease of the islet isolation process (Vrochides et al. 2009; Wang et al. 2013).

Given the limitations associated with current islet isolation and allotransplantation techniques, there are three major factors that affect islet transplant. These factors include the associated adverse effects of current immunosuppressive protocols, the rejection associated with allotransplantation, and the limited supply of donor tissue (Titus et al. 2000). To overcome these limitations, current studies have turned their focus to xenotransplantation, with the porcine model being the most widely studied due to the genetic similarities of porcine and human insulin (Han and Tuch 2001). Porcine insulin has also been administered to diabetic patients for decades with successful patient outcomes (Richter and Neises 2002; Greene et al. 1983), and in theory, porcine islets should be able to provide a sustained and dynamic release of insulin required to maintain euglycemia in T1D patients.

Xenotransplantation as a Solution

What Is Xenotransplantation?

Xeno or "foreign" transplantation can be defined as the implantation, transplantation, or infusion of living cells, tissues, or organs from one species to another (National Health and Medical Research Council 2013; World Health Organization 2005).

Xenotransplantation includes a variety of different procedures that can be classified into three categories; external animal therapies, solid organ transplant, and tissue and cellular therapies, and transplants (HumanXenoTransplant 2013).

External animal therapies are procedures that are performed outside the body of the human patient, involving the use of animal cells, tissue, or organ. Liver perfusion involving the introduction of pig cells for the purification of blood and the growing of human skin over a layer of animal skin in a laboratory for later skin transplants into clinical patients are some examples of clinically relevant external animal therapies.

Solid organ transplantation can be defined as the transplantation of solid viscera into a human recipient in order to replace a diseased or damaged organ. Examples include the following: liver, heart, kidneys, pancreas, and even skin.

Tissue and cellular therapies and transplants include the implantation, infusion, or transplantation of animal cells into a human recipient to replenish or compensate for the patient's dysfunctional or diseased cells or tissue. Examples of tissue and cell therapies include the use of pancreatic islets, neurons, bone marrow, and stem cells.

Although xenotransplantation has the potential to resolve the problem of human organ shortage, it also has the potential for transmitting infectious diseases, both known and undiscovered, from animals to humans (U.S. Food and Drug Administration 2013).

Potential of Xenografts in Organ Transplantation

Due to the limited availability of suitable human organs and cells for clinical transplantation, pigs have been investigated as a potential alternative source. Xenotransplantation using porcine tissue could offer a scalable source of organs and cells, which have been shown to be physiological similar to humans (Soto-Gutierrez et al. 2012). Over the years there has been an increase in the use of porcine organs and cells in transplantation research, largely due to the increasing availability of specialized pig models (Cooper and Ayares 2011). In nonhuman primates, porcine heterotopic heart grafts were reported to have a survival rate of up to 8 months; primates that received porcine kidney transplants showed function for 3 months (Cooper and Ayares 2011). Although there has been significant advancement in

this field, several barriers still exist, including immunity and organ rejection that must be overcome before this can become a standard therapy in the clinic.

One such barrier involving host immunity is the acute organ rejection that occurs after transplantation from pigs to baboons. It is related to the coagulation dysfunction triggered by activation of the host's humoral immune system that results in thrombotic microangiopathy (Kuwaki et al. 2004, 2005; Tseng et al. 2005; Ierino et al. 1998) and consumptive coagulopathy secondary to antibody deposition in the donor organs (Kozlowski et al. 1999; Buhler et al. 2000; Lin et al. 2010). This coagulation dysfunction may be related to molecular incompatibilities in the coagulation systems between pigs and humans (Bulato et al. 2012). Current efforts are focusing on the genetic modification of the cell source or of the complete organ itself (Ekser and Cooper 2010). Studies out of the Massachusetts General Hospital and Harvard Medical School have shown that the use of genetically modified pigs as a donor source for transplant can show improvements in graft function and survival (Kuwaki et al. 2004). Cowan et al. also showed that the transplantation of transgenic porcine kidneys that express human complement factors CD55 and CD59, along with the intravenous treatment of antithrombin III, has a significantly higher graft survival rate when compared to controls receiving normal porcine kidneys (Cowan et al. 2002). The transplantation of transgenic porcine islets has also shown encouraging results when transplanted into nonhuman primate models. Current studies have shown that diabetic nonhuman primates transplanted with Gal-deficient islets expressing human CD46, human CD39, and/or human TFPI can maintain graft survival and function and establish normoglycemia for up to a year post transplant (Ayares et al. 2013).

Xenotransplantation has also explored the use of encapsulation to prevent rejection of the graft by the recipient's immune system and protect recipient tissue by creating a biocompatible barrier around the transplanted tissue. Current cases using encapsulated islets have demonstrated long-term graft survival, without the use of harmful immunosuppression regimes (Chhabra and Brayman 2011). The most promising encapsulation technology in use involves creating an immunoisolation barrier around transplanted cells using sodium alginate. Current alginate encapsulated islet experiments using porcine islets transplanted into diabetic nonhuman primates have shown a significant reduction in daily insulin requirements when compared to un-transplanted controls (Elliott et al. 2005). A study by Dufrane et al. showed survival and function of encapsulated porcine islet grafts up to 6 months post transplant into Cynomolgus maccacus, with detectable C-peptide levels and anti-pig IgM/IgG 1 month after transplantation (Dufrane et al. 2006a). Unfortunately, even though the results were promising, the number of surviving functional islets was insufficient to make a significant impact on the required daily insulin dose after the first year. A major obstacle reported with transplant encapsulation of islets is opacified or cloudy capsules. Reports suggest that capsule opacification may affect the functional longevity of the islets, thus negatively impacting islet longevity (Elliott et al. 2007; Clayton et al. 1993).

Potential Sources of Islet Donors for Clinical Xenotransplantation

Piscine Islets

Although fish may not be the first thought when thinking of islet transplantation, but studies show that tilapia may be a perfect factory fish because it breeds easily, and studies have shown that tilapia islets, known as Brockmann bodies (BBs), can be easily and inexpensively harvested (Yang et al. 1997; Wright et al. 2004). They are currently being explored as an alternative islet source for xenotransplantation (Wright et al. 2004). BBs are separate organs located near the liver within a triangular region of adipose tissue (Wright and Yang 1997; Yang and Wright 1995; Dickson et al. 2003; Yang et al. 1997). According to reports by Wright et al., when BBs are transplanted into streptozotocin-induced diabetic mice under the kidney capsule, function is restored immediately and the animals are able to maintain long-term normoglycemia (>50 days) (Wright et al. 1992, 2004). However, BBs do have a few drawbacks. Tilapia insulin differs from human insulin by 17 amino acids; thus, it is required to be "humanized" prior to transplant in order to become biologically active in the human recipient (Alexander et al. 2006; Wright et al. 1992, 2004; Wright and Yang 1997). Furthermore, the xenotransplantation of fish BBs into humans would require the need for encapsulation to prevent recipient's immunological rejection of the foreign tissue.

Bovine Islets

Bovine islets can be isolated by a procedure similar to human islet isolation, using collagenase digestion and density gradient purification, and reports have shown graft function when they have been transplanted into diabetic nude mice (Lanza et al. 1995a). In addition, bovine islets can be cultured up to 4 weeks while maintaining glucose responsiveness (Marchetti et al. 1995). Encapsulated bovine islets have also been shown to restore euglycemia in non-immunosuppressed immunocompetent diabetic mice (Lanza et al. 1995b) and rats (Lanza et al. 1991). These results demonstrate the potential use of bovine islets as islet donors, because they are readily available and in high volume in the USA. However promising bovine islets are, they also possess a few drawbacks. Bovine islets are difficult and expensive to isolate and require an experienced team to be isolated (Figliuzzi et al. 2000; Friberg 2011). Studies have shown that bovine insulin differs from human insulin by three amino acids, which causes a different response to glucose levels compared to human insulin when used in exogenous therapies, and reports have shown that it can be more immunogenic (Derewenda and Dodson 1993).

Porcine Islets

Porcine islets have received the most attention as a possible source for islet transplantation. Porcine insulin differs from human insulin by only one amino acid (Storms et al. 1987), and current studies support the use of these islets in the clinical setting. In the early 1990s, studies by Lanza et al. showed that encapsulated islets that were isolated from adult pigs and then transplanted into the peritoneal

cavity of diabetic Lewis rats could establish euglycemia within the first month and maintain it for a prolonged period of time (Lanza et al. 1991).

Another study by Sun et al. reported that euglycemia was maintained for up to 10 months post transplant in diabetic Balb/c mice that received a transplant of encapsulated adult porcine islets (Sun et al. 1992). Another study by Foster et al. used fetal pig islet-like clusters and reported 20 weeks of normoglycemia following transplantation into diabetic mice (Foster et al. 2007), while another study found that encapsulated neonatal porcine islets maintained euglycemia for more than 174 days (Omer et al. 2003). The study of porcine islets as a plausible islet source continues, and a variety of groups, using adult, fetal, and neonatal pig islets, also support the findings of Lanza, Sun, and Foster that porcine islets can maintain normal glycemia for extended periods of time (Calafiore et al. 2004; Cruise et al. 1999; Dufrane et al. 2006b; Duvivier-Kali et al. 2004; Foster et al. 2007; Lanza et al. 1993, 1999; Omer et al. 2003; Safley et al. 2008).

Although all of these show promising results and have stimulated interest in using porcine islets as a clinical source for transplant, several obstacles still remain. Adult porcine islets are shown to be difficult and expensive to isolate, and the islets tend to fragment during the isolation and purification processes (Falorni et al. 1996; Kuo et al. 1993). Conversely, neonatal and fetal islet isolation are much less difficult; however, studies have shown these islets require time to function in vivo (Mancuso et al. 2010). Even though these drawbacks can be addressed through changes in processes, all transplanted porcine islets need to be either encapsulated, within an immunoprotective layer to circumvent the species barrier, thus protect the islets from graft rejection, or the recipients receiving them must be given adequate immunosuppressive therapy, with numerous adverse effects (Hering et al. 2006; Buhler et al. 2002).

Limitations of Xenotransplantation

Xenotransplantation has a variety of limitations, most notably the immunologic and physiologic incompatibilities between animals and humans (Ibrahim et al. 2006). Another cause for concern is the potential of transplanted animal tissue to transmit endogenous retroviruses and other infectious agents across the species barrier, posing significant risk to the patient's health (Boneva et al. 2001; Fano et al. 1998).

According to the United States Federal Drug Administration's guidelines for xenotransplantation process, the potential health risk to the patient and community may include the possible transmission of infectious agents, which may not be detectable or infectious to the animal host. With the possibility of transmission, the FDA's report also outlines that there is an elevated risk of infection when transplanted into immunocompromised recipients, and there is a potential for recombination of animal and human genetic material, within the recipient, thus creating new pathogenic forms of microorganisms ((CBER) CfBEaR 2003).

Pig to Human Infections

Due to the similarities in overall physiology, there is a potential for pathogens to be transmitted from pigs to humans (Fano et al. 1998). There has been a special interest in a group of viruses known as porcine endogenous retroviruses, or PERVs. This interest is, namely, because of their potential to infect humans post transplant, shown by their ability to infect human cells in vitro and their continued presence in the donor animal's genome (Clark 1999; Denner 1998; Vanderpool 1999; Wilson 2008). Even though the potential of transmission exists, extensive investigations have uncovered no evidence of a human infection with PERV (Cozzi et al. 2005; Denner and Tonjes 2012).

Even though there has yet to be a recorded human infection with PERV or other porcine-transmitted microorganisms, there is still the possibility to infect and harm the transplant patient, which could extend additional risk to the transplant hospital personnel, family members, and to the wider community (Fishman 1997; Fishman and Patience 2004; Bach et al. 1998).

Due to the risk factors associated with the transmission of infectious agents, there must be well-outlined and regulated standards implemented long before any clinical trial can commence. In 2004 the International Xenotransplant Association (IXA) Ethics Committee released a position paper stating that there must be strict regulations in order to minimize the risks of contracting animal pathogens during and after transplantation, including the need to ensure appropriate animal husbandry, which entails the use of barrier-contained breeding facilities and stringent controls for surgical procedures and screening of cells or organs for transplant. All of these regulations must also be overseen by federal or national agencies to be effective in minimizing health risks (Sykes et al. 2003).

These standards will hopefully help minimize the use of xenotransplantation without clear governmental regulation and increase the understanding for national guidelines to minimize any potential health risk to the recipient and their community.

Facilities and Microbial Safety

Microbial safety is an important focus when it comes to xenotransplantation, and many facilities and transplant centers are planning to create standards for animal housing, surgery, islet isolations, and clinical transplant, to minimize the transmission of pathogens. Many centers have implemented current good manufacturing practices (cGMP) for islet processing facilities to provide a clean and safe environment for clinical transplants (Larijani et al. 2012; Korbutt 2009). Many studies have focused on improving these facilities and determining the microbial output they may have. One study performed reported that there was a 31 % microbial contamination in the transplant solution of human pancreases, but

through the process of surface decontamination and islet processing, there was a 92 % clearance in microbial contamination (Kin et al. 2007). Even though the original solution was positive for contamination, the risk of a positive culture after processing is low but still possible within a cGMP facility. From these results in cadaveric human islet transplantation, many centers are trying to develop sterile animal housing facilities for breeding and pancreas procurement, along with established clean rooms used for islet processing and final product release (Schuurman 2009).

One of the first pathogen-free pig farms was implemented in New Zealand and led by Living Cell Technologies (LCT) (2013). The LCT farm holds a herd of rare pathogen-free Auckland Island pigs. The current facility, under the regulation of the New Zealand government, houses over 1,000 virus- and disease-free pigs for breeding and islet isolation (Living Cell Technologies 2013).

The Spring Point Project (SPP), in Minneapolis Minnesota, currently has a pathogen-free, medical grade porcine facility capable of breeding, housing, and pancreas procurement and has received the Class V medical license from the FDA in Aug 2012 (Bosserman 2010).

Facilities like LCT and SPP require extensive resources in order to develop. Stable farm sentinel pigs must be tested monthly for a variety of pathogens, including *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*, and all products or animals leaving the facilities must be tested to confirm a non-disease state (Muirhead and Alexander 1997).

The Public Health Service (PHS) Guidelines state that extensive preclinical data must be obtained in order to progress to clinical trials and screening must be performed on all source herds and the final product for transplantation, along with stringent testing of clinical patients (Schuurman 2008).

Despite stringent testing being implemented and improved facilities being designed, the risk of infection still exists. To remove this risk, these pathogens must be removed from the source animal's genome. PHS states that a virus-free herd and final product are needed to ensure no pathogens are transferred from the source to the patient (Allan 1995), but this process will take time and is still in the early stages.

Islet Xenotransplantation Transplant Studies: Large Animals and Clinical Trials

Canine Islet Xenotransplantation

Large animal xenotransplantation trials help collect the requisite preclinical data necessary to justify proceeding into clinical trials because of the metabolic and immunological similarities between larger animals and humans. Canines have been used as large animal models because of the ease of training

and also because they are a well-established diabetes model, either by chemical induction (streptozotocin or alloxan) or by surgical pancreatectomy (Anderson et al. 1993; Guptill et al. 2003). Many groups have shown positive results using encapsulated porcine islets transplanted into diabetic canines. In 2009 a group from Argentina microencapsulated adult pig islets and reported decreased insulin requirements and glycosylated hemoglobin (HbA1c) for up to 6–12 months post transplantation (Abalovich et al. 2009). Previous articles have reported that encapsulated porcine islets transplanted into diabetic canines can maintain euglycemia for >100 days with detectable porcine C-peptide levels in the serum (Kin et al. 2002; Monaco et al. 1991; Petruzzo et al. 1991).

With these reports and continued interest among researchers, canines offer a valid model for preclinical xeno-islet transplantation studies.

Nonhuman Primate Islet Xenotransplantation

Nonhuman primate (NHP) transplant models offer a stepping stone to the clinic by offering the closest model to a human for transplant, due to the similarities they share with humans, genetically, immunologically, and physically (Dufrane and Gianello 2012). Studies suggest that there is the potential for long-term survival and function of xenotransplanted, in contrast to studies performed with primate solid organ xenotransplantation (Cozzi 2009).

However, a study out of the University of Minnesota reported that when adult porcine islets were transplanted into NHP, the C-peptide levels were lower than NHPs transplanted with cadaveric human islets, thus suggesting lower glucose-stimulated insulin release by the transplanted porcine islets (Graham et al. 2011). Although encouraging, these results must be further addressed if porcine islets are to be used as a viable source for transplant and lead to the development of an improved, functional porcine islet type. A study in 2010 by Dufrane et al. reported that, without encapsulation, adult porcine islets are rejected after 7 days when transplanted under the kidney capsule of NHPs without immunosuppression, while the same islets can maintain viability and function in vivo for up to 6 months, if encapsulated first (Dufrane et al. 2010).

In 2012, a comprehensive review in Diabetes reported that several research centers have had successes with pig to NHP islet transplants, with normoglycemia maintained for up to 6 months post transplant. Out of the six reported studies, five reported the combination of transplants and immunosuppression with porcine donor tissue of varying ages ranging from adult to fetal islet tissue. Furthermore, one study even transplanted GTKO neonatal porcine islets with two out of the six groups within this study used encapsulation techniques (van der Windt et al. 2012).

It is still unknown which porcine islet model and age is “best” for clinical transplant and whether or not there is a need for encapsulation or immunosuppression regimes at this stage.

Clinical Islet Xenotransplanation

With all the current support for xenotransplantation and the NHP data to support it, this procedure has been implemented in many countries. However, efforts within the United States have yet to result in meaningful change. As of today, several countries all over the world are performing clinical trials using porcine islets (Table 1).

In 1994, Groth et al. found that isolated fetal porcine islets transplanted into diabetic kidney transplant patients, either intraportally or under the kidney capsule, were able to produce porcine C-peptide for up to 400 days post transplantation (Groth et al. 1994). This study, along with current findings, offers encouragement that the use of optimized islet preparations and encapsulation techniques will lead to improved clinical outcomes and result in long-term survival of transplanted islets (Elliott et al. 2007).

One of the most notable and well-documented clinical trials are the trials being performed by Living Cell Technologies (LCT). LCT is a company based in Australia that focuses on encapsulated cellular therapies for human therapeutics and currently has clinical trials in three countries for treating and curing T1D (Living Cell Technologies 2013). LCT's clinical trials use pathogen-free, medical grade fetal pigs that have been encapsulated with their "Diabecell" device. The first clinical trial lead by LCT was conducted in Australia. This trial was to demonstrate the safety and function of the "Diabecell" device (Living Cell Technologies 2012a). This trial reported that patients showed improvement in HbA1c levels and decreases in hypoglycemia unawareness (Living Cell Technologies 2012b). The results of the first trial from Australia led to a phase I/IIa clinical trial in New Zealand, where a total of 14 patients were transplanted. They were classified into groups based on the dose of islets they received: 5,000, 10,000, 15,000, and 20,000 IEQ/kg doses. A reduction in daily insulin requirements in all groups, with a statistically significant improvement in hypoglycemic unawareness events in the groups transplanted with 5,000 and 10,000IEQ was reported. In addition, there was no evidence of any transmissible zoonotic infection in the recipients (Living Cell Technologies 2012a).

Then in 2011 LCT commenced a third phase I/IIa clinical trial in Argentina. Patients enrolled in this trial received a dose of either 10,000 IEQ/kg ($n = 4$) or two implants (12 weeks apart) of 5,000 IEQ/kg each ($n = 4$). Reductions in hypoglycemic events, HbA1c levels, and daily insulin requirements were noted in both groups after 24 weeks post transplant (Living Cell Technologies 2012c). The results from all of these studies are still underway, but the results from all of them have been overwhelmingly positive. Patients demonstrated lower HbA1c levels and lower daily blood glucose levels.

With an increasing number of clinical trials being performed all over the world in the field of xenotransplantation, there has been increased interest in further developments and future applications. Even though the majority of patients have seen improvements in their diabetes management, without infection of evidence of PERV injection noted to date, long-term studies are still required to confirm improvements in recipient quality of life (Denner and Tonjes 2012; Argaw et al. 2004; Delwart and Li 2012; Garkavenko et al. 2004; Boneva et al. 2001).

Table 1 Xenotransplant clinical trials: pig to human transplant. Summary of clinical islet xenotransplantation for type 1 diabetes treatment (National Health and Medical Research Council 2013; Sgroi et al. 2010)

Tissue type	Country	Trial period	# Patients (age)	Encapsulation type	Company/author	Results
Fetal pig islets	New Zealand	2010–present	20+ (adult)	Alginate capsules	LCT (R.B Elliot) (Living Cell Technologies 2012b)	Improved HbA1c and reduction in hyperglycemia. Two patients were able to stop insulin after 4 months
Fetal pig islets	Russia	2007–present	8 (21–68)	Alginate capsules	LCT (R.B Elliot) (Living Cell Technologies 2010)	Six out of the eight patients had improved blood glucose control, with reduction in both insulin requirements and HbA1c. Two patients discontinued insulin for 8 months
Fetal pig islets	Argentina	2011–present	8 (adult), 20 recruited	Alginate capsules	LCT (R.B Elliot) (Living Cell Technologies 2012c)	No reports yet
Porcine islets	Mexico	2002 and 2005	12 (14.7 mean years)	Collagen devise w/2 steel mesh tubes	Valdes Gonzales (2002)	All patients infection free and two became insulin independent for several months; one continues to be insulin free after 2 years
Pig islets	Ukraine	1995, in progress	Unknown	None	I.S. Turchin (1995)	Unknown
Fetal pig islet like clusters	Sweden	1979–1993	10	None	C Gustav Groth (1994)	No C-peptide detected in plasma, but detected in urine after 460 days. Kidney biopsies showed viable cells
Pig islets	China	2005	22	None; with immunosupression	W. Wang (2011)	Reduction of insulin requirements and lowering of HbA1c. Detection of porcine C-peptide, immunosuppressants stopped after 1 year

Recent Developments and Future Applications

Genetically Modified Porcine Islets

Genetically modified porcine islets could offer advantages in graft function and limit the host's immune response to the transplant. Current studies focus on gene transfer techniques in order to decrease the host response to the graft. The genetic transduction of genes like antiapoptotic and antinecrotic Bcl-2 into porcine islets has shown to decrease the release of natural xenoreactive antibodies and cell lysis by the complement cascade (Contreras et al. 2001). Other molecules, like MSPEG (PEG-mono-succimidyl-succinate) and DSPEG (PEG-di-succimidyl-succinate), in combination with Bcl-2 transduction have shown no negative effect on islet viability, function, or morphology (Hui et al. 2005). Results from studies using these methods have also shown that these combinations have positive effects, with a reduction in lactate dehydrogenase release, a marker for cellular necrosis, when transplanted into animal recipients (Contreras et al. 2004). The use of T-cell inhibitors to limit the graft rejection by the adaptive immune system has also been studied. In a study by Klymiuk et al., neonatal porcine islets were isolated from transgenetic pigs that expressed LEA29Y, which inhibits T-cell costimulation. Isolated islets were then transplanted into diabetic immunocompetent mice and resulted in complete protection from being rejected by the host (Klymiuk et al. 2012).

These approaches and the development of genetically modified porcine islets may allow for transplantation without the need for toxic immunosuppressive regimens allowing for better survival of the islet graft and better overall health of the transplant recipient.

Gal-Deficient Galactosyl Transferase Knockout and Pathogen-Free Hybrids

The first target in antibody-mediated rejection in the pig to human transplant paradigm is the carbohydrate galactose-α1,3-galactose (α-gal). In porcine islets, expression levels varied depending on the size of the islet and not the age of the pig donor (Dufrane et al. 2005). Since nearly all porcine islets express α-gal, there have been recent developments targeted at the creation of a Gal knockout pig (GTKO) strain. GTKO pigs are deficient in α 1,3 galactosyl transferase, a strategy that addresses antibody-mediated α-Gal-based rejection. Studies characterizing GTKO pig islets have demonstrated insulin independence and establishment of normoglycemia (Thompson et al. 2011). A study in 2011 compared wild-type neonatal porcine islets (non-GTKO islets) with neonatal-GTKO islets and found an 80 % insulin independence with the GTKO islets compared to 20 % insulin independence with the wild type (Thompson et al. 2011). This experiment and the development of GTKO pigs allow for improved consistency and overall transplantation outcomes for the treatment of T1D.

Along with GTKO pigs, researchers have also been focusing on limiting or eradicating the occurrence of porcine-specific pathogens, thus creating pathogen-free pigs (Denner and Tonjes 2012; Vanrompay et al. 2005). Controlled environment breeding and housing facilities of porcine donors along with sterile rooms for pancreas procurement allow for a decreased risk of microbial contamination from outside sources and enhanced overall transplantation success (Gouin et al. 1997). Specific pathogen-free (SPF) pig farms have also created a suitable and reliable source for islet transplant that limit the risk of porcine microbe transmission but are limited because they do not limit PERVs because they are so well integrated into the pigs genome (Denner and Tonjes 2012). Without the development of a cross-species model, the study and validation of PERV transmission rates is nearly impossible (Denner et al. 2009). Thus, the creation of a PERV-free pig strain remains crucial to further research in this field.

Immunoisolation for Islet Xenotransplantation

The implementation of xenogeneically sourced islets for transplant therapy of T1D requires a solution to tackle the problem of acute rejection (Lanza et al. 1997; Platt 2000). While immunosuppression has previously been the standard therapy to prevent rejection in human islet allotransplantation, immunoisolation has been studied as an alternative for xenograft islets due to the presence of cell surface antigens such as α-gal (La Flamme et al. 2007; Wang et al. 1997).

A successful immunoisolation technique can allow islets to be transplanted without any immunosuppression. One such in vivo study utilizing porcine islets that have been immunoisolated using encapsulation in alginate showed remarkable longevity in the reversal of diabetes, showing blood glucose management up to 550 days when transplanted into diabetic rats (Meyer et al. 2008). There are several factors that influence this potential for success. One major factor involved in transplant success is the quality and purity of the islets that have been isolated because a purer preparation of islets reduces the chance of extraneous exocrine tissue to protrude out of the encapsulation layer (Narang and Mahato 2006; Omer et al. 2005). Another factor involved is the chemical composition, and purity of the alginate of greater purity has shown to improve the biocompatibility of the capsules, such that the immunoisolated islets are not recognized as foreign bodies (King et al. 2003; Hernandez et al. 2010).

Future Direction of Islet Xenotransplantation

The future direction of xenotransplantation may be headed in the development of a genetically engineered donor animal in order to reduce immunogenicity post transplant or through the development of immunoisolating materials such as alginate to allow the transplanted xenograft to remain undetected by the host immune

system. Improvements in these technologies could make xenotransplantation a viable treatment option to circumvent the current donor tissue shortage that truly limits our ability to treat the diabetic population.

Cross-References

- ▶ Advances in Clinical Islet Isolation
- ▶ Islet Encapsulation
- ▶ Successes and Disappointments with Clinical Islet Transplantation
- ▶ The Comparative Anatomy of Islets

References

(CBER) CfBEaR (2003) Guidance for industry: source animal, product, preclinical, and clinical issues concerning the use of xenotransplantation products in humans. Food and Drug Administration. http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/xenotransplantation/ucm074354.htm#CLINICALISSUESINXENOTRANSPLANTATION. Accessed 27 Aug 2013

Abalovich AG, Bacque MC, Grana D, Milei J (2009) Pig pancreatic islet transplantation into spontaneously diabetic dogs. Transplant Proc 41(1):328–330

Alexander EL, Dooley KC, Pohajdak B, Xu BY, Wright JR Jr (2006) Things we have learned from tilapia islet xenotransplantation. Gen Comp Endocrinol 148(2):125–131

Allan JS (1995) Xenograft transplantation of the infectious disease conundrum. ILAR J/Natl Res Counc Inst Lab Anim Resour 37(1):37–48

Anderson HR, Stitt AW, Gardiner TA, Lloyd SJ, Archer DB (1993) Induction of alloxan/streptozotocin diabetes in dogs: a revised experimental technique. Lab Anim 27(3):281–285

Argaw T, Colon-Moran W, Wilson CA (2004) Limited infection without evidence of replication by porcine endogenous retrovirus in guinea pigs. J Gen Virol 85(Pt 1):15–19

Ayares D, Phelps C, Vaught T, Ball S, Monahan J, Walters A, Giraldo A, Bertera S, van der Windt D, Wijkstrom M, Cooper D, Bottino R, Trucco M (2013) Multi-transgenic pigs for xenoislet transplantation. Xenotransplantation 20(1):46

Bach FH, Fishman JA, Daniels N, Proimos J, Anderson B, Carpenter CB, Forrow L, Robson SC, Fineberg HV (1998) Uncertainty in xenotransplantation: individual benefit versus collective risk. Nat Med 4(2):141–144

Boneva RS, Folks TM, Chapman LE (2001) Infectious disease issues in xenotransplantation. Clin Microbiol Rev 14(1):1–14

Bosserman J (2010) Animal care facility receives highest accreditation. Spring Point Project. http://www.springpointproject.org/wp-content/uploads/2012/07/July-2010-Animal-care-facility-receives-highest-accreditation.pdf. Accessed 27 Aug 2013

Buhler L, Basker M, Alwayn IP, Goepfert C, Kitamura H, Kawai T, Gojo S, Kozlowski T, Ierino FL, Awwad M, Sachs DH, Sackstein R, Robson SC, Cooper DK (2000) Coagulation and thrombotic disorders associated with pig organ and hematopoietic cell transplantation in nonhuman primates. Transplantation 70(9):1323–1331

Buhler L, Deng S, O'Neil J, Kitamura H, Koulmanda M, Baldi A, Rahier J, Alwayn IP, Appel JZ, Awwad M, Sachs DH, Weir G, Squifflet JP, Cooper DK, Morel P (2002) Adult porcine islet transplantation in baboons treated with conventional immunosuppression or a non-myeloablative regimen and CD154 blockade. Xenotransplantation 9(1):3–13

Bulato C, Radu C, Simioni P (2012) Studies on coagulation incompatibilities for xenotransplantation. Methods Mol Biol 885:71–89

Calafiore R, Basta G, Luca G, Calvitti M, Calabrese G, Racanicchi L, Macchiarulo G, Mancuso F, Guido L, Brunetti P (2004) Grafts of microencapsulated pancreatic islet cells for the therapy of diabetes mellitus in non-immunosuppressed animals. Biotechnol Appl Biochem 39(Pt 2):159–164

Chhabra P, Brayman KL (2011) Current status of immunomodulatory and cellular therapies in preclinical and clinical islet transplantation. J Transplant 2011:637692

Clark MA (1999) This little piggy went to market: the xenotransplantation and xenozoonose debate. J Law Med Ethics 27(2):137–152

Clayton HA, James RF, London NJ (1993) Islet microencapsulation: a review. Acta Diabetol 30(4):181–189

Cnop M, Welsh N, Jonas JC, Jorns A, Lenzen S, Eizirik DL (2005) Mechanisms of pancreatic β-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes 54(Suppl 2):S97–S107

Contreras JL, Bilbao G, Smyth C, Eckhoff DE, Xiang XL, Jenkins S, Cartner S, Curiel DT, Thomas FT, Thomas JM (2001) Gene transfer of the Bcl-2 gene confers cytoprotection to isolated adult porcine pancreatic islets exposed to xenoreactive antibodies and complement. Surgery 130(2):166–174

Contreras JL, Xie D, Mays J, Smyth CA, Eckstein C, Rahemtulla FG, Young CJ, Anthony Thompson J, Bilbao G, Curiel DT, Eckhoff DE (2004) A novel approach to xenotransplantation combining surface engineering and genetic modification of isolated adult porcine islets. Surgery 136(3):537–547

Cooper DK, Ayares D (2011) The immense potential of xenotransplantation in surgery. Int J Surg 9(2):122–129

Cowan PJ, Aminian A, Barlow H, Brown AA, Dwyer K, Filshie RJ, Fisicaro N, Francis DM, Gock H, Goodman DJ, Katsoulis J, Robson SC, Salvaris E, Shinkel TA, Stewart AB, d'Apice AJ (2002) Protective effects of recombinant human antithrombin III in pig-to-primate renal xenotransplantation. Am J Transplant 2(6):520–525

Cozzi E (2009) On the road to clinical xenotransplantation. Transpl Immunol 21(2):57–59

Cozzi E, Bosio E, Seveso M, Vadori M, Ancona E (2005) Xenotransplantation-current status and future perspectives. Br Med Bull 75–76:99–114

Cruise GM, Hegre OD, Lamberti FV, Hager SR, Hill R, Scharp DS, Hubbell JA (1999) In vitro and in vivo performance of porcine islets encapsulated in interfacially photopolymerized poly (ethylene glycol) diacrylate membranes. Cell Transplant 8(3):293–306

Delwart E, Li L (2012) Rapidly expanding genetic diversity and host range of the Circoviridae viral family and other Rep encoding small circular ssDNA genomes. Virus Res 164(1–2): 114–121

Denner J (1998) Immunosuppression by retroviruses: implications for xenotransplantation. Ann N Y Acad Sci 862:75–86

Denner J, Tonjes RR (2012) Infection barriers to successful xenotransplantation focusing on porcine endogenous retroviruses. Clin Microbiol Rev 25(2):318–343

Denner J, Schuurman HJ, Patience C (2009) The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes – chapter 5: strategies to prevent transmission of porcine endogenous retroviruses. Xenotransplantation 16(4):239–248

Derewenda U, Dodson GG (1993) Molecular structures in biology. Oxford University Press, Oxford, pp 260–277

Dickson BC, Yang H, Savelkoul HF, Rowden G, van Rooijen N, Wright JR Jr (2003) Islet transplantation in the discordant tilapia-to-mouse model: a novel application of alginate microencapsulation in the study of xenograft rejection. Transplantation 75(5):599–606

Dufrane D, Gianello P (2012) Pig islet for xenotransplantation in human: structural and physiological compatibility for human clinical application. Transplant Rev 26(3):183–188

Dufrane D, Goebbels RM, Guiot Y, Gianello P (2005) Is the expression of Gal-α1,3Gal on porcine pancreatic islets modified by isolation procedure? Transplant Proc 37(1):455–457
Dufrane D, Goebbels RM, Saliez A, Guiot Y, Gianello P (2006a) Six-month survival of microencapsulated pig islets and alginate biocompatibility in primates: proof of concept. Transplantation 81(9):1345–1353
Dufrane D, Steenberghe M, Goebbels RM, Saliez A, Guiot Y, Gianello P (2006b) The influence of implantation site on the biocompatibility and survival of alginate encapsulated pig islets in rats. Biomaterials 27(17):3201–3208
Dufrane D, Goebbels RM, Gianello P (2010) Alginate macroencapsulation of pig islets allows correction of streptozotocin-induced diabetes in primates up to 6 months without immunosuppression. Transplantation 90(10):1054–1062
Duvivier-Kali VF, Omer A, Lopez-Avalos MD, O'Neil JJ, Weir GC (2004) Survival of microencapsulated adult pig islets in mice in spite of an antibody response. Amer J Transplant 4(12):1991–2000
Dvornik D (1978) Chapter 17. Chronic complications of diabetes. In: Annual reports in medicinal chemistry, pp 159–166
Ekser B, Cooper DK (2010) Overcoming the barriers to xenotransplantation: prospects for the future. Expert Rev Clin Immunol 6(2):219–230
Elliott RB, Escobar L, Tan PL, Garkavenko O, Calafiore R, Basta P, Vasconcellos AV, Emerich DF, Thanos C, Bambra C (2005) Intraperitoneal alginate-encapsulated neonatal porcine islets in a placebo-controlled study with 16 diabetic cynomolgus primates. Transplant Proc 37(8):3505–3508
Elliott RB, Escobar L, Tan PL, Muzina M, Zwain S, Buchanan C (2007) Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. Xenotransplantation 14(2):157–161
Falorni A, Basta G, Santeusanio F, Brunetti P, Calafiore R (1996) Culture maintenance of isolated adult porcine pancreatic islets in three-dimensional gel matrices: morphologic and functional results. Pancreas 12(3):221–229
Fano A, Cohen MJ, Cramer M, Greek R, Kaufman SR (1998) Of pigs, primates, and plagues: a layperson's guide to the problems with animal-to-human organ transplants. Med Res Modernization Comm, http://www.mrmcmed.org/pigs.html. Accessed August 27, 2013 2013
Figliuzzi M, Zappella S, Morigi M, Rossi P, Marchetti P, Remuzzi A (2000) Influence of donor age on bovine pancreatic islet isolation. Transplantation 70(7):1032–1037
Fishman JA (1997) Xenosis and xenotransplantation: addressing the infectious risks posed by an emerging technology. Kidney Int Suppl 58:S41–S45
Fishman JA, Patience C (2004) Xenotransplantation: infectious risk revisited. Ame J Transplant 4(9):1383–1390
Foster JL, Williams G, Williams LJ, Tuch BE (2007) Differentiation of transplanted microencapsulated fetal pancreatic cells. Transplantation 83(11):1440–1448
Frank A, Deng S, Huang X, Velidedeoglu E, Bae YS, Liu C, Abt P, Stephenson R, Mohiuddin M, Thambipillai T, Markmann E, Palanjian M, Sellers M, Naji A, Barker CF, Markmann JF (2004) Transplantation for type I diabetes: comparison of vascularized whole-organ pancreas with isolated pancreatic islets. Ann Surg 240(4):631–640; discussion 640–633
Friberg A (2011) Standardization of islet isolation and transplantation variables. Digit Compr Summ Upps Diss Fac Med 669:76
Garkavenko O, Croxson MC, Irgang M, Karlas A, Denner J, Elliott RB (2004) Monitoring for presence of potentially xenotic viruses in recipients of pig islet xenotransplantation. J Clin Microbiol 42(11):5353–5356
Gouin E, Rivereau AS, Darquy S, Cariolet R, Jestin A, Reach G, Sai P (1997) Minimisation of microbial contamination for potential islet xenografts using specific pathogen-free pigs and a protected environment during tissue preparation. Diabetes Metab 23(6):537–540

Graham ML, Bellin MD, Papas KK, Hering BJ, Schuurman HJ (2011) Species incompatibilities in the pig-to-macaque islet xenotransplant model affect transplant outcome: a comparison with allotransplantation. Xenotransplantation 18(6):328–342

Greene SA, Smith MA, Cartwright B, Baum JD (1983) Comparison of human versus porcine insulin in treatment of diabetes in children. British Med J 287(6405):1578–1579

Groth CG, Korsgren O, Tibell A, Tollemar J, Moller E, Bolinder J, Ostman J, Reinholt FP, Hellerstrom C, Andersson A (1994) Transplantation of porcine fetal pancreas to diabetic patients. Lancet 344(8934):1402–1404

Guptill L, Glickman L, Glickman N (2003) Time trends and risk factors for diabetes mellitus in dogs: analysis of veterinary medical data base records (1970–1999). Vet J 165(3):240–247

Han X, Tuch BE (2001) Cloning and characterization of porcine insulin gene. Comp Biochem Physiol B Biochem Mol Biol 129(1):87–95

Heller SR (2008) Minimizing hypoglycemia while maintaining glycemic control in diabetes. Diabetes 57(12):3177–3183

Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, Matsumoto I, Ihm SH, Zhang HJ, Parkey J, Hunter DW, Sutherland DE (2005) Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. JAMA 293(7):830–835

Hering BJ, Wijkstrom M, Graham ML, Hardstedt M, Aasheim TC, Jie T, Ansite JD, Nakano M, Cheng J, Li W, Moran K, Christians U, Finnegan C, Mills CD, Sutherland DE, Bansal-Pakala P, Murtaugh MP, Kirchhof N, Schuurman HJ (2006) Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. Nat Med 12(3):301–303

Hernandez RM, Orive G, Murua A, Pedraz JL (2010) Microcapsules and microcarriers for in situ cell delivery. Adv Drug Deliv Rev 62(7–8):711–730

Hui H, Khoury N, Zhao X, Balkir L, D'Amico E, Bullotta A, Nguyen ED, Gambotto A, Perfetti R (2005) Adenovirus-mediated XIAP gene transfer reverses the negative effects of immunosuppressive drugs on insulin secretion and cell viability of isolated human islets. Diabetes 54 (2):424–433

HumanXenoTransplant (2013) Inventory of human xenotransplantation practices. http://www.humanxenotransplant.org/home/index.php. Accessed 26 Aug 2013

Ibrahim Z, Busch J, Awwad M, Wagner R, Wells K, Cooper DK (2006) Selected physiologic compatibilities and incompatibilities between human and porcine organ systems. Xenotransplantation 13(6):488–499

Ierino FL, Kozlowski T, Siegel JB, Shimizu A, Colvin RB, Banerjee PT, Cooper DK, Cosimi AB, Bach FH, Sachs DH, Robson SC (1998) Disseminated intravascular coagulation in association with the delayed rejection of pig-to-baboon renal xenografts. Transplantation 66 (11):1439–1450

Kin T, Iwata H, Aomatsu Y, Ohyama T, Kanehiro H, Hisanaga M, Nakajima Y (2002) Xenotransplantation of pig islets in diabetic dogs with use of a microcapsule composed of agarose and polystyrene sulfonic acid mixed gel. Pancreas 25(1):94–100

Kin T, Rosichuk S, Shapiro AM, Lakey JR (2007) Detection of microbial contamination during human islet isolation. Cell Transplant 16(1):9–13

King A, Strand B, Rokstad AM, Kulseng B, Andersson A, Skjak-Braek G, Sandler S (2003) Improvement of the biocompatibility of alginate/poly-L-lysine/alginate microcapsules by the use of epimerized alginate as a coating. J Biomed Mater Res A 64(3):533–539

Klymiuk N, van Buerck L, Bahr A, Offers M, Kessler B, Wuensch A, Kurome M, Thormann M, Lochner K, Nagashima H, Herbach N, Wanke R, Seissler J, Wolf E (2012) Xenografted islet cell clusters from INSLEA29Y transgenic pigs rescue diabetes and prevent immune rejection in humanized mice. Diabetes 61(6):1527–1532

Korbutt GS (2009) The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes–chapter 3: pig islet product manufacturing and release testing. Xenotransplantation 16(4):223–228

Kozlowski T, Shimizu A, Lambrigts D, Yamada K, Fuchimoto Y, Glaser R, Monroy R, Xu Y, Awwad M, Colvin RB, Cosimi AB, Robson SC, Fishman J, Spitzer TR, Cooper DK, Sachs DH (1999) Porcine kidney and heart transplantation in baboons undergoing a tolerance induction regimen and antibody adsorption. Transplantation 67(1):18–30

Kuo CY, Myracle A, Burghen GA, Herrod HG (1993) Neonatal pig pancreatic islets for transplantation. In Vitro Cell Dev Biol Anim 29A(9):677–678

Kuwaki K, Knosalla C, Dor FJ, Gollackner B, Tseng YL, Houser S, Mueller N, Prabharasuth D, Alt A, Moran K, Cheng J, Behdad A, Sachs DH, Fishman JA, Schuurman HJ, Awwad M, Cooper DK (2004) Suppression of natural and elicited antibodies in pig-to-baboon heart transplantation using a human anti-human CD154 mAb-based regimen. Am J Transplant 4(3):363–372

Kuwaki K, Tseng YL, Dor FJ, Shimizu A, Houser SL, Sanderson TM, Lancos CJ, Prabharasuth DD, Cheng J, Moran K, Hisashi Y, Mueller N, Yamada K, Greenstein JL, Hawley RJ, Patience C, Awwad M, Fishman JA, Robson SC, Schuurman HJ, Sachs DH, Cooper DK (2005) Heart transplantation in baboons using α1,3-galactosyltransferase gene-knockout pigs as donors: initial experience. Nat Med 11(1):29–31

La Flamme KE, Popat KC, Leoni L, Markiewicz E, La Tempa TJ, Roman BB, Grimes CA, Desai TA (2007) Biocompatibility of nanoporous alumina membranes for immunoisolation. Biomaterials 28(16):2638–2645

Lanza RP, Butler DH, Borland KM, Staruk JE, Faustman DL, Solomon BA, Muller TE, Rupp RG, Maki T, Monaco AP et al (1991) Xenotransplantation of canine, bovine, and porcine islets in diabetic rats without immunosuppression. Proc Natl Acad Sci U S A 88(24):11100–11104

Lanza RP, Beyer AM, Staruk JE, Chick WL (1993) Biohybrid artificial pancreas. Long-term function of discordant islet xenografts in streptozotocin diabetic rats. Transplantation 56(5):1067–1072

Lanza RP, Kuhtreiber WM, Ecker DM, Marsh JP, Chick WL (1995a) Transplantation of porcine and bovine islets into mice without immunosuppression using uncoated alginate microspheres. Transplant Proc 27(6):3321

Lanza RP, Kuhtreiber WM, Ecker D, Staruk JE, Chick WL (1995b) Xenotransplantation of porcine and bovine islets without immunosuppression using uncoated alginate microspheres. Transplantation 59(10):1377–1384

Lanza RP, Cooper DK, Chick WL (1997) Xenotransplantation. Sci Am 277(1):54–59

Lanza RP, Jackson R, Sullivan A, Ringeling J, McGrath C, Kuhtreiber W, Chick WL (1999) Xenotransplantation of cells using biodegradable microcapsules. Transplantation 67(8):1105–1111

Larijani B, Arjmand B, Amoli MM, Ao Z, Jafarian A, Mahdavi-Mazdah M, Ghanaati H, Baradar-Jalili R, Sharghi S, Norouzi-Javidan A, Aghayan HR (2012) Establishing a cGMP pancreatic islet processing facility: the first experience in Iran. Cell Tissue Bank 13(4):569–575

Lin CC, Ezzelarab M, Shapiro R, Ekser B, Long C, Hara H, Echeverri G, Torres C, Watanabe H, Ayares D, Dorling A, Cooper DK (2010) Recipient tissue factor expression is associated with consumptive coagulopathy in pig-to-primate kidney xenotransplantation. Am J Transplant 10(7):1556–1568

Living Cell Technologies (2010) Report from LCT's Russian phase I/IIa diabetes trial. Living Cell Technologies, New Zealand

Living Cell Technologies (2012a) Diabecell clinical trials update. Living Cell Technologies, New Zealand

Living Cell Technologies (2012b) LCT announces positive DIABECELL Phase I/IIa trial. Living Cell Technologies, New Zealand

Living Cell Technologies (2012c) Strong interim results in Argentinian DIABECELL® trial. Living Cell Technologies, New Zealand

Living Cell Technologies (2013) LCT: the cell implant company. http://www.lctglobal.com. Accessed 27 Aug 2013

Mancuso F, Calvitti M, Luca G, Nastruzzi C, Baroni T, Mazzitelli S, Becchetti E, Arato I, Boselli C, Ngo Nselel MD, Calafiore R (2010) Acceleration of functional maturation and differentiation of neonatal porcine islet cell monolayers shortly in vitro cocultured with microencapsulated sertoli cells. Stem Cells Int 2010:587213

Marchetti P, Giannarelli R, Cosimi S, Masiello P, Coppelli A, Viacava P, Navalesi R (1995) Massive isolation, morphological and functional characterization, and xenotransplantation of bovine pancreatic islets. Diabetes 44(4):375–381

Meyer T, Hocht B, Ulrichs K (2008) Xenogeneic islet transplantation of microencapsulated porcine islets for therapy of type I diabetes: long-term normoglycemia in STZ-diabetic rats without immunosuppression. Pediatr Surg Int 24(12):1375–1378

Monaco AP, Maki T, Ozato H, Carretta M, Sullivan SJ, Borland KM, Mahoney MD, Chick WL, Muller TE, Wolfrum J et al (1991) Transplantation of islet allografts and xenografts in totally pancreatectomized diabetic dogs using the hybrid artificial pancreas. Ann Surg 214(3):339–360, discussion 361–332

Muirhead M, Alexander T (1997) The health status of the herd managing pig health and the treatment of disease

Narang AS, Mahato RI (2006) Biological and biomaterial approaches for improved islet transplantation. Pharmacol Rev 58(2):194–243

National Health and Medical Research Council (2013) Animal to human transplantation research (Xenotransplantation). http://www.nhmrc.gov.au/health-ethics/ethical-issues/animal-human-transplantation-research-xenotransplantation. Accessed 12 Apr 2013

National Kidney Disease Education Program (2013) Slow progression and reduce complications. http://nkdep.nih.gov/identify-manage/manage-patients/slow-progression.shtml. Accessed 26 Aug 2013

Omer A, Duvivier-Kali VF, Trivedi N, Wilmot K, Bonner-Weir S, Weir GC (2003) Survival and maturation of microencapsulated porcine neonatal pancreatic cell clusters transplanted into immunocompetent diabetic mice. Diabetes 52(1):69–75

Omer A, Duvivier-Kali V, Fernandes J, Tchipashvili V, Colton CK, Weir GC (2005) Long-term normoglycemia in rats receiving transplants with encapsulated islets. Transplantation 79(1):52–58

Petruzzo P, Pibiri L, De Giudici MA, Basta G, Calafiore R, Falorni A, Brunetti P, Brotzu G (1991) Xenotransplantation of microencapsulated pancreatic islets contained in a vascular prosthesis: preliminary results. Transpl Int 4(4):200–204

Platt JL (2000) Immunobiology of xenotransplantation. Transpl Int 13(Suppl 1):S7–S10

Richter B, Neises G (2002) 'Human' insulin versus animal insulin in people with diabetes mellitus. Cochrane Database Syst Rev (3):CD003816

Ris F, Toso C, Veith FU, Majno P, Morel P, Oberholzer J (2004) Are criteria for islet and pancreas donors sufficiently different to minimize competition? Amer J Transplant 4(5):763–766

Safley SA, Cui H, Cauffiel S, Tucker-Burden C, Weber CJ (2008) Biocompatibility and immune acceptance of adult porcine islets transplanted intraperitoneally in diabetic NOD mice in calcium alginate poly-L-lysine microcapsules versus barium alginate microcapsules without poly-L-lysine. J Diabetes Sci Technol 2(5):760–767

Schuurman HJ (2008) Regulatory aspects of pig-to-human islet transplantation. Xenotransplantation 15(2):116–120

Schuurman HJ (2009) The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes – chapter 2: source pigs. Xenotransplantation 16(4):215–222

Sgroi A, Buhler LH, Morel P, Sykes M, Noel L (2010) International human xenotransplantation inventory. Transplantation 90(6):597–603

Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 343(4):230–238

Soto-Gutierrez A, Wertheim JA, Ott HC, Gilbert TW (2012) Perspectives on whole-organ assembly: moving toward transplantation on demand. J Clin Invest 122(11):3817–3823

Storms FE, Lutterman JA, van't Laar A (1987) Comparison of efficacy of human and porcine insulin in treatment of diabetic ketoacidosis. Diabetes Care 10(1):49–55

Sun AM, Vacek I, Sun YL, Ma X, Zhou D (1992) In vitro and in vivo evaluation of microencapsulated porcine islets. ASAIO J 38(2):125–127

Sutherland DE, Gruessner RW, Dunn DL, Matas AJ, Humar A, Kandaswamy R, Mauer SM, Kennedy WR, Goetz FC, Robertson RP, Gruessner AC, Najarian JS (2001) Lessons learned from more than 1,000 pancreas transplants at a single institution. Ann Sur 233(4):463–501

Sykes M, Dapice A, Sandrin M, Committee IXAE (2003) Position paper of the Ethics Committee of the International Xenotransplantation Association. Xenotransplantation 10(3):194–203

Thompson P, Badell IR, Lowe M, Cano J, Song M, Leopardi F, Avila J, Ruhil R, Strobert E, Korbutt G, Rayat G, Rajotte R, Iwakoshi N, Larsen CP, Kirk AD (2011) Islet xenotransplantation using gal-deficient neonatal donors improves engraftment and function. Am J Transplant 11(12):2593–2602

Titus T, Badet L, Gray DW (2000) Islet cell transplantation for insulin-dependent diabetes mellitus: perspectives from the present and prospects for the future. Expert Rev Mol Med 2(6):1–28

Tseng YL, Kuwaki K, Dor FJ, Shimizu A, Houser S, Hisashi Y, Yamada K, Robson SC, Awwad M, Schuurman HJ, Sachs DH, Cooper DK (2005) α1,3-Galactosyltransferase gene-knockout pig heart transplantation in baboons with survival approaching 6 months. Transplantation 80(10):1493–1500

Turchin IS, Tronko MD, Komissarenko VP (1995) Experience of 1.5 thousand transplantation of β-cells cultures to patients with diabetes mellitus. In: 5th IPITA Congress, Miami, 1995

U.S. Food and Drug Administration (2013) Guidance, compliance & regulatory information (Biologics). http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/default.htm. Accessed 26 Aug 2013

Valdes R (2002) Xenotransplantation trials. Lancet 359(9325):2281

van der Windt DJ, Bottino R, Kumar G, Wijkstrom M, Hara H, Ezzelarab M, Ekser B, Phelps C, Murase N, Casu A, Ayares D, Lakkis FG, Trucco M, Cooper DK (2012) Clinical islet xenotransplantation: how close are we? Diabetes 61(12):3046–3055

Vanderpool HY (1999) Commentary: a critique of Clark's frightening xenotransplantation scenario. J Law Med Ethics 27(2):153–157

Vanrompay D, Hoang TQ, De Vos L, Verminnen K, Harkinezhad T, Chiers K, Morre SA, Cox E (2005) Specific-pathogen-free pigs as an animal model for studying Chlamydia trachomatis genital infection. Infect Immun 73(12):8317–8321

Vrochides D, Paraskevas S, Papanikolaou V (2009) Transplantation for type 1 diabetes mellitus. Whole organ or islets? Hippokratia 13(1):6–8

Wang T, Lacik I, Brissova M, Anilkumar AV, Prokop A, Hunkeler D, Green R, Shahrokhi K, Powers AC (1997) An encapsulation system for the immunoisolation of pancreatic islets. Nat Biotechnol 15(4):358–362

Wang W, Mo Z, Ye B, Hu P, Liu S, Yi S (2011) A clinical trial of xenotransplantation of neonatal pig islets for diabetic patients. Zhong Nan Da Xue Xue Bao Yi Xue Ban 36(12):1134–1140

Wang Y, Daneilson KK, Ropski A, Harvat T, Barbar B, Paushter D, Qi M, Oberholzer J (2013) Systematic analysis of donor and isolation factor's impact on human islet yield and size distribution. Cell Transplant. doi:10.3727/096368912X662417

Wilson CA (2008) Porcine endogenous retroviruses and xenotransplantation. Cell Mol Life Sci 65(21):3399–3412

World Health Organization (2005) Xenotransplantation. http://www.who.int/transplantation/xeno/en/. Accessed 12 Apr 2013

Wright JR Jr, Yang H (1997) Tilapia Brockmann bodies: an inexpensive, simple model for discordant islet xenotransplantation. Ann Transplant 2(3):72–75

Wright JR Jr, Polvi S, MacLean H (1992) Experimental transplantation with principal islets of teleost fish (Brockmann bodies). Long-term function of tilapia islet tissue in diabetic nude mice. Diabetes 41(12):1528–1532

Wright JR Jr, Pohajdak B, Xu BY, Leventhal JR (2004) Piscine islet xenotransplantation. ILAR J/Natl Res Counc Inst Lab Anim Resour 45(3):314–323

Yang H, Wright JR Jr (1995) A method for mass harvesting islets (Brockmann bodies) from teleost fish. Cell Transplant 4(6):621–628

Yang H, O'Hali W, Kearns H, Wright JR Jr (1997) Long-term function of fish islet xenografts in mice by alginate encapsulation. Transplantation 64(1):28–32

Islet Encapsulation

46

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Contents

Abstract

Type 1 diabetes is an autoimmune disorder that destroys the insulin-producing cells of the pancreas. The mainstay of treatment is replacement of insulin through injectable exogenous insulin. Improvements in islet isolation techniques and immunosuppression regimens have made islet transplants a treatment option

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_29,

for select patients. Islet transplants have improved graft function over the years; however, graft function beyond year two is rare and notably these patients require immunosuppression to prevent rejection. Cell encapsulation has been proposed for numerous cell types, but it has found increasing enthusiasm for islets. Since islet transplants have experienced a myriad of success, the next step is to improve graft function and avoid systemically toxic immunosuppressive regimens. Cell encapsulation hopes to accomplish this goal. Encapsulation involves placing cells in a semipermeable biocompatible hydrogel that allows the passage of nutrients and oxygen and however blocks immune regulators from destroying the cell, thus avoiding systemic drugs. Several advances in encapsulation engineering and cell viability promise to make this a revolutionary discovery. This paper provides a comprehensive review of cell encapsulation of islets for the treatment of type 1 diabetes including a historical outlook, current research, and future studies.

Keywords
Cell encapsulation • Cell engineering • Islet transplants • Microcapsules • Type 1 diabetes

Introduction

Islet transplantation to treat type 1 diabetes has achieved vast improvements over the years, with more recipients able to achieve insulin independence for a longer period of time (Barton et al. 2012). Unfortunately, the lack of available donor organs and the use of antirejection medications continue to impede further progress. Encapsulation of islets for transplantation into diabetic recipients aims to provide a solution to these problems. Cell encapsulation is a revolutionary method of enveloping cells in a biocompatible matrix that provides a gradient for the diffusion of oxygen and nutrients but prevents large immune molecules from reaching the cell, thus avoiding rejection. This idea has been described since the 1930s but notable achievements have occurred over the last decade. This chapter aims to provide a review including a historical perspective, current research, and future applications of cell encapsulation of islets for the treatment of type 1 diabetes.

History

Over 25 million people in the United States (US) suffer from diabetes with approximately 5 % characterized as type 1. Diabetes is ranked as the seventh leading cause of death in the United States (CDC 2012). Type 1 diabetes (T1DM) is characterized as an autoimmune destruction of the β-cells of the pancreas with resultant insulin deficiency (ADA 2004). Currently, the mainstay of treatment for T1DM is glycemic control through injectable insulin. However, improvements in islet transplantation continue to occur, hopefully changing the treatment paradigm

into cell replacement rather than supportive care. According to the Collaborative Islet Transplant Registry (CITR), there have been a total of 677 islet transplant recipients from 1999 to 2010. The percentage of recipients that achieve insulin independence for 3 years is 44 % between 2007 and 2010 compared to 27 % from 1999 to 2002 (Barton et al. 2012). Various immunosuppressive regimens have been implemented in islet transplant centers to maintain graft function. However, like other solid organ transplants, immune rejection medications are implicated in numerous adverse effects to the patient, as well as toxicity to the graft (Hafiz et al. 2005; Niclauss et al. 2011). To circumvent these issues, cell encapsulation has been proposed as the next treatment option for islet transplants with the goal of elimination of immunosuppression. Although cell encapsulation has been tested in other disease processes, such as neurodegenerative diseases, pain, and epilepsy to name a few, by far the greatest achievement using this technology has been in the encapsulation of islets for the treatment of T1DM (Bachoud-Lévi et al. 2000; Jeon 2011; Eriksdotter-Jönhagen et al. 2012; Fernández et al. 2012; Huber et al. 2012). It is clear that insulin independence can be achieved through the infusion of isolate islets, but improvements in graft viability and avoidance of systemically toxic drugs can be accomplished through encapsulation (Hearing and Ricordi 1999; Shapiro et al. 2003). The following paragraph will discuss advances in encapsulated islet technology.

Current Research

Animal and Human Trials

The first researcher to be accredited with transplantation of encapsulated tissue was Biscegeli in 1933. He placed mouse tumor cells in a polymer matrix into the abdomen of a guinea pig and was able to achieve survival without rejection (Bisceglie 1933). This idea was not replicated until approximately 50 years later when Lim and Sun were the first to use encapsulation for islet transplants in diabetic animals. They placed 2,000–3,000 islet equivalent (IEQ) in an alginate hydrogel for transplantation into the peritoneum of diabetic rats to achieve normoglycemia for up to 3 weeks compared to only 8 days in unencapsulated islets (Lim and Sun 1980). Currently, there are a number of achievements in encapsulating islets seen in small and large animal studies, as well as in early-phase clinical trials. In studies performed by Kobayashi et al. in 2003, NOD mice were used as both donor and recipient. The authors used a 5 % agarose microcapsule encasing 1,500–2,000 islet equivalents (IEQ) per mouse for intraperitoneal implantation as well as omental pouch transplants and witnessed prolonged euglycemia for a period of 100 days compared to 8 days for unencapsulated islet transplants (Kobayashi et al. 2003). The same authors repeated the study in 2006 and observed the same period of normoglycemia in the recipients; however, they also retrieved the devices after a period of 400 days and noted viable islets were recovered with a small percentage of necrotic cells (Kobayashi et al. 2006). In a more recent study, Dong et al. performed

syngeneic transplants into streptozotocin (STZ)-induced BALB/c mice using polyethylene glycol-polylactic-co-glycolic acid (PEG-PLGA) nanoparticles with only 500–600 IEQ and revealed that over half of the mice achieved normal glucose levels for up to 100 days (Dong et al. 2012).

Less consistent but still notable results were accomplished with large animal models. Shoon-Shiong performed several encapsulated islet transplants into diabetic canines. In one publication in 1993, islets at a dose of 15,000–20,000 islets/kg were encapsulated in a microcapsule made of alginate into the intraperitoneal cavity and witnessed independence from exogenous insulin for a period of 110 days, as well as the presence of C-peptide in the blood for an average of 483 days (Soon-Shiong et al. 1993). In 2010, Dufrane used nonhuman primates as donor and recipients for subcutaneous and kidney capsule transplants of alginate micro- and macroencapsulated islets using 30,000 IEQ/kg. The authors noted correction of diabetes for a period of up to 28 weeks (Dufrane et al. 2010). In another study using cynomolgus monkeys as recipients by Elliott et al., neonatal pig islets were isolated (10,000 IEQ/Kg) and encapsulated in alginate microcapsules resulting in a more than 40 % reduction in injectable insulin dose compared to preimplantation (Elliott et al. 2005). Based on some noteworthy achievements in large animal studies, several researchers have been granted approval for stage one and two human clinical trials. Due to the previous success by Shoon-Shiong using a canine model, the authors were authorized for the first human clinical trial in 1994. A 38-year-old male, with type 1 diabetes and end-stage renal disease status postoperative kidney transplant, maintained on low-dose immunosuppression, became the first recipient of an encapsulated islet transplant. The patient initially received 10,000 IEQ/kg of cadaver islets encapsulated in an alginate microcapsule followed by a repeat infusion of 5,000 IEQ/kg 6 months later. The patients' insulin requirements were reduced to 1–2 insulin units per day, and eventually he was able to discontinue all exogenous insulin after 9 months (Soon-Shiong et al. 1994). In 2006 Calafiore et al. reported on their encapsulated islet transplants. Human cadavers (400,000–600,000 IEQ) were isolated for encapsulation into sodium alginate beads for intraperitoneal injection. The patients experienced improved daily glucose levels and a decline in daily exogenous insulin intake; however, neither patient became insulin independent (Calafiore et al. 2006).

Living Cell Technologies, a company out of Australia, has achieved the best outcomes for encapsulated islet transplants. In one arm of their studies, xenotransplants from fetal pigs were isolated from a pathogen-free farm in New Zealand. The islets were encapsulated in alginate microcapsules for intraperitoneal injections into human recipients. Several early-phase clinical trials have been performed from this group with promising results. The most significant achievement has been in the reduction of hypoglycemic episodes to around 40 %. Several patients achieved improvements in daily glucose levels and a reduction in exogenous insulin dosing; two patients became insulin independent after 4 months (Elliott et al. 2007, 2010, Elliott and Living cell technologies Ltd-LCT 2011). These are promising results and achievements; however, not all researchers have

been able to achieve such results, and the lack of reproducibility is threatening to dampen enthusiasm. For example, a human clinical trial by Tuch et al. used alginate microcapsules for human cadaveric islet transplants and noted the presence of plasma C-peptide levels for up to 2.5 years; however, there was no change in insulin requirements for the recipients (Tuch et al. 2009). Likewise, in a follow-up publication by Elliot et al., from Living Cell Technologies, one recipient experienced early success with a 30 % reduction in insulin dosing; however, at follow-up at 49 weeks, the patient was back on his original insulin dosing regimen (Elliott et al. 2007). Although the purpose of these early-phase clinical trials is to assure safety and determine dosing, it is notable that most encapsulated islet recipients do not achieve sustainable insulin independence. Likewise, there is yet to be a standardized protocol for the type of biomaterial used and the dose of islets to be infused. It is clear, however, based on novel in vivo studies that the type of biomaterial does impact graft survival. King et al. tested several encapsulation methods using alginate with poly-L-lysine (PLL) or without as well as with high glucuronic acid (G) or high mannuronic acid (M) in mouse recipients and revealed that significant results were achieved with PLL-free high M microcapsules, with sustained normoglycemia for 8 weeks (King et al. 2003). Likewise, Lanza et al. revealed that improved capsule integrity and graft function could be achieved by altering the concentration of alginate in their xenotransplants into diabetic Lewis rats (Lanza et al. 1999). Although no consensus is achieved regarding the best encapsulation vehicles for islets transplants, no review would be complete without a discussion regarding biomaterials.

Biomaterials in Transplantation

Chang et al. were one of the first to describe the use of a semipermeable membrane for encapsulation. They postulated that liver enzymes or cells can be delivered in a polymer membrane for treatment of a disorder (Chang 1964). Now 50 years later, several types of encapsulation methods have been developed over the years however; currently the most common employed method are alginate microcapsules (King et al. 2003; Zimmermann et al. 2007; Krishnamurthy and Gimi 2011; Khanna et al. 2012). Capsule vehicles have taken the form of vascular shunts and macro-, micro-, and nanoscale devices. The original vascular device was developed over three decades ago as capillary fibers in culture-coated medium (Chick et al. 1975). Maki et al. performed several studies with vascular devices implanted as arterio-venous shunts into diabetic canines. These devices showed promising results with several canines achieving reduced insulin requirements (Maki et al. 1991, 1996). Ultimately, the difficulty with these devices was the ability to provide enough islets to coat the fibers. When attempts were made to elongate the fibers to include more islets, the devices resulted in clotting and fibrosis. This eventually led to its disuse as the higher dose of islets needed to achieve insulin independence in humans would result in the requirement for multiple devices to be employed Lanza et al. (1992). Macroscale devices have been used by a relative few, due to their

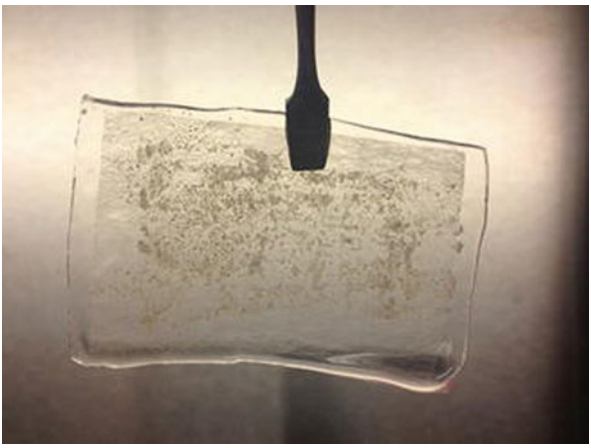

Fig. 1 Macroencapsulated islets. Islets were isolated from young piglets (average 20 days), matured for 8 days, and injected into a prefabricated islet sheet made of ultrapure high M alginate. Islets were placed in the center of the sheet with a scrim added for greater stability. Encapsulation design by Islet Science, LLC (Picture provided by Dr. Jonathan RT Lakey, UCI)

increased immunogenicity as well as the larger diffusion parameters required for oxygen and nutrients to reach the cell; however, they offer several advantages including implantation ease and retrievability. In a study using an alginate macroscaled sheet, diabetic canines achieved improved glucose levels for over 80 days (Storrs et al. 2001) (Fig. 1). Nanoencapsulation offers the advantage of improved diffusion parameters, improving the response of insulin to rising blood glucose levels. PEG has been used for nanoencapsulation devices, and when exposed to UV or visible light, it can be cross-linked with minimal damage to the inner cell. However, PEG is not as biocompatible as other hydrogels and does not provide complete protection from cytokines (Jang et al. 2004). Despite these concerns, some success has been attained with these gels (Dong et al. 2012). By far the most common encapsulation device is a microscale vehicle. These beads have mechanical stability, have an improved surface area to volume ratio, and have enhanced immunologic profiles (Krishnamurthy and Gimi 2011; Borg and Bonifacio 2011), but more importantly several companies make so-called encapsulators that can produce uniform capsules using air jet-driven droplet technology (Fiszman et al. 2002; Sun 1988) (Fig. 2). This is highly important when discussing using these capsules for clinical use as standardization, safety, and cost-effectiveness are going to be important aspects for its widespread clinical use. All of the clinical trials discussed in the previous paragraph used microcapsules for encapsulating islets. Along with the different capsule size, various materials have also been tested. Extracellular matrixes have included both synthetic and biologic materials. The most common synthetic agents employed in encapsulation engineering include polyethylene oxide, polyacrylic acid, polyvinyl alcohol, polyphosphazene, polypeptides, and derivatives. Naturally occurring hydrogels include gelatin, fibrin, agarose, hyaluronate, chitosan, and alginate (Nicodemus and Bryant 2008; Lee and Mooney 2000). Polyglycolic and lactic acid polymers continue to be the most commonly used synthetic material used in medical devices. However, several concerns are raised when using synthetic materials as a scaffold,

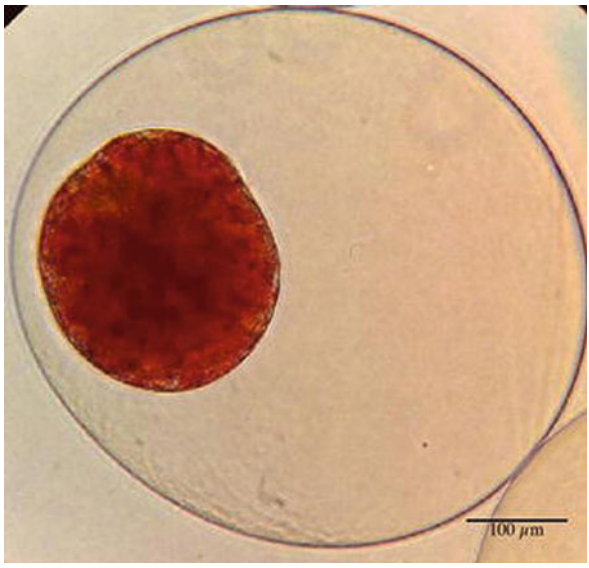

Fig. 2 Microencapsulated islets. Islets were isolated from young pigs (range 18–26 days), matured for 8 days, and then encapsulated in ultrapure high M (UPLVM) alginate microcapsule, using an electrostatic encapsulator. Encapsulated islets were then stained with dithizone. Sample image is an isolated islet, approximately 150 μm in diameter, encapsulated in a 500 μm microcapsule. Scale 100 μm (Picture provided by Dr. Jonathan RT Lakey, UCI)

due to the possible potential that the foreign material will elicit a greater response by the body leading to fibrosis and loss of the encased cells. The production of these synthetic constituents would need to occur with nontoxic materials, and these materials would need to be purified in a method that is gentle enough on the cells and the transplant site. These capsules typically are heavily modified so that they can interact with the environment and degrade under physiologic conditions. Consequently, biologic materials are being used with an increasing incidence, with collagen being the most widely used naturally derived polymer in medical devices today. However, these gels exhibit poor strength, are expensive, and display significant variations between collagen batches making standardization of the process a problem (Lee and Mooney 2000). Therefore, alginate has become increasingly utilized for encapsulation due to its excellent biocompatibility, hydrophilic properties, easy gelation process, stable architecture, and relatively low cost. Alginate is a polysaccharide derived from seaweed which has to undergo extensive purification to improve its immunologic profile (O'Sullivan et al. 2010). Impure alginate has been implicated in islet cell necrosis and recruitment of inflammatory mediators (De Vos et al. 1997). Alginate is made up of chains of mannuronic (M) and guluronic (G) acid; the sequence of these units determines many of the encapsulation properties such as mechanical strength, durability, and permeability. For example, high G alginates form gels which are smaller and stronger than high M (O'Sullivan et al. 2010). Wang et al. tested over 200 capsule designs for their islet transplants finally deciding on a polymethyl coguanidine-cellulose sulfate/poly-L-lysine-sodium alginate (PMCG)-CS/PLL (Wang et al. 1997) for their canine syngeneic transplants resulting in normoglycemia achieved for approximately 160 days, with one canine achieving euglycemia for 214 days (Wang et al. 2008). The use of polycations and anions has been shown to

provide improved permeability and mechanical strength; however, they also tend to result in an increase biologic response. Some counteract this response by adding another thin layer of alginate to provide a barrier (O'Sullivan et al. 2010). In broad terms, the process of gelation involves cross-linking by covalent, ionic, or physical bonds. For example, alginate converts into a gel form by ionic cross-linking with bivalent cations such as calcium, magnesium, and more commonly barium (King et al. 2001). The diffusion gradient that provides the bidirectional flow of materials is established by the degree of cross-linking. Mesh or pore size is typically much smaller than the encased cells however; hydrogels do not result in uniform pore size, and permselectability has never been clearly defined (O'Sullivan et al. 2010). What is apparent is that an increase in the degree of cross-linking results in gels that have superior mechanical strength but inversely reduces the size of the pores available for diffusion.

Several components need to be considered in engineering scaffolds that are safe for the encapsulated cells and the surrounding environment. The process of gelation needs to be mild; the capsule structure and chemistry should be nontoxic and reproducible. The degradation process must follow physiologic tissue growth, and its products must not adversely affect the coated cells or the body. Above all, the process of hydrogel engineering needs to be easily scaled up for industry, acceptable to surgeons, patients, and regulatory committees.

Clearly, capsule engineering is of vital importance for promoting graft survival and function, and many steps need to be considered in capsule construction to promote islet transplantations. Many advances have been achieved, but some studies have illustrated the difficulty with this technology. In animal studies performed by Suzuki et al., Tze, and Duvivier-Kali as well as the human clinical trial by Tuch, capsule fibrosis was a significant problem encountered (Suzuki et al. 1998; Tze et al. 1998; Duvivier-Kali et al. 2004; Tuch et al. 2009). Theoretically immune isolation is achieved by encapsulating cells, however, in these studies; clearly, some component of rejection was experienced. Likewise, oxygen and nutrients are able to freely diffuse across a matrix; however, studies illustrated by De Vos and Xin noted the absence of fibrosis, however, retrieved capsules containing necrotic islets, indicating the lack of appropriate oxygen to promote graft survival (De Vos et al. 1999; Xin et al. 2005). Due to these issues, some researchers have gone on to improve capsule engineering by means of co-encapsulation. These advances will be discussed in future applications as most of these undertakings are currently being performed in vitro.

Future Applications

Improved Capsule Engineering

Co-encapsulation is the process of adding additional molecules to the capsule to enhance the performance of the enveloped cell. In a novel study performed by Bunge, islets encapsulated with dexamethasone witnessed improved islet survival

in mice recipients compared to those without the steroid (Bunger et al. 2005). In another study, Baruch encapsulated mouse monocyte macrophage cells and hamster kidney cells with ibuprofen and witnessed improved cell survival in vitro and in vivo (Baruch et al. 2009). Although cells are protected from large molecules such as antibodies, proinflammatory cytokines have a smaller molecular weight and can diffuse across most hydrogel gradients; thus, protection from these cytokines may promote capsule survival. In an in vitro study performed by Leung, capsules with anti-TNF α were able to remove active TNF α from a culture media (Leung et al. 2008). In order to improve oxygen supply to the cell, the access to a rich vascular bed is essential. As such, Khanna showed that the angiogenic factor, fibroblast growth factor 1, was able to be encapsulated and revealed continuous release for a 1 month period in vitro (Khanna et al. 2010). In another study, Pedraza et al. was able to encapsulate solid peroxide within polydimethylsiloxane resulting in sustained oxygen generation for approximately 6 weeks (Pedraza et al. 2012). Clearly, co-encapsulation is possible; however, most of these studies are still in the early phases of investigation, so we are yet to see whether this will improve graft function in vivo. The latest forefront for encapsulation cell technology for the treatment of diabetes involves the use of stem cells as a source of islets. Although some researchers have begun doing in vivo studies, the results have been varied.

Stem Cells

Stem cells are an attractive addition to cell encapsulation as the availability of human cadaveric donors for islet transplants continues to be a major problem. A company out of San Diego, Viacyte LLC, has performed the bulk of encapsulated stem cell-related transplants. Human embryonic stem cells directed down a pancreatic lineage were encapsulated in a device, called TheraCyte, which is made of a double membrane of polytetrafluoroethylene. In a study by Kroon et al., diabetic mice achieved normalization of glucose levels after 2 months (Kroon et al. 2008). Likewise, Lee et al. noted initially low glucose responses and plasma C-peptide levels after 12 weeks; however, after 5 months, improvements in glucose and C-peptide levels were evident, indicating differentiation continued to occur while encapsulated (Lee et al. 2009). In contrast, Matveyenko et al. did not achieve such outcomes, and in fact, their devices were noted to be encased in fibrotic tissue and upon retrieval did not stain positive for endocrine cells (Matveyenko et al. 2010). In a study using mesenchymal stem cells from human amniotic membranes, Kadam et al. were able to produce functional islet-like clusters which were encapsulated in polyurethane-polyvinylpyrrolidone microcapsules for transplantation into diabetic mice which resulted in euglycemia after day 15 until approximately day 30 (Kadam et al. 2010). Although, no study has been able to accomplish sustainable insulin independence using stem cells, improvements in stem cell differentiation are being accomplished and will hopefully improve upon this method (Blyszczuk et al. 2003).

Conclusion

Cell encapsulation of islets for the treatment of T1DM is a promising field that aims to revolutionize the treatment paradigm for diabetics. Although significant achievements have occurred, there are several obstacles that need to be addressed before achieving widespread use of this technology. Improvements in graft viability, biomaterial engineering, and islet isolation techniques will continue to promote success in this field.

References

American Diabetes Association (2004) Diagnosis and classification of diabetes mellitus. Diabetes Care 1:s5–s10

Bachoud-Lévi A, Déglon N, Nguyen JP, Bloch J, Bourdet C, Winkel L, Rémy P, Goddard M, Lefaucheur J, Brugières P, Baudic S, Cesaro P, Peschanski M, Aebischer P (2000) Neuroprotective gene therapy for Huntington's disease using a polymer encapsulated BHK cell line engineered to secrete human CNTF. Hum Gene Ther 11:1723–1729

Barton FB, Rickels MR, Alejandro R, Hering B, Wease S, Naziruddin B et al (2012) Improvement in outcomes of clinical islet transplantation. Diabetes Care 35:1436–1445

Baruch L, Benny O, Gilbert A, Ukobnik O (2009) Alginate-PLL cell encapsulation system co-entrapping PLGA-microspheres for the continuous release of anti-inflammatory drugs. Biomed Microdevices 11:1103–1113

Bisceglie V (1933) Uber die antineoplastische Immunität; heterologe Einpflanzung von Tumoren in Hühner-embryonen. Z Krebsforsch 40:122–140

Blyszczuk P, Czyz J, Kania G, Wagner M, Roll U, St-Onge L, Wobus AM (2003) Expression of Pax4 in embryonic stem cells promotes differentiation of nestin-positive progenitor and insulin-producing cells. Proc Natl Acad Sci U S A 100:998–1003

Borg DJ, Bonifacio E (2011) The use of biomaterials in islet transplantation. Curr Diab Rep 11:434–444

Bunger CM, Tiefenbach B, Jahnkea A, Gerlacha C, Freier T, Schmitz KP, Hopt UT, Schareck W, Klar E, De Vos P (2005) Deletion of the tissue response against alginate-pll capsules by temporary release of co-encapsulated steroids. Biomaterials 26:2353–2360

Calafiore R, Basta G, Luca G, Lemmi A, Montanucci P, Calabrese G, Racanicchi L, Mancuso F, Brunetti P (2006) Microencapsulated pancreatic islet allografts into non immunosuppressed patients with type diabetes: first two cases. Diabetes Care 29:1137–1139

Centers for Disease Control and Prevention (2012) Centers for Disease Control and Prevention: Diabetes Public Health Resources. http://www.cdc.gov/diabetes. Accessed 1 Aug 2012

Chang T (1964) Semipermeable microcapsules. Science 156:524–525

Chick WL, Like AA, Lauris V (1975) 8-cell culture on synthetic capillaries: an artificial endocrine pancreas. Science 187:847–849

De Vos P, De Haan BJ, Wolters GH, Strubbe JH, Van Schilfgaarde R (1997) Improved biocompatibility but limited graft survival after purification of alginate for microencapsulation of pancreatic islets. Diabetologia 40:262–270

De Vos P, Van Straaten JF, Nieuwenhuizen AG, De Groot M, Ploeg RJ, De Haan BJ, Van Schilfgaarde R (1999) Why do microencapsulated islet grafts fail in the absence of fibrotic overgrowth? Diabetes 48:1381–1388

Dong H, Fahmy TM, Metcalfe SM, Morton SL, Dong X, Inverardi L, Adams DB, Gao W, Wang H (2012) Immuno-isolation of pancreatic islet allografts using pegylated nanotherapy leads to long-term normoglycemia in full MHC mismatch recipient mice. PLoS One 7:e50265

Dufrane D, Goebbels RM, Gianello P (2010) Alginate macroencapsulation of pig islets allows correction of streptozotocin-induced diabetes in primates up to 6 months without immunosuppression. Transplantation 90:1054–1062

Duvivier-Kali VF, Omer A, Lopez-Avalos MD, O'Neil JJ, Weir GC (2004) Survival of microencapsulated adult pig islets in mice in spite of an antibody response. Am J Transplant 12:1991–2000

Elliott RB, Living cell technologies Ltd-LCT (2011) In: International pancreas and islet transplant association meeting, Prague

Elliott RB, Escobar L, Tan PL, Garkavenko O, Calafiore R, Basta P, Vasconcellos AV, Emerich DF, Thanos C, Bambra C (2005) Intraperitoneal alginate-encapsulated neonatal porcine islets in a placebo-controlled study with 16 diabetic cynomolgus primates. Transplant Proc 37:3505–3508

Elliott RB, Escobar L, Tan PL et al (2007) Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. Xenotransplantation 14:157–161

Elliott RB, Garkavenko O, Tan P, Skaletsky NN, Guliev A, Draznin B (2010) Transplantation of microencapsulated neonatal porcine islets in patients with type 1 diabetes: safety and efficacy.70th scientific sessions. American Diabetes Association, Orlando

Eriksdotter-Jönhagen M, Linderoth B, Lind G, Aladellie L, Almkvist O, Andreasen N, Blennow K, Bogdanovic N, Jelic V, Kadir A, Nordberg A, Sundström E, Wahlund LO, Wall A, Wiberg M, Winblad B, Seiger A, Almqvist P, Wahlberg L (2012) Encapsulated cell biodelivery of nerve growth factor to the basal forebrain in patients with Alzheimer's disease. Dement Geriatr Cogn Disord 33:18–28

Fernández M, Barcia E, Fernández-Carballido A, Garcia L, Slowing K, Negro S (2012) Controlled release of rasagiline mesylate promotes neuroprotection in a rotenone-induced advanced model of Parkinson's disease. Int J Pharm 438:266–278

Fiszman GL, Karara AL, Finocchiaro LM, Glikin GC (2002) A laboratory scale device for microencapsulation of genetically engineered cells into alginate beads. Electron J Biotechnol 5:23–24

Hafiz MM, Faradji RN, Froud T, Pileggi A, Baidal DA, Cure P, Ponte G, Poggioli R, Cornejo A, Messinger S, Ricordi C, Alejandro R (2005) Immunosuppression and procedure-related complications in 26 patients with type 1 diabetes mellitus receiving allogeneic islet cell transplantation. Transplantation 80:1718–1728

Hearing B, Ricordi C (1999) Islet transplantation for patients for patients with type 1 diabetes; results, research priorities, and reasons for optimism. Graft 2:12–27

Huber A, Padrun V, Deglon N, Aebischer P, Hanns M, Boison D (2012) Grafts of adenosine-releasing cells suppress seizures in kindling epilepsy. Proc Natl Acad Sci U S A 98:7611–7616

Jang JY, Lee DY, Park SJ, Byun Y (2004) Immune reactions of lymphocytes and macrophages against PEG-grafted pancreatic islets. Biomaterials 25:3663–3669

Jeon Y (2011) Cell based therapy for the management of chronic pain. Korean J Anesthesiol 60:3–7

Kadam S, Muthyala S, Nair P, Bhonde R (2010) Human placenta-derived mesenchymal stem cells and islet-like cell clusters generated from these cells as a novel source for stem cell therapy in diabetes. Rev Diabet Stud 7:168–182

Khanna O, Moya EC, Opara EC, Brey EM (2010) Synthesis of multilayered alginate microcapsules for the sustained release of fibroblast growth factor-1. J Biomed Mater Res A 95:632–640

Khanna O, Larson JC, Moya ML, Opara EC, Brey EM (2012) Generation of alginate microspheres for biomedical applications. J Vis Exp 66:e3388

King S, Dorian R, Storrs R (2001) Requirements for encapsulation technology and the challenges for transplantation of islets of Langerhans. Graft 4:491–499

King A, Lau J, Nordin A, Sandler S, Andersson A (2003) The effect of capsule composition in the reversal of hyperglycemia in diabetic mice transplanted with microencapsulated allogeneic islets. Diabetes Technol Ther 5:653–663

Kobayashi T, Aomatsu Y, Iwata H, Kin T, Kanehiro H, Hisanaga M, Ko S, Nagao M, Nakajima Y (2003) Indefinite islet protection from autoimmune destruction in nonobese diabetic mice by agarose microencapsulation without immunosuppression. Transplantation 75:619–625

Kobayashi T, Aomatsu Y, Iwata H, Kin T, Kanehiro H, Hisanga M, Ko S, Nagao M, Harb G, Nakajima Y (2006) Survival of microencapsulated islets at 400 days posttransplantation in the omental pouch of NOD mice. Cell Transplant 15:359–365

Krishnamurthy NV, Gimi B (2011) Encapsulated cell grafts to treat cellular deficiencies and dysfunction. Crit Rev Biomed Eng 39:473–491

Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE (2008) Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 26:443–452

Lanza RP, Borland KM, Lodge P, Carretta M, Sullivan SJ, Muller TE, Solomon BA, Maki T, Monaco AP, Chick WL (1992) Treatment of severely diabetic, pancreatectomized dogs using a diffusion-based hybrid pancreas. Diabetes 41:886–889

Lanza RP, Jackson R, Sullivan A, Ringeling J, McGrath C, Kühtreiber W, Chick WL (1999) Xenotransplantation of cells using biodegradable microcapsules. Transplantation 67:1105–1111

Lee K, Mooney D (2000) Hydrogels for tissue engineering. Chem Rev 101:1869–1879

Lee SH, Hao E, Savinov AY, Geron I, Strongin AY, Itkin-Ansari P (2009) Human β-cell precursors mature into functional insulin-producing cells in an immunoisolation device: implications for diabetes cell therapies. Transplantation 87:983–991

Leung A, Lawrie G, Nielson LK, Trau M (2008) Synthesis and characterization of alginate/poly-L-ornithine/alginate microcapsules for local immunosuppression. J Microencapsul 25:387–398

Lim F, Sun AM (1980) Microencapsulated islets as bioartificial pancreas. Science 210:908

Maki T, Ubhi CS, Sanchez-Farpon H, Sullivan SJ, Borland K, Muller TE, Solomon BA, Chick WL, Monaco AP (1991) Successful treatment of diabetes with the biohybrid artificial pancreas in dogs. Transplantation 51:43–51

Maki T, Otsu I, O'Neil JJ, Dunleavy K, Mullon CJ, Solomon BA, Monaco AP (1996) Treatment of diabetes by xenogeneic islets without immunosuppression. Use of a vascularized bioartificial pancreas. Diabetes 45:342–347

Matveyenko AV, Georgia S, Bhushan A, Butler PC (2010) Inconsistent formation and nonfunction of insulin-positive cells from pancreatic endoderm derived from human embryonic stem cells in athymic nude rats. Am J Physiol Endocrinol Metab 299:13–20

Niclauss N, Bosco D, Morel P, Giovannoni L, Berney T, Parnaud G (2011) Rapamycin impairs proliferation of transplants islet β cells. Transplantation 91:714–722

Nicodemus G, Bryant S (2008) Cell encapsulation in biodegradable hydrogels for tissue engineering applications. Tissue Eng Part B Rev 14:149–165

O'Sullivan ES, Johnson AS, Omer A, Hollister-Lock J, Bonner-Weir S, Colton CK, Weir GC (2010) Rat islet cell aggregates are superior to islets for transplantation in microcapsules. Diabetologia 53:937–945

Pedraza E, Coronel MM, Fraker CA, Ricordi C, Stabler CL (2012) Preventing hypoxia-induced cell death in β cells and islets via hydrolytically activated, oxygen-generating biomaterials. Proc Natl Acad Sci U S A 109:4245–4250

Shapiro AM, Nanji SA, Lakey JR (2003) Clinical islet transplant: current and future directions towards tolerance. Immunol Rev 196:219–236

Soon-Shiong P, Feldman E, Nelson R, Heintz R, Yao Q, Yao Z, Zheng T, Merideth N, Skjak-Braek G, Espevik T et al (1993) Long-term reversal of diabetes by the injection of immunoprotected islets. Proc Natl Acad Sci U S A 90:5843–5847

Soon-Shiong P, Heintz RE, Merideth N, Yao QX, Yao Z, Zheng T, Murphy M, Moloney MK, Schmehl M, Harris M et al (1994) Insulin independence in a type 1 diabetic patient after encapsulated islet transplantation. Lancet 343:950–951

Storrs R, Dorian R, King SR, Lakey J, Rilo H (2001) Preclinical development of the islet sheet. Ann N Y Acad Sci 944:252–266

Sun AM (1988) Microencapsulation of pancreatic islet cells: a bioartificial endocrine pancreas. Methods Enzymol 137:575–580

Suzuki K, Bonner-Weir S, Trivedi N, Yoon KH, Hollister-Lock J, Colton CK, Weir GC (1998) Function and survival of macroencapsulated syngeneic islets transplanted into streptozocin-diabetic mice. Transplantation 66:21–28

Tuch BE, Keogh GW, Williams LJ et al (2009) Safety and viability of microencapsulated human islets transplanted into diabetic humans. Diabetes Care 32:1887–1889

Tze WJ, Cheung SC, Tai J, Ye H (1998) Assessment of the in vivo function of pig islets encapsulated in uncoated alginate microspheres. Transplant Proc 30:477–478

Wang T, Lacík I, Brissová M, Anilkumar AV, Prokop A, Hunkeler D, Green R, Shahrokhi K, Powers AC (1997) An encapsulation system for the immunoisolation of pancreatic islets. Nat Biotechnol 15:358–362

Wang T, Adcock J, Kühtreiber W, Qiang D, Salleng KJ, Trenary I, Williams P (2008) Successful allotransplantation of encapsulated islets in pancreatectomized canines for diabetic management without the use of immunosuppression. Transplantation 85:331–337

Xin ZL, Ge SL, Wu XK, Jia YJ, Hu HT (2005) Intracerebral xenotransplantation of semipermeable membrane-encapsuled pancreatic islets. World J Gastroenterol 11:5714–5717

Zimmermann H, Shirley SG, Zimmermann U (2007) Review Alginate-based encapsulation of cells: past, present, and future. Curr Diab Rep 7:314–320

Stem Cells in Pancreatic Islets

47

Erdal Karaöz and Gokhan Duruksu

Contents

Abstract

Adult stem cells are unspecialized cells with the capacity to differentiate into many different cell types in the body. These cells together with the progenitor cells involve in the tissue repair processes and maintain the functionality of the tissue in which they are found. During the onset and progression of the diabetes mellitus type 1, the β cells in Langerhans islets undergo progressive and selective destruction. To restore the normal insulin levels to regulate glucose homeostasis, the dropping number of β cells is replaced by progenitor/stem cells in the environment of pancreatic islets. Due to the complexity of the organ organization, these cells could originate from different sources. In this chapter, the types of the stem cells derived from pancreatic environment with the differentiation capacity into pancreatic islets and their cells were mainly focused. Some of these cells do not only involve in the regeneration of insulin-producing cells, but they

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_31,

also function in the preservation of β cell viability. Besides providing an alternative renewable source for β cell replacement, the interaction of pancreatic stem cells with immune system in diabetes mellitus type 1 is also discussed.

Keywords
β cells • Differentiation • Immune regulatory • Regeneration • Stem cells

Abbreviations

ACTA2	Actin α 2 (smooth muscle actin)
CHIBs	Cultured human islet buds
ECFP	Cyan fluorescent protein
FACS	Fluorescence-activated cell sorting
FGF	Fibroblast growth factors
GLP-1	Glucagon-like peptide-1
HGF	Hepatocyte growth factor
ICAM-1	Intercellular adhesion molecule 1 (CD54)
IFN-γ	Interferon γ
IL-	Interleukin
MSCs	Mesenchymal stem cells
NG2	Neuron-glial antigen 2
NOD	Nonobese diabetic
PDGFRβ	Platelet-derived growth factor receptor, β polypeptide
PDX-1	Pancreatic duodenal homeobox-containing factor-1
PI-SCs	Pancreatic islet-derived stem cells
TGF-α	Transforming growth factor α
TGF-β	Transforming growth factor β
TNF-α	Tumor necrosis factor α
VCAM-1	Vascular cell adhesion molecule 1 (CD106)

Introduction

The imbalance between the generation and destruction of β cells has been seen as one of the probable causes of diabetes. According to this view, improving the regenerative potential of β cells might be an alternative approach for the treatment of type 1 diabetes by increasing the number of β cells in islets to compensate for the damage of autoimmunity (Banerjee and Bhonde 2003; Guz et al. 2001; Lechner et al. 2002). For this purpose, many experimental studies were carried out in diabetic animal models, and all the studies shared three common targets regardless of which experimental strategies they preferred:

- To demonstrate the continuation of existence of stem/progenitor cells in pancreas or islets of Langerhans in diabetes animal models
- To clarify the role of this stem/progenitor cells in β cell regeneration in pancreatic islets in response to changing conditions

- To determine the possible effect of β cell regeneration (or neogenesis) on manifestations of diabetes mellitus

Adult pancreatic β cells were previously thought to be the terminal cells (cells in last stage of differentiation) with limited regeneration/self-renewal capacity. However, the replication of adult islet cells was shown to induce in response to stimulators, like glucose, certain hormones, and growth factors, especially HGF which initiates the maturation of neonatal islet cells (Hayek et al. 1995; Peck and Ramiya 2004; Swenne 1992). In addition, the reversible dynamic alterations in the mass of pancreatic endocrine cells were commonly observed to meet the requirements during pregnancy and obesity throughout the life. Even during the growth and development of body, the increase in cell mass was reported (Bonner-Weir et al. 1989; Brelje et al. 1993; Marynissen et al. 1983; Peck and Ramiya 2004; Street et al. 2004).

Based on these data, Finegood et al. developed a mathematical model to evaluate the dynamics of pancreatic β cell transformation (Finegood et al. 1995). They suggested the existence of a balance between the division, development, and loss of β cells in healthy individuals. Despite the number of β cells closely linked with their replication rate, their average life expectancy was reported to be varying from 1 to 3 months. In another words, 1–4 % of β cells in adult pancreas were regenerated daily. These data demonstrate that the β cells significantly regenerated during the whole lifetime.

The Type of Stem Cells in the Pancreatic Tissue

In the experimental animal models of pancreatic damage, many evidences were collected for regeneration of mature islet cells by differentiation of pancreas stem or progenitor cells. The development of new islets from pancreatic duct epithelium or neighboring cells after partial pancreatectomy in rodents is a well-documented example (Bonner-Weir et al. 1993). In another damage model, alloxan and streptozotocin, which are known for their potential toxic effect on β cells, were observed to induce the regeneration of endocrine cells from intra-islet precursor cells (Korcakova 1971; Cantenys et al. 1981). In other studies, the role of pancreatic islet progenitor/stem cells in regeneration or neogenesis of islets was reported in the cases of partial duct obstruction (Rosenberg and Vinik 1992); copper deficiency (Rao et al. 1989); soybean trypsin inhibitor therapy (Weaver et al. 1985); caerulein-induced pancreatitis (Elsässer et al. 1986); transient hyperglycemia (Bonner-Weir et al. 1989); overexpression of IFN-γ (Gu and Sarvetnick 1993), Reg1 (Yamaoka et al. 2000), and TGF-α (Sandgren et al. 1990); and after injection of steroids (Kem and Logothetopoulos 1970), insulin antibodies (Logothetopoulos and Bell 1966), and growth factors (Otonkoski et al. 1994).

After the demonstration of the progenitor/stem cells' role in completion of insufficient pancreatic islet functions after damage to meet the requirements,

studies focused on the characterization of these cells with pancreatic islets' or islet cells' producing capacity. In many in vitro and in vivo studies, these cells could be classified with respect to their location, morphological, immunophenotypic, and gene expression profile characteristics as the following:

- Pancreatic duct stem cells
- Acinar cells
- Pancreatic stellate cells (fibroblast-like cells)
- Nestin-positive islet-derived progenitor cells

Pancreatic Duct-Derived Stem/Progenitor Cells

The duct epithelium-derived regeneration of islets under defined conditions was demonstrated for the first time in 1993 (Bonner-Weir et al. 1993). Two years later, Peck et al. published that the isolated islet-producing stem cells from pancreatic channel developed functional islets in long-term culture (Cornelius et al. 1997; Peck and Cornelius 1995; Peck and Ramiya 2004). These cells were obtained from duct epithelial cell cultures prepared by partial digestion of fresh pancreas tissue of prediabetic NOD (nonobese diabetic) mouse or human donors. With the recent improvements, this protocol consists of four main steps: first, duct epithelial cells and islets are isolated from digested pancreas, and they are cultured in the media with the supporting capacity for monolayer-forming epithelial cells with neuroendocrine cell-like phenotype. Epithelial-like islet precursor cells were observed to colonize in single layers, and as they continue to proliferate, these units turned into either sphenoid structures or single-cell suspensions depending on the type of culture and culture conditions. In the third step, islet-derived precursor cells were stimulated by high glucose concentrations and growth factors. By this procedure, it was aimed to support the cell maturation and to induce the generation of multicellular islet-like structures containing endocrine cells in various maturation stages. At the end, the formed islet-like structures were cultured in vivo (e.g., under the kidney capsule) to mature.

Many reports are available on the differentiation of duct cells into islet-like cells or islets by using different protocols (Wang et al. 1995; Fernandes et al. 1997; Rosenberg 1998; Finegood et al. 1999; Sharma et al. 1999; Xu et al. 1999; Yamamoto et al. 2000; Guz et al. 2001; Bonner-Weir et al. 2004; Ogata et al. 2004; Rooman and Bouwens 2004; Bouwens and Rooman 2005; Holland et al. 2005; Oyama et al. 2006; Wang et al. 2008). In the study by Ramiya et al. (2000), duct cells were derived from pancreas of prediabetic NOD mice by enzymatic digestion with collagenase, and they were cultured for 3 years (there are no information available for the culture period of duct cells from human sources). The islet number obtained from five mice pancreatic tissues in 3 years in secondary cultures was equivalent to the number of 10,000 pancreatic islets.

In the other study by Bonner-Weir et al., a monolayer of epithelial cells formed three-dimensional channel cystic structures in a diameter of 50–150 μm after the culturing of duct cells obtained by a similar protocol from human tissues

(Bonner-Weir et al. 2000), and from these structures, islet-like cell clusters comprising pancreas endocrine cells were budded. After 3–4 weeks of culture, the formation of islet-like structures (annotated as *cultured human islet buds* or *CHIBs*) was observed, and both duct and endocrine cells were characterized after detailed analyses. Moreover, uncharacterized cells with ungranulated cytoplasm were detected in culture. Significant increase in insulin secretion was measured, such that 10–15-fold increases in the amount of insulin were measured in each culture. Bonner-Weir later suggested that these duct cells could function as an important precursor cell source for both islet and acinar tissues and duct epithelia could be considered as *facultative stem cells*, consequently (Bonner-Weir et al. 2004).

Important issue in this approach is that every produced islet should be matured and checked for their functionality. For this purpose, the islet-like structures were generally transplanted under the kidney capsule (subcapsular) or subcutaneous region of diabetic or nondiabetic animals. To test the produced islets after the last in vivo maturation phases, Ramiya et al. (2000) transferred mouse islet-like clusters into the kidney subcapsular region of syngeneic female diabetic NOD mice (300 islets per subject). Within 1 week, the blood sugar levels of transplanted mice were decreased to 180–220 mg/dl, and they become insulin independent.

Another interesting result of syngeneic mouse islet implantation study was the lack of the reactive autoimmune response generation against in vitro-produced islets. During the histological examination of implant location, the absence of immune cells in this region was accepted as the evidence for this unresponsiveness (Ramiya et al. 2000; Peck and Ramiya 2004). Researchers usually connected these observations with:

- Loss of β cell autoantigens' expression in in vitro culture
- Development of peripheral tolerance by newly formed transplanted islets after re-induction of autoimmune response
- Lack of time required for the resumption of an autoimmune response
- Characteristics of the implant location

The origin of islets, which were derived from duct epithelial stem cells in this study, could be associated with the unresponsiveness of autoimmune system in transplanted diabetic mouse. Whatever the reason, the important conclusion of this study was that the duct stem/progenitor cells might be accepted as a potential islet/β cell source in cell replacement therapy of diabetes type 1 due to the lack of reactivation of autoimmune response in this system.

All of these studies indicate the existence of progenitor/stem cells with the differentiation capacity into both exocrine and endocrine cells within the population of pancreatic duct cells. Since the first hypothesis of islet regeneration from duct epithelium under certain conditions by Bonner-Weir et al. (1993), there have been many attempts to obtain functional islets and/or β cells from human or rodent duct cells by different techniques and approaches including gene transfer (Leng and Lu 2005; Kim et al. 2006). Considering the molecular and structural development stages of pancreas, all endocrine, acinar, and duct cells were developed from the same endodermal epithelial tissue derived from embryonic foregut (duodenal)

region, and adult duct cells share some common features with embryonic primitive channels. As a result, pancreatic duct cells may contribute to the production of endocrine cells in adulthood.

Multipotent precursor/stem cells were isolated from islet and duct cell culture expressing markers of both neuronal and pancreatic precursor cells. These cells have the ability to differentiate into neuronal and glial cells as well as into pancreatic lineage cells (Seaberg et al. 2004). Because of their expression of distinctive markers excluding the mesoderm or neural crest characters, the existence of an intrinsic pancreatic precursor cell population was suggested. The argument about the origin of these cells being other tissue was eliminated by labeling the non-endocrine pancreas epithelial cells and eliminating contaminating mesenchymal cells with selection for drug resistance (Hao et al. 2006).

These progenitor/stem cells were isolated by FACS (fluorescence-activated cell sorting) according to the negative selection for CD45, Ter119, c-Kit (CD117), and Flk-1 (hematopoietic markers) and the positive selection for c-Met (hepatocyte growth factor receptor). Their differentiation into exocrine and endocrine pancreatic cell lineages was observed both in vitro and in vivo (Suzuki et al. 2004). In most of the studies on the recovery of pancreatic islets after the experimental damage, the number of β cells was increased. However, the origin of the β cells was not clearly defined, whether they differentiated from progenitor/stem cells into β cells or generated from β cells. To solve this problem, Ngn3-positive progenitor/stem cells in duct epithelium were studied. In the specific culture conditions, these cells were shown to differentiate into islet-/β cell-like cells. After pancreatic duct ligation, the number of Ngn3-positive cells was increased in mice (Xu et al. 2008). The silencing of Ngn3 in these cells resulted in the impairment of β/islet regeneration after duct ligation. On the other hand, the transplantation of these cells into Ngn3-knockout mice induced the generation of islet-like cells in embryonic pancreas. These evidences indicate the important role of Ngn3-positive multipotent cells in islet/β regeneration after duct ligation, but their origin is still unknown, whether they are duct cells or derived from a subpopulation of the duct epithelial cells.

Acinar Cells

There are many manuscripts that reported the differentiation of pancreatic exocrine/acinar cells into pancreatic duct and endocrine cells (transdifferentiation), the molecular marker expression of three important pancreas tissue components (exocrine, pancreas endocrine, and duct cells) of these stem/progenitor cells, and their supporting roles in pancreas regeneration and islet neogenesis.

Following the experimental pancreas damaged by various factors (duct ligation, pancreatectomy, and chemical agents), pancreatic acinar cells were induced to differentiate primarily into duct cell-like cells (*acino-ductal metaplasia*) and involved in the neogenesis of islets in some of these studies (Gu et al. 1994, 1997; Gmyr et al. 2000; De Haro-Hernandez et al. 2004; Holland et al. 2004;

Sphyris et al. 2005). These observations were supported by identification of *transition cells* expressing specific markers of endocrine/exocrine cells and duct cells.

Lipsett and Finegood (2002) reported that the acinar cells were directly responsible for increased β cell neogenesis in long-term experimental hyperglycemia model. Interestingly in the immunohistochemical examination of pancreatic tissues from dexamethasone-treated animals with pancreatic duct ligation, Lardon et al. detected the acino-insular structures (transition from acinar into islet) consisted of amylase and insulin-expressing transitional cells (Lardon et al. 2004). Some evidences were provided for the differentiation of adult mouse pancreatic cells into insulin-producing cells by cell line monitoring with Cre/Lox-based techniques (Minami et al. 2005). The newly formed cells possessed all the essential requirements for glucose-stimulated insulin secretion, which is the fundamental mechanism of insulin secretion. In this lineage monitoring by Cre-loxP recombination techniques, enhanced cyan fluorescent protein (ECFP) was expressed under the control of mouse amylase-2 and rat elastase-1 promoters. Pancreatic acinar cells, obtained from mice with R26R-ECFP reporter, were transdifferentiated into insulin-producing cells.

Similar observations were reported for the transdifferentiation of acinar cells into endocrine pancreas lineage cells (Baeyens et al. 2005; Minami et al. 2005; Okuno et al. 2007). Under defined conditions, acinar to duct cell transdifferentiation was detected (Means et al. 2005), whereas the acinar to β cell transdifferentiation was not (Desai et al. 2007). With the reprogramming of mouse acinar cells by inducing the expression of Ngn3, Pdx-1, and MafA, β cell transdifferentiation was induced in these cells (Zhou et al. 2008). The detailed analyses on transcription factors for β cell transdifferentiation showed that Pdx-1, Ngn3, and MafA expressions were critical for both differentiation and function. Indirect evidences were collected for acinar to β cells transdifferentiation in other studies (Lipsett and Finegood 2002; Rooman and Bouwens 2004). The capacity of pancreatic oval cells to differentiate into pancreatic lineages has not been proven yet.

Pancreatic Stellate Cells

The presence of stem cell-like cells in the pancreas, which was morphologically and functionally similar to Ito cells (known as fat-storing cells, vitamin A-storing fibroblast-like cells, or as myofibroblast-like cells) or stellate cells in the liver, was recently reported.

These cells, also called as pancreatic stellate cells, were first discovered in 1982 in mice (Watari et al. 1982) and 8 years later in human tissue (Ikejiri 1990). In the following studies, these cells were shown to be responsible for pancreatic fibrogenesis (Saotome et al. 1997). In the detailed examination, it was determined that they share many common features (like morphological and immunohistochemical properties, and synthesis capacity of extracellular matrix) with the hepatic stellate cells in the space of Disse. Hepatic stellate cells are localized in the interlobular septa and

interacinar (periacinar) areas and have a pathogenic role in fibrosis (Haber et al. 1999). Pancreatic stellate cells were observed to support the proliferation of acinar cells and tubular structures in trypsin-induced necrohemorrhagic pancreatitis (Lechene et al. 1991). Recently, they were reported to involve in the regeneration of early stages of acute pancreatitis in humans (Zimmermann et al. 2002). All these findings point out that those pancreatic stellate cells not only are involved in fibrogenesis but also contribute in the tissue remodeling at the same time.

After reporting the role of pancreatic stellate cells in regeneration process to various pathogenic conditions, Kruse et al. (2004) isolated pancreas stellate cell-like cells from rodent exocrine pancreas, and these cells were cultured for a long time (at least 20 passages) without differentiation. These cells formed three-dimensional cell clusters, called *organoid bodies* (similar to the embryoid bodies), following culturing in droplets for 2 days in vitro. After incubation of these organoid bodies in tissue culture dishes for 7 weeks, they developed cell colonies, which formed new organoid bodies spontaneously. Without any induction, these bodies were observed to differentiate into various cell types of all three germ layers (smooth muscle cells, neurons, glial cells, epithelial cells, chondrocytes, and pancreatic exocrine and endocrine cells). The formation of embryonic cell line from organoid bodies of undifferentiated adult stem cells and their spontaneous differentiation into cell lineages of endoderm, mesoderm, and ectoderm lead some researchers to consider these cells as the new class of undifferentiated pluripotent adult stem cells.

Nestin-Positive Islet-Derived Progenitor Cells

Before the study by Zulewski et al., pancreatic islet precursor cells were believed to be found only in pancreatic ducts, and the related studies were focused consequently on this region (Zulewski et al. 2001). Due to the common features of β cells and neural cells in the early stages of mammalian embryonic development, and their similar characteristics, the expression of neural stem cell-specific marker, nestin, in rat and human pancreatic islet cells was explored. After all, these intermediate filament protein nestin-expressing cells were isolated from human and rat islets and propagated in culture conditions. These cells were named as nestin-positive islet-derived progenitor cells, and after induction for differentiation, they were transformed into islet cells with insulin, glucagon, and Pdx-1 gene expressions. Besides the expression of other neuroendocrine cells, hepatic and pancreatic exocrine genes were shown by RT-PCR.

Another study of this research group revealed the increase of the differentiation ratio into islet-like insulin-producing cell clusters in response to GLP-1 in vitro (Abraham et al. 2002). With this recent study, the researchers showed the presence of GLP-1 receptor in nestin-positive islet precursor cells. Therefore, their differentiation into insulin-producing cells was induced by binding of GLP-1 to appropriate receptors. The effect of GLP-1 in vivo is quite intriguing, if it significantly involved in β cell differentiation in vitro. Assuming the induction of β cell regeneration by

local production of GLP-1 in pancreas, patients with type 2 diabetes were treated by GLP-1 injection. The upregulation in insulin secretion was observed afterwards, and the insulin-mediated sugar utilization was increased (Meneilly et al. 2001, 2003a). Similar study was also performed in patients with type 1 diabetes, but the upregulatory effect of GLP-1 on insulin-mediated glucose utilization was not observed in this case (Meneilly et al. 2003b). Despite the ineffectiveness in type 1diabetes, the positive results of GLP-1, with which clinical trials are still ongoing, are very important in type 2 diabetes. In addition, GLP-1 agonists have been shown to induce β cell development and differentiation besides their cell protective role and antiapoptotic effects on β cells. In accordance with these findings, pancreatic stem/progenitor cells might be used to produce β cells in vitro by stimulatory effect of GLP-1 and might be used in replacement therapy (List and Habener 2004).

One to three percent of these islet-derived nestin-positive precursor cells were observed to have similar immunophenotypic features of undifferentiated bone marrow stem cells and were defined as side population. In parallel with these findings, nestin-positive precursor cells were suggested to contain a subpopulation of immature stem cells with differentiation potential (Lechner et al. 2002). The detection of cells with immunophenotypic markers specific for bone marrow-derived cells in pancreatic islets was another important point in these studies. These cells, also possess specific immunophenotypic markers of pancreatic islet stem cells, were considered to migrate from bone marrow (Lechner and Habener 2003). On the other hand, some evidences were reported that these cells could not originate from hematopoietic cell lineages. For example, Poliakova et al. defined similar cells in the pancreases of human and monkey, but the vast majority of these cells (95 %) were negative for hematopoietic stem cell-specific markers, like CD34 and CD45 (Poliakova et al. 2004). In addition, a study was reported that no evidence was found on the development of endocrine cells from nestin-positive stem cells (Gao et al. 2003).

Other studies on the islet progenitor or stem cells mostly focused on demonstrating the presence of these adult pancreas-derived insulin-producing cells with the production capacity of βcell-like cells by using different culture conditions. These cells are called by different names, like intra-islet precursor cells (Guz et al. 2001; Banerjee and Bhonde 2003), pancreatic stem cells (Schmied et al. 2001; Suzuki et al. 2004), small cells (Petropavlovskaia and Rosenberg 2002), islet-derived progenitor cells (Wang et al. 2004; Linning et al. 2004; von Mach et al. 2004), multipotent stem cell (Choi et al. 2004), or β stem cells (Duvillie et al. 2003). Although these cells commonly differentiated into β-like insulin-producing cells in vitro and in vivo, they were also observed to form other types of cells, like central nervous system and multiple cell types with phenotypic characteristics of neural crista (Choi et al. 2004), exocrine cells (Schmied et al. 2001), and liver, stomach, and intestinal cells (Suzuki et al. 2004), under defined media in vitro. Their differentiation capacity into acinar (Suzuki et al. 2004) and liver cells (von Mach et al. 2004) was demonstrated in vivo.

In the culture of the postmortem adult human islets, mesenchymal-type cells exhibiting fibroblast morphology were characterized. Although these cells lack the

ability of insulin secretion in response to glucose stimuli, they can be induced to differentiate into hormone-expressing islet-like cells (Gershengorn et al. 2004, 2005). Initially, they were thought to be the precursor cells of insulin-expressing cells undergone to mesenchymal-to-epithelial transition, but the human islet-derived precursor cells were later demonstrated to have the mesenchymal stem cell characteristics (Gershengorn et al. 2005; Davani et al. 2007). On the other hand, the inability of the epithelial-to-mesenchymal-to-epithelial transition by mouse β cells was revealed (Atouf et al. 2007; Morton et al. 2007; Meier et al. 2006). Some studies suggested the dedifferentiation of pancreatic islet cells into precursor cells, and the redifferentiation into the pancreatic lineage cells might be the source of the regenerated β cells (Lechner et al. 2005; Ouziel-Yahalom et al. 2006; Banerjee and Bhonde 2003).

Another clonogenic cell population, nestin-positive progenitor/stem cells, was isolated from islets showing bone marrow MSC phenotypic markers (Table 1) and differentiating into insulin-producing cells in vitro (Lechner et al. 2002; Gallo et al. 2007; Gershengorn et al. 2005; Zhang et al. 2005; Atouf et al. 2007; Davani et al. 2007; Karaoz et al. 2010a; Carlotti et al. 2010; Montanucci et al. 2011). These cells were mainly negative for c-peptide and Pdx-1 in the culture, but under serum-free conditions, these cells lost their MSC phenotype, formed islet-like clusters, and were able to secrete insulin. Although the endocrine hormones were not expressed in the normal culture conditions, these cells have the tendency to form clusters, which induce the expression of insulin, glucagon, and somatostatin genes in serum-free medium (Carlotti et al. 2010). Like mesenchymal stem cells (MSCs), they express lineage-specific markers, i.e., CD13, CD29, CD44, CD54, CD73, CD90, CD105, nestin, and vimentin, while they are negative for hematopoietic, endothelial, and ductal cell markers (Fig. 1) and differentiate into osteogenic, adipogenic, and chondrogenic cell lineages after induction (Fig. 2). The expression of ACTA2, CD146, NG2, and PDGFRβ by these cells indicates their pericyte characteristics. Besides, the expression of pluripotency markers by rat pancreatic islet-derived progenitor/stem cells, i.e., Oct-4, Rex-1, and Sox-2, was shown (Karaoz et al. 2010a). For that reason, these nestin-positive cells were called as pancreatic islet-derived stem cells (PI-SCs) (Karaoz et al. 2010a). Montanucci et al. also reported the existence of insulin-producing progenitor cells with the expression of Oct-4, Sox-2, Nanog, ABCG2, Klf-4, and CD117 in human islets (Montanucci et al. 2011). Similarly, they showed differentiation capacity like MSCs, including the differentiation ability into insulin-secreting endocrine cells.

The differentiation of nestin-positive progenitor/stem cells into insulin-secreting, β-like cells might be a promising approach to compensate for β cell loss in diabetes. Recently, the studies on MSCs were focused on their immunosuppressive activity as well. MSCs derived from bone marrow were demonstrated to have regulatory function on the components of immune system (Guo et al. 2006; Spaggiari et al. 2008; Comoli et al. 2008). These cells control the immune response by modifying their cytokine secretion profiles to show anti-inflammatory effect or tolerant phenotype (Chang et al. 2006). IFN-γ secreted by T and NK cells and IL-1 group cytokines by mononuclear cells were found to activate the suppressive

Table 1 Immunocytochemical properties of undifferentiated rPI-SCs in passage 3

Marker molecule	rPI-SCs[a]	Marker molecule	rPI-SCs[a]
β3Tubulin	+	Glut2	∅
Actin beta (ACTβ)	+	HNK-1ST	∅
ACTA2	+	IL-10	∅
BDNF	+	IL-1β	−/+
BMP-2	+	IL-1ra	+
BMP-4	+	IL-4	∅
BrdU	+	Insulin	∅
CD 31 (PECAM-1)	∅	Ki67	+
CD 34 (HPCA1)	∅	MAP 2a,b	+
CD 45 (PTP)	∅	MPO	+
CD 71 (TfR1)	∅	MyoD	∅
CD105 (Endoglin)	+	Myogenin	+
CD146 (MCAM)	+	Myosin IIa	+
c-Fos	+	Nestin	+
CNTF	∅	NSE (Eno2)	+
Collagen Ia1	−/+	Osteocalcin	+
Collagen II	+	Osteonectin	+
Connexin43	+	Osteopontin	+[b]
C-Peptide	∅	PCNA	+
Cytokeratin 18	∅	PDX-1	∅
Cytokeratin 19	∅	S100	+
Desmin	+	Somatostatin	∅
EP3	+	STAT3	+
Fibronectin	+	Vimentin	+
GFAP	+	vWF	∅
Glucagon	∅	β-tubulin	+

[a]Expression: +: positive, ∅: lack, −/+: weak
[b]Immunoreactivity was positive in 10–20 % of the cells

mechanisms in MSCs. Besides the soluble factors, like IL-10 (Krampera et al. 2003), IL-6 (Noel et al. 2007), TGF-β (Di Nicola et al. 2002; Aggarwal and Pittenger 2005), and HGF (Rasmusson 2006), cell-to-cell contact was shown to have role in regulation (Krampera et al. 2003; Di Nicola et al. 2002).

Similar to bone marrow-derived MSCs, pancreatic islet MSCs could also suppress proliferation of stimulated T cells. In the study by Kim et al. (2012), pancreatic islet MSCs showed higher IL-1β, IL-6, STAT3, and FGF9 expressions compared to bone marrow-derived MSC and less IL-10. As bone marrow-derived MSCs and pancreatic islet-derived stem cells share many common features, both types of cells were expected to have immune regulatory effect. For this aim, islet stem cells were cocultured with chemically stimulated T cells, and cellular responses were analyzed (unpublished data). After coculture, the proliferation of stimulated T cells was decreased, and the number of apoptotic cells was increased. The expressions of pro-inflammatory cytokines (IL-1a, IL-2, IL-12b, IFN-γ, and

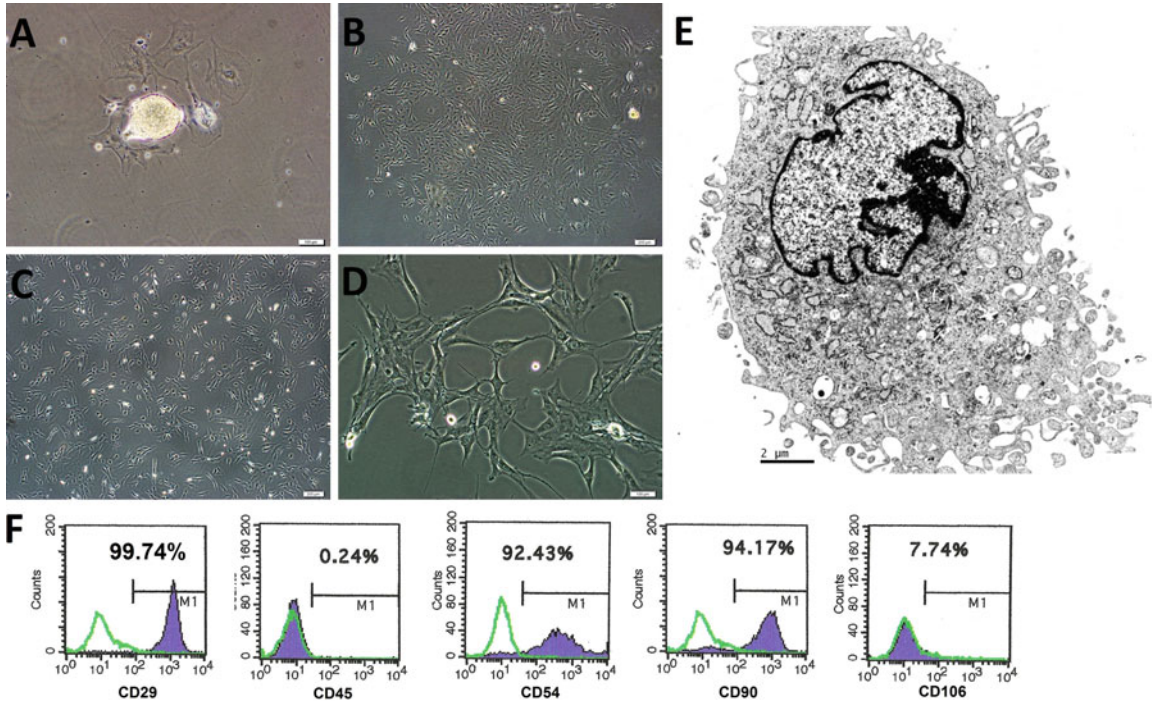

Fig. 1 Colony formation and characterization of rat pancreatic islet-derived stem cells. During the culture, cells with mesenchymal morphology expanded out from the pancreatic islet (**a**) and form colonies (**b**). Later, the cells exhibited large, flattened, or fibroblast-like morphology (**c**, **d**). The ultrastructural analysis of the cells revealed their eccentric and irregularly shaped large nuclei with multiple nucleoli (**e**). Chromatin-formed thin and dense layer inside the perinuclear cisternae. The cytoplasms had many rough endoplasmic reticulum cisternae. The plasma membrane of pancreatic islet stem cells has several thick pseudopodia-like structures (Scale Bars: 2 µm). The cell maker analysis showed typical mesenchymal marker expression for these cells, i.e., positive for CD29, CD54, and CD90 and negative for hematopoietic marker, CD45. CD106 (Vcam1) expression was partially positive

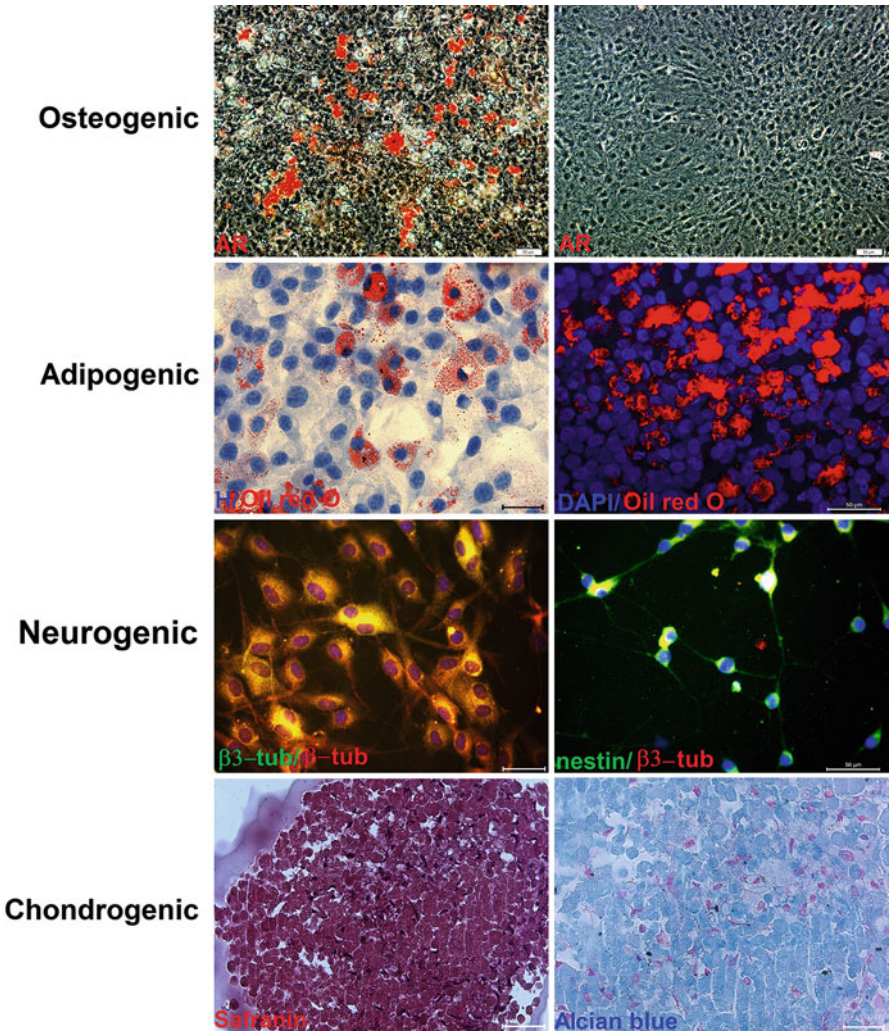

Fig. 2 Differentiation of pancreatic islet-derived stem cells into different cell lineages. After the culture in specific differentiation media, stem cells were characterized with respect to histochemical and immunofluorescence staining. Osteogenic differentiation was shown by staining of mineral deposits with *Alizarin red S*. Staining was not observed in the undifferentiated cells. Adipogenic differentiation was proven by staining of cytoplasmic oil deposits with *Oil Red O*. Neurogenic differentiation was determined both by alteration in cell morphology and staining for neurogenic markers (β-tubulin, β 3-tubulin, and nestin). Chondrogenic differentiation was shown by safranin and alcian blue staining

TNF-a) decreased while the level of anti-inflammatory cytokines (IL-4, IL-6, and IL-10) increased during the cocultures. Moreover, the number of T_{reg} significantly increased during the the presence of rPI-SCs, which is known to suppress the T-cell activity. The expression of IL-1ra in pancreatic islet stem cells blocks the

inflammatory responses by inhibiting the activity of IL-1a and IL-1β (Jiang et al. 2005; Ye et al. 2008). The expression levels of ICAM-1, VCAM-1, IL-4, IL-6, IL-10, IL-13, TGF-β, and HGF increased in both direct and indirect cocultures. Their increased expression in the cocultures of rPI-SCs with T cells may be the sign for the immunosuppressive mechanism (Glennie et al. 2005; English et al. 2009; Zheng et al. 2004). Because of the immune regulatory effect, these cells were suggested to be used in organ transplants to decrease the rejection or in β cell protection from the immune attacks during the onset of diabetes. A very important question arouse consequently. If those stem cells in pancreatic islets were effectively modulating the immune response, then why can they not function effectively in the diabetic persons? The reasonable explanation for this might be the limited number of these cells for the regulation effects.

After stimulation with IFN-γ, mesenchymal stem cells were shown to act as antigen-presenting cells (Stagg et al. 2006; Chan et al. 2006; Morandi et al. 2008). This phenomenon is quite similar to the antigen-presenting cells in pancreatic islets that are considered to be the initiator of type 1 diabetes by presenting βcell-derived peptides to immune cells (Lacy et al. 1979; Nauta et al. 2006). In these studies, the antigen presentation was observed in the bone marrow-derived MSCs, but it is still unclear whether pancreatic islet-derived stem cells exhibit the similar activity. Indirect evidences were collected in the analyses of these cells. Pancreatic islet-derived stem cells showed CD40 and CD80 expressions (Klein et al. 2005) but not CD86. Similar case was also reported by Lei et al. (2005), in which murine keratinocyte stem cells expressed CD80 but not CD86, indicating that these cells could act as antigen-presenting cells (Lei et al. 2005). Furthermore, MHC II was also not expressed by pancreatic islet-derived stem cells, which makes them nonprofessional antigen-presenting cells. Remarkably, the expression of CX3CR1, which is typically expressed by monocytes, dendritic cells, natural killer cells, and helper T cells (type 1), was observed in pancreatic islet stem cells (Fraticelli et al. 2001; Nishimura et al. 2002; Dichmann et al. 2001). The similar characteristics of pancreatic islet-derived stem cells with antigen-presenting cells make them an important target in the early onset of the diabetes type 1.

In this context, the interaction of endocrine cells, mainly β cells, with islet-derived stem cells was analyzed at the microscopic level following the preparation of single-cell suspension of rat pancreatic islets (Fig. 3). Endocrine cells could be cultured for more than 30 days in appropriate culture conditions in the presence of islet-derived stem cells. However, the removal of these stem cells from the culture caused the decrease in the viability that made the long-term culture of endocrine cells impossible under defined condition (unpublished data). According to this observation, islet-derived stem cells might have similar protective effect like none marrow-derived stem cells (Karaoz et al. 2010b). Considering there could be similar interactions in vivo, the role of islet-derived stem cells was studied in the apoptosis of β cells. After coculture of damaged pancreatic islets with pancreatic islet-derived stem cells, the expression of regulatory proteins in apoptosis, like Bcl3, TNIP1 (TNFAIP3 interacting protein 1), and MAPKAPK2, was increased under stress in pancreatic islets (unpublished data). The number of viable cells and

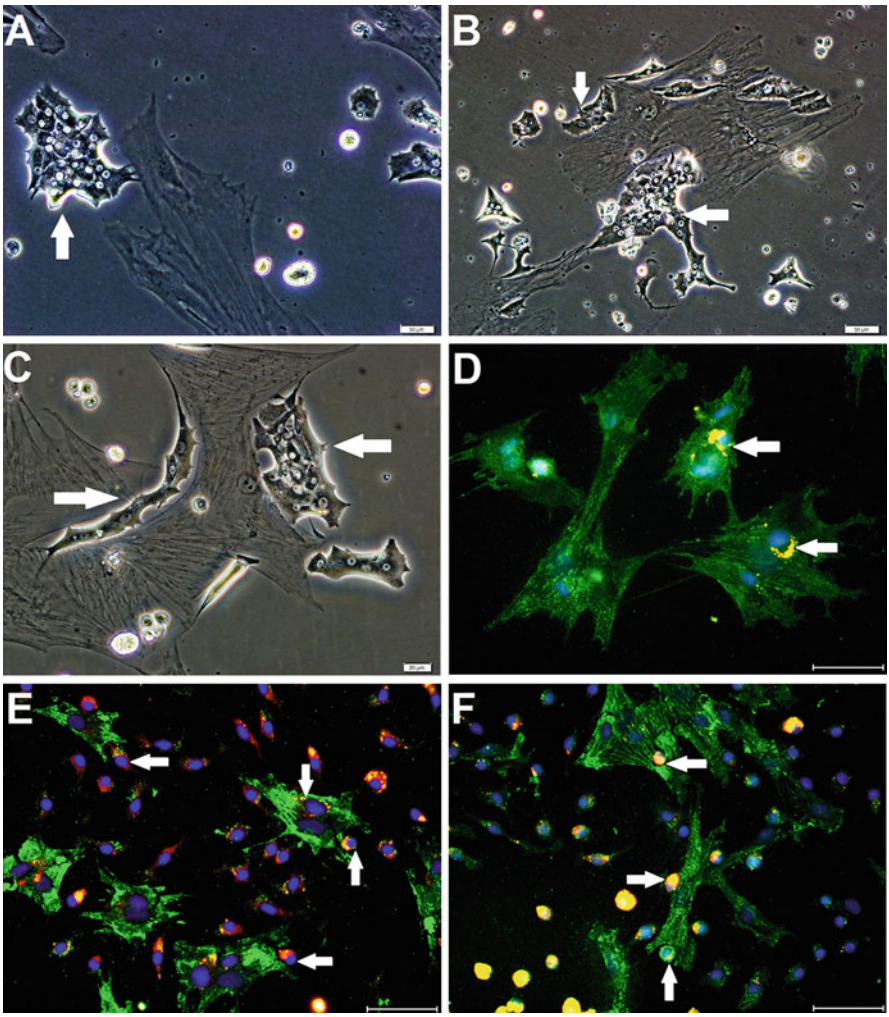

Fig. 3 Interaction of pancreatic islet endocrine cells with stem cells derived from pancreatic islets. Single-cell suspension of endocrine cells, prepared by enzymatic digestion of rat pancreatic islets, was cultured for 30 days without any significant loss of their viability together with stem cells derived from pancreatic islets. The endocrine cells, mostly β cells, interact closely with the stem cells in the culture (*white arrow*, **a**–**c**). Pancreatic islet-derived stem cells were identified by staining with antibodies against α-Actin-2 (**d**; *green*) and β-actin (**e**, **f**; *green*). The single-cell suspension of endocrine cells from islets with high number of insulin-positive cells (*red*) signifies that the suspension mostly composed of β cells. Stem cells in the environment of the islets support the β cell viability in vitro, and similar effect is also expected to exist

insulin-secretion capacity was preserved in the coculture with stem cells, whereas necrotic bodies were formed in the absence of the stem cells.

By direct and indirect mechanisms, islet stem cells have a protective role on pancreatic islet cells. Although a number of molecules involve in the antiapoptotic

effect, the expression of IL-6 and TGF-β was significantly increased during the coculture (unpublished data). By the reverse experimental approach, in which the effect of IL-6 was neutralized by antibody, the pivotal role of IL-6 in the preserving the β-cell viability was shown. On the other hand, significant effect of IL-6 supplementation to the culture of damaged islets was not observed. The same experiment with TGF-β supplementation increased the cell viability, but the effect remained limited. These findings indicate that there are many key molecules in the regulation of apoptosis with complex interactions, although their expression levels are not as high as IL-6 or TGF-β. In the gene expression analysis of the cocultures after IL-6 neutralization, the expression levels of IL-2 and IL-9 were decreased considerably. The IL-2- and IL-9-mediated regulation of apoptosis in pancreatic islets was mainly mediated by JAK/STAT signaling, which controls numerous important biologic responses like immune function and cellular growth (Demoulin et al. 1996; Rabinovitch et al. 2002; Chentoufi et al. 2011; Tang et al. 2008).

The origin of progenitor/stem cells in pancreatic islets is still a controversial issue. Gong et al. (2012) reported that the nestin-positive cells involve in repair of damage in acinar tissues and islets. They take part only in the supporting of damaged islets, and functional differentiation of these cells was not observed. The expression of c-Kit (CD117) by these cells, and rarely by normal pancreatic tissues, becomes the main point of their suggestion that the origin of pancreatic islet stem cells might be the bone marrow stem cells. Those cells were reported to locate only in the islets and involve only in the repair process of islets damaged. For that reason, they were defined as progenitor cells rather stem cells (Gong et al. 2012). In another study, pancreatic islet-derived stem cells were pointed out to involve in endocrine pancreatic regeneration with a lower frequency (Joglekar et al. 2007).

β Cell Replication

Until recently, the β-cells regeneration was thought to be very limited. However, the report by a group of researchers from Harvard University published in 2004 changed this notion. The research group lead by Douglas Melton observed the self-renewal and regeneration of β cells in mice after partial pancreatectomy by the method of genetic lineage tracing (Dor et al. 2004; Dor and Melton 2004; Nir and Dor 2005). In the following studies, they demonstrated the replication of β-cells both in vivo and in vitro (Georgia and Bhushan 2004; Meier et al. 2006). Another evidence for β-cell regeneration by replication of mature β-cells was provided by Gershengorn et al. (2004, 2005). The newly formed cells were reported to be originated from nestin-positive mesenchymal-like progenitor cells that were generated by dedifferentiation of previously existing β cells, also called epithelial-mesenchymal transition process. Interestingly, the supporting studies came from two independent research groups later (Lechner et al. 2005; Ouziel-Yahalom et al. 2006). Both groups reported that the β-cell regeneration in human pancreatic islets occurs by de- and redifferentiation of these cells.

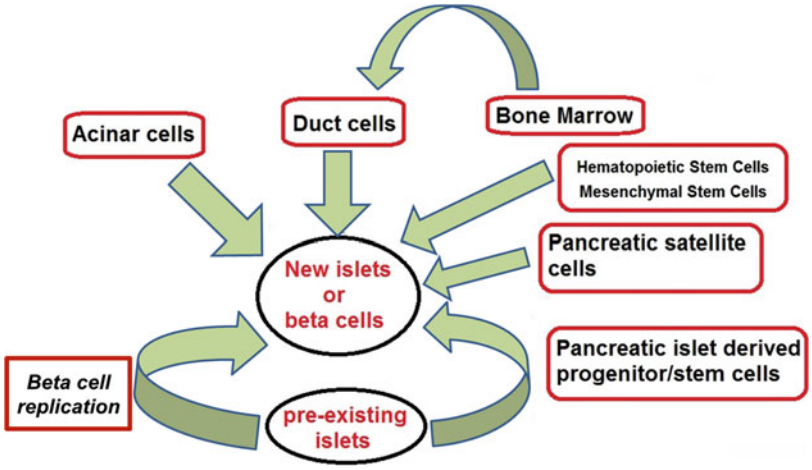

Fig. 4 Schematic drawing of the possible sources of regenerated pancreatic islets and β-cells

All these results indicated the generation of new islet or β cells occurred by the normal and pathological processes in adult pancreatic islets (Fig. 4). These cells might be the stem cells mobilized from bone marrow, cells originated from duct cells that homed in islets, undifferentiated stem cell cells from the early stage of development, or cells de- and redifferentiated in response to various physiological signals. Regardless of their source, the pancreatic islet stem cells promise by the demonstration of β-cell regeneration in adulthood the development of radical treatment procedures for type 1 diabetes in the future.

Concluding Remarks

As pancreatic islet-derived stem cells carry both immune suppressive and antiapoptotic features, they might have important roles in pathogenesis in diabetes type 1. Under normal circumstances, these cells are expected to function as protector of β-cells and other endocrine cells in islets maintaining both their viability and functionality. However, the weakness in their function during the onset of the diabetes is crucial in this case. Therefore, the interaction between stem cells and endocrine cells in pancreatic islets should be focused in the future more in detail for better understanding of the disease. In vitro and in vivo studies are required to explain why pancreatic islet-derived stem cells may not suppress attacks of autoreactive immune cells towards pancreatic islets in people with diabetes type 1. The comparison of stem cells from pancreatic islets of diseased people with healthy individuals at both genomic and proteomic levels would benefit to find alternative treatment approaches.

Cross-References

► Generating Pancreatic Endocrine Cells from Pluripotent Stem Cells
► Regulation of Pancreatic Islet Formation

References

Abraham EJ, Leech CA, Lin JC, Zulewski H, Habener JF (2002) Insulinotropic hormone glucagon-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells. Endocrinology 143:3152–3161

Aggarwal S, Pittenger MF (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105:1815–1822

Atouf F, Park CH, Pechhold K, Ta M, Choi Y, Lumelsky NL (2007) No evidence for mouse pancreatic β-cell epithelial-mesenchymal transition in vitro. Diabetes 56:699–702

Baeyens L, De Breuck S, Lardon J, Mfopou JK, Rooman I, Bouwens L (2005) İn vitro generation of insulin-producing β cells from adult exocrine pancreatic cells. Diabetologia 48:49–57

Banerjee M, Bhonde RR (2003) Islet generation from intra islet precursor cells of diabetic pancreas: in vitro studies depicting in vivo differentiation. JOP 4:137–145

Bonner-Weir S, Deery D, Leahy JL, Weir GC (1989) Compensatory growth of pancreatic β-cells in adults after short-term glucose infusion. Diabetes 38:49–53

Bonner-Weir S, Baxter LA, Schuppin GT, Smith FE (1993) A second pathway for regeneration of adult exocrine and endocrine pancreas. A possible recapitulation of embryonic development. Diabetes 42:1715–1720

Bonner-Weir S, Taneja M, Weir GC, Tatarkiewicz K, Song KH, Sharma A, O'Neil JJ (2000) In vitro cultivation of human islets from expanded ductal tissue. Proc Natl Acad Sci USA 97:7999–8004

Bonner-Weir S, Toschi E, Inada A, Reitz P, Fonseca SY, Aye T, Sharma A (2004) The pancreatic ductal epithelium serves as a potential pool of progenitor cells. Pediatr Diabetes 5:16–22

Bouwens L, Rooman I (2005) Regulation of pancreatic β-cell mass. Physiol Rev 85:1255–1270

Brelje TC, Scharp DW, Lacy PE, Ogren L, Talamantes F, Robertson M, Friesen HG, Sorenson RL (1993) Effect of homologous placenta lactogens, prolactins, and growth hormones on islet β-cell division and insulin secretion in rat, mouse, and human islets: implication for placental lactogen regulation of islet function during pregnancy. Endocrinology 132:879–887

Cantenys D, Portha B, Dutrillaux MC, Hollande E, Roze C, Picon L (1981) Histogenesis of the endocrine pancreas in newborn rats after destruction by streptozotosin. An immunocytochemical study. Virchows Arch B Cell Pathol Incl Mol Pathol 35:109–122

Carlotti F, Zaldumbide A, Loomans CJ, van Rossenberg E, Engelse M, de Koning EJ, Hoeben RC (2010) Isolated human islets contain a distinct population of mesenchymal stem cells. Islets 2:164–173

Chan JL, Tang KC, Patel AP, Bonilla LM, Pierobon N, Ponzio NM, Rameshwar P (2006) Antigen-presenting property of mesenchymal stem cells occurs during a narrow window at low levels of interferon-γ. Blood 107:4817–4824

Chang CJ, Yen ML, Chen YC, Chien CC, Huang HI, Bai CH, Yen BL (2006) Placenta-derived multipotent cells exhibit immunosuppressive properties that are enhanced in the presence of interferon-γ. Stem Cells 24:2466–2477

Chentoufi AA, Gaudreau S, Nguyen A, Sabha M, Amrani A, Elghazali G (2011) Type I diabetes-associated tolerogenic properties of interleukin-2. Clin Dev Immunol 2011:289343

Choi Y, Ta M, Atouf F, Lumelsky N (2004) Adult pancreas generates multipotent stem cells and pancreatic and nonpancreatic progeny. Stem Cells 22:1070–1084

Comoli P, Ginevri F, Maccario R, Avanzini MA, Marconi M, Groff A, Cometa A, Cioni M, Porretti L, Barberi W, Frassoni F, Locatelli F (2008) Human mesenchymal stem cells

inhibit antibody production induced in vitro by allostimulation. Nephrol Dial Transplant 23:1196–1202
Cornelius JG, Tchemev V, Kao KJ, Peck AB (1997) İn vitro generation of islets in long-term cultures of pluripotent stem cells from adult mouse pancreas. Horm Metab Res 29:271–277
Davani B, Ikonomou L, Raaka BM, Geras-Raaka E, Morton RA, Marcus-Samuels B, Gershengorn MC (2007) Human islet-derived precursor cells are mesenchymal stromal cells that differentiate and mature to hormone-expressing cells in vivo. Stem Cells 25:3215–3222
De Haro-Hernandez R, Cabrera-Munoz L, Mendez JD (2004) Regeneration of β-cells and neogenesis from small ducts or acinar cells promote recovery of endocrine pancreatic function in alloxan-treated rats. Arch Med Res 35:114–120
Demoulin JB, Uyttenhove C, Van Roost E, DeLestré B, Donckers D, Van Snick J, Renauld JC (1996) A single tyrosine of the interleukin-9 (IL-9) receptor is required for STAT activation, antiapoptotic activity, and growth regulation by IL-9. Mol Cell Biol 16:4710–4716
Desai BM, Oliver-Krasinski J, De Leon DD, Farzad C, Hong N, Leach SD, Stoffers DA (2007) Preexisting pancreatic acinar cells contribute to acinar cell, but not islet β cell, regeneration. J Clin Invest 117:971–977
Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM (2002) Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 99:3838–3843
Dichmann S, Herouy Y, Purlis D, Rheinen H, Gebicke-Harter P, Norgauer J (2001) Fractalkine induces chemotaxis and actin polymerization in human dendritic cells. Inflamm Res 50:529–533
Dor Y, Melton DA (2004) How important are adult stem cells for tissue maintenance? Cell Cycle 3:1104–1106
Dor Y, Brown J, Martinez OI, Melton DA (2004) Adult pancreatic β-cells are formed by self-duplication rather than stem-cell differentiation. Nature 429:41–46
Duvillie B, Attali M, Aiello V, Quemeneur E, Scharfmann R (2003) Label-retaining cells in the rat pancreas: location and differentiation potential in vitro. Diabetes 52:2035–2042
Elsässer HP, Adler G, Kern HF (1986) Time course and cellular source of pancreatic regeneration following acute pancreatitis in the rat. Pancreas 1:421–429
English K, Ryan JM, Tobin L, Murphy MJ, Barry FP, Mahon BP (2009) Cell contact, prostaglandin E 2 and transforming growth factor β 1 play non-redundant roles in human mesenchymal stem cell induction of CD4 CD25 high forkhead box P3 regulatory T cells. Clin Exp Immunol 156:149–160
Fernandes A, King LC, Guz Y, Stein R, Wright CV, Teitelman G (1997) Differentiation of new insulin-producing cells is induced by injury in adult pancreatic islets. Endocrinology 138:1750–1762
Finegood DT, Scaglia L, Bonner-Weir S (1995) Dynamics of β-cell mass in the growing rat pancreas. Estimation with a simple mathematical model. Diabetes 44:249–256
Finegood DT, Weir GC, Bonner-Weir S (1999) Prior streptozotocin treatment does not inhibit pancreas regeneration after 90% pancreatectomy in rats. Am J Physiol 276:E822–E827
Fraticelli P, Sironi M, Bianchi G, D'Ambrosio D, Albanesi C, Stoppacciaro A, Chieppa M, Allavena P, Ruco L, Girolomoni G, Sinigaglia F, Vecchi A, Mantovani A (2001) Fractalkine (CX3CL1) as an amplification circuit of polarized Th1 responses. J Clin Invest 107:1173–1181
Gallo R, Gambelli F, Gava B, Sasdelli F, Tellone V, Masini M, Marchetti P, Dotta F, Sorrentino V (2007) Generation and expansion of multipotent mesenchymal progenitor cells from cultured human pancreatic islets. Cell Death Differ 14:1860–1871
Gao R, Ustinov J, Pulkkinen MA, Lundin K, Korsgren O, Otonkoski T (2003) Characterization of endocrine progenitor cells and critical factors for their differentiation in human adult pancreatic cell culture. Diabetes 52:2007–2015
Georgia S, Bhushan A (2004) β cell replication is the primary mechanism for maintaining postnatal β cell mass. J Clin Invest 114:963–968

Gershengorn MC, Hardikar AA, Wei C, Geras-Raaka E, Marcus-Samuels B, Raaka BM (2004) Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells. Science 306:2261–2264

Gershengorn MC, Geras-Raaka E, Hardikar AA, Raaka BM (2005) Are better islet cell precursors generated by epithelial-to-mesenchymal transition? Cell Cycle 4(3):380–382

Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F (2005) Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood 105:2821–2827

Gmyr V, Kerr-Conte J, Belaich S, Vandewalle B, Leteurtre E, Vantyghem MC, Lecomte-Houcke-M, Proye C, Lefebvre J, Pattou F (2000) Adult human cytokeratin 19-positive cells reexpress insulin promoter factor 1 in vitro: further evidence for pluripotent pancreatic stem cells in humans. Diabetes 49:1671–1680

Gong J, Zhang G, Tian F, Wang Y (2012) Islet-derived stem cells from adult rats participate in the repair of islet damage. J Mol Histol 43:745–750

Gu D, Sarvetnick N (1993) Epithelial cell proliferation and islet neogenesis in IFN-γ transgenic mice. Development 118:33–46

Gu D, Lee MS, Krahl T, Sarvetnick N (1994) Transitional cells in the regenerating pancreas. Development 120:1873–1881

Gu D, Arnush M, Sarvetnick N (1997) Endocrine/exocrine intermediate cells in streptozotocin-treated Ins-IFN-γ transgenic mice. Pancreas 15:246–250

Guo Z, Li H, Li X, Yu X, Wang H, Tang P, Mao N (2006) In vitro characteristics and in vivo immunosuppressive activity of compact bone-derived murine mesenchymal progenitor cells. Stem Cells 24:992–1000

Guz Y, Nasir I, Teitelman G (2001) Regeneration of pancreatic β cells from intra-islet precursor cells in an experimental model of diabetes. Endocrinology 142:4956–4968

Haber PS, Keogh GW, Apte MV, Moran CS, Stewart NL, Crawford DH, Pirola RC, McCaughan GW, Ramm GA, Wilson JS (1999) Activation of pancreatic stellate cells in human and experimental pancreatic fibrosis. Am J Pathol 155:1087–1095

Hao E, Tyrberg B, Itkin-Ansari P, Lakey JR, Geron I, Monosov EZ, Barcova M, Mercola M, Levine F (2006) β-cell differentiation from nonendocrine epithelial cells of the adult human pancreas. Nat Med 12:310–316

Hayek A, Beattie GM, Cirulli V, Lopez AD, Ricordi C, Rubin JS (1995) Growth factor/matrix-induced proliferation of human adult β cells. Diabetes 44:1458–1460

Holland AM, Gonez LJ, Harrison LC (2004) Progenitor cells in the adult pancreas. Diabetes Metab Res Rev 20:13–27

Holland AM, Góñez LJ, Naselli G, Macdonald RJ, Harrison LC (2005) Conditional expression demonstrates the role of the homeodomain transcription factor Pdx1 in maintenance and regeneration of β-cells in the adult pancreas. Diabetes 54:2586–2595

Ikejiri N (1990) The vitamin A-storing cells in the human and rat pancreas. Kurume Med J 37:67–81

Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, Mao N (2005) Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 105:4120–4126

Joglekar MV, Parekh VS, Hardikar AA (2007) New pancreas from old: microregulators of pancreas regeneration. Trends Endocrinol Metab 18:393–400

Karaoz E, Ayhan S, Gacar G, Aksoy A, Duruksu G, Okçu A, Demircan PC, Sariboyaci AE, Kaymaz F, Kasap M (2010a) Isolation and characterization of stem cells from pancreatic islet: pluripotency, differentiation potential and ultrastructural characteristics. Cytotherapy 12:288–302

Karaoz E, Genç ZS, Demircan PÇ, Aksoy A, Duruksu G (2010b) Protection of rat pancreatic islet function and viability by coculture with rat bone marrow-derived mesenchymal stem cells. Cell Death Dis 1:e36

Kem H, Logothetopoulos J (1970) Steroid diabetes in the guinea pig. Studies on islet-cell ultrastructure and regeneration. Diabetes 19:145–154

Kim BM, Kim SY, Lee S, Shin YJ, Min BH, Bendayan M, Park IS (2006) Clusterin induces differentiation of pancreatic duct cells into insulin-secreting cells. Diabetologia 49:311–320

Kim J, Breunig MJ, Escalante LE, Bhatia N, Denu RA, Dollar BA, Stein AP, Hanson SE, Naderi N, Radek J, Haughy D, Bloom DD, Assadi-Porter FM, Hematti P (2012) Biologic and immunomodulatory properties of mesenchymal stromal cells derived from human pancreatic islets. Cytotherapy 14:925–935

Klein D, Barbé-Tuana F, Pugliese A, Ichii H, Garza D, Gonzalez M, Molano RD, Ricordi C, Pastori RL (2005) A functional CD40 receptor is expressed in pancreatic β cells. Diabetologia 48:268–276

Korcakova L (1971) Mitotic division and its significance for regeneration of granulated β-cells in the islets of Langerhans in allozan-diabetic rats. Folia Morphol (Praha) 19:24–30

Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, Dazzi F (2003) Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood 101:3722–3729

Kruse C, Birth M, Rohwedel J, Assmuth K, Goepel A, Wedel T (2004) Pluripotency of adult stem cells derived from human and rat pancreas. Appl Phys A 79:1617–1624

Lacy PE, Davie JM, Finke EH (1979) Prolongation of islet allograft survival following in vitro culture (24 degrees C) and a single injection of ALS. Science 204:312–313

Lardon J, Huyens N, Rooman I, Bouwens L (2004) Exocrine cell transdifferentiation in dexamethasone-treated rat pancreas. Virchows Arch 444:61–65

Lechene de la Porte P, Iovanna J, Odaira C, Choux R, Sarles H, Berger Z (1991) Involvement of tubular complexes in pancreatic regeneration after acute necrohemorrhagic pancreatitis. Pancreas 6:298–306

Lechner A, Habener JF (2003) Stem/progenitor cells derived from adult tissues: potential for the treatment of diabetes mellitus. Am J Physiol Endocrinol Metab 284:E259–E266

Lechner A, Leech CA, Abraham EJ, Nolan AL, Habener JF (2002) Nestin-positive progenitor cells derived from adult human pancreatic islets of Langerhans contain side population (SP) cells defined by expression of the ABCG2 (BCRP1) ATP-binding cassette transporter. Biochem Biophys Res Commun 293:670–674

Lechner A, Nolan AL, Blacken RA, Habener JF (2005) Redifferentiation of insulin-secreting cells after in vitro expansion of adult human pancreatic islet tissue. Biochem Biophys Res Commun 327:581–588

Lei J, Cheng J, Li Y, Li S, Zhang L (2005) CD80, but not CD86, express on cultured murine keratinocyte stem cells. Transplant Proc 37:289–291

Leng SH, Lu FE (2005) Induction of pancreatic duct cells of neonatal rats into insulin-producing cells with fetal bovine serum: a natural protocol and its use for patch clamp experiments. World J Gastroenterol 11:6968–6974

Linning KD, Tai MH, Madhukar BV, Chang CC, Reed DN Jr, Ferber S, Trosko JE, Olson LK (2004) Redox-mediated enrichment of self-renewing adult human pancreatic cells that possess endocrine differentiation potential. Pancreas 29:e64–e76

Lipsett M, Finegood DT (2002) β-cell neogenesis during prolonged hyperglycemia in rats. Diabetes 51:1834–1841

List JF, Habener JF (2004) Glucagon-like peptide 1 agonists and the development and growth of pancreatic β-cells. Am J Physiol Endocrinol Metab 286:E875–E881

Logothetopoulos J, Bell EG (1966) Histological and autoradiographic studies of the islets of mice injected with insulin antibody. Diabetes 15:205–211

Marynissen G, Aerts L, Van Assche FA (1983) The endocrine pancreas during pregnancy and lactation in the rat. J Dev Physiol 5:373–381

Means AL, Meszoely IM, Suzuki K, Miyamoto Y, Rustgi AK, Coffey RJ Jr, Wright CV, Stoffers DA, Leach SD (2005) Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates. Development 132:3767–3776

Meier JJ, Ritzel RA, Maedler K, Gurlo T, Butler PC (2006) Increased vulnerability of newly forming β cells to cytokine-induced cell death. Diabetologia 49:83–89

Meneilly GS, McIntosh CH, Pederson RA, Habener JF, Gingerich R, Egan JM, Finegood DT, Elahi D (2001) Effect of glucagon-like peptide 1 on non-insulin-mediated glucose uptake in the elderly patient with diabetes. Diabetes Care 24:1951–1956

Meneilly GS, Greig N, Tildesley H, Habener JF, Egan JM, Elahi D (2003a) Effects of 3 months of continuous subcutaneous administration of glucagon-like peptide 1 in elderly patients with type 2 diabetes. Diabetes Care 26:2835–2841

Meneilly GS, McIntosh CH, Pederson RA, Habener JF, Ehlers MR, Egan JM, Elahi D (2003b) Effect of glucagon-like peptide 1 (7–36 amide) on insulin-mediated glucose uptake in patients with type 1 diabetes. Diabetes Care 26:837–842

Minami K, Okuno M, Miyawaki K, Okumachi A, Ishizaki K, Oyama K, Kawaguchi M, Ishizuka N, Iwanaga T, Seino S (2005) Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. Proc Natl Acad Sci USA 102:15116–15121

Montanucci P, Pennoni I, Pescara T, Blasi P, Bistoni G, Basta G, Calafiore R (2011) The functional performance of microencapsulated human pancreatic islet-derived precursor cells. Biomaterials 32:9254–9262

Morandi F, Raffaghello L, Bianchi G, Meloni F, Salis A, Millo E, Ferrone S, Barnaba V, Pistoia V (2008) Immunogenicity of human mesenchymal stem cells in HLA-class I-restricted T-cell responses against viral or tumor-associated antigens. Stem Cells 26:1275–1287

Morton RA, Geras-Raaka E, Wilson LM, Raaka BM, Gershengorn MC (2007) Endocrine precursor cells from mouse islets are not generated by epithelial-to-mesenchymal transition of mature β cells. Mol Cell Endocrinol 270:87–93

Nauta AJ, Kruisselbrink AB, Lurvink E, Willemze R, Fibbe WE (2006) Mesenchymal stem cells inhibit generation and function of both $CD34^{+}$−derived and monocyte-derived dendritic cells. J Immunol 177:2080–2087

Nir T, Dor Y (2005) How to make pancreatic β cells – prospects for cell therapy in diabetes. Curr Opin Biotechnol 16:524–529

Nishimura M, Umehara H, Nakayama T, Yoneda O, Hieshima K, Kakizaki M, Dohmae N, Yoshie O, Imai T (2002) Dual functions of fractalkine/CX3C ligand 1 in trafficking of perforin+/granzyme B+ cytotoxic effector lymphocytes that are defined by CX3CR1 expression. J Immunol 168:6173–6180

Noel D, Djouad F, Bouffı C, Mrugala D, Jorgensen C (2007) Multipotent mesenchymal stromal cells and immune tolerance. Leuk Lymphoma 48:1283–1289

Ogata T, Li L, Yamada S, Yamamoto Y, Tanaka Y, Takei I, Umezawa K, Kojima I (2004) Promotion of β-cell differentiation by conophylline in fetal and neonatal rat pancreas. Diabetes 53:2596–2602

Okuno M, Minami K, Okumachi A, Miyawaki K, Yokoi N, Toyokuni S, Seino S (2007) Generation of insulin-secreting cells from pancreatic acinar cells of animal models of type 1 diabetes. Am J Physiol Endocrinol Metab 292:E158–E165

Otonkoski T, Beattie GM, Rubin JS, Lopez AD, Baird A, Hayek A (1994) Hepatocyte growth factor/scatter factor has insulinotropic activity in human fetal pancreatic cells. Diabetes 43:947–953

Ouziel-Yahalom L, Zalzman M, Anker-Kitai L, Knoller S, Bar Y, Glandt M, Herold K, Efrat S (2006) Expansion and redifferentiation of adult human pancreatic islet cells. Biochem Biophys Res Commun 341:291–298

Oyama K, Minami K, Ishizaki K, Fuse M, Miki T, Seino S (2006) Spontaneous recovery from hyperglycemia by regeneration of pancreatic β-cells in Kir6.2G132S transgenic mice. Diabetes 55:1930–1938

Peck AB, Cornelius JG (1995) İn vitro growth of mature pancreatic islets of Langerhans from single, pluripotent stem cells isolated from pre-diabetic adult pancreas. Diabetes 44:10A

Peck AB, Ramiya V (2004) İn vitro-generation of surrogate islets from adult stem cells. Transpl Immunol 12:259–272

Petropavlovskaia M, Rosenberg L (2002) Identification and characterization of small cells in the adult pancreas: potential progenitor cells? Cell Tissue Res 310:51–58

Poliakova L, Pirone A, Farese A, MacVittie T, Farney A (2004) Presence of nonhematopoietic side population cells in the adult human and nonhuman primate pancreas. Transplant Proc 36:1166–1168

Rabinovitch A, Suarez-Pinzon WL, Shapiro AM, Rajotte RV, Power R (2002) Combination therapy with sirolimus and interleukin-2 prevents spontaneous and recurrent autoimmune diabetes in NOD mice. Diabetes 51:638–645

Ramiya VK, Maraist M, Arfors KE, Schatz DA, Peck AB, Cornelius JG (2000) Reversal of insulin dependent diabetes using islets generated in vitro from pancreatic stem cells. Nat Med 6:278–282

Rao MS, Dwivedi RS, Yeldandi AV, Subbarao V, Tan XD, Usman MI, Thangada S, Nemali MR, Kumar S, Scarpelli DG, Reddy JK (1989) Role of periductal and ductular epithelial cells of the adult rat pancreas in pancreatic hepatocyte lineage. A change in the differentiation commitment. Am J Pathol 134:1069–1086

Rasmusson I (2006) Immun modulation by mesenchymal stem cells. Exp Cell Res 312:2169–2179

Rooman I, Bouwens L (2004) Combined gastrin and epidermal growth factor treatment induces islet regeneration and restores normoglycaemia in C57Bl6/J mice treated with alloxan. Diabetologia 47:259–265

Rosenberg L (1998) Induction of islet cell neogenesis in the adult pancreas: the partial duct obstruction model. Microsc Res Tech 43:337–346

Rosenberg L, Vinik AL (1992) Trophic stimulation of the ductular-islet cell axis: a new approach to the treatment of diabetes. Adv Exp Med Biol 321:95–104

Sandgren EP, Luetteke NC, Palmiter RD, Brinster RL, Lee DC (1990) Overexpression of TGF α in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. Cell 61:1121–1135

Saotome T, Inoue H, Fujimiya M, Fujiyama Y, Bamba T (1997) Morphological and immunocytochemical identification of periacinar fibroblast-like cells derived from human pancreatic acini. Pancreas 14:373–382

Schmied BM, Ulrich A, Matsuzaki H, Ding X, Ricordi C, Weide L, Moyer MP, Batra SK, Adrian TE, Pour PM (2001) Transdifferentiation of human islet cells in a long-term culture. Pancreas 23:157–171

Seaberg RM, Smukler SR, Kieffer TJ, Enikolopov G, Asghar Z, Wheeler MB, Korbutt G, van der Kooy D (2004) Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. Nat Biotechnol 22:1115–1124

Sharma A, Zangen DH, Reitz P, Taneja M, Lissauer ME, Miller CP, Weir GC, Habener JF, Bonner-Weir S (1999) The homeodomain protein IDX-1 increases after an early burst of proliferation during pancreatic regeneration. Diabetes 48:507–513

Spaggiari GM, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, Moretta L (2008) Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood 111:1327–1333

Sphyris N, Logsdon CD, Harrison DJ (2005) Improved retention of zymogen granules in cultured murine pancreatic acinar cells and induction of acinar-ductal transdifferentiation in vitro. Pancreas 30:148–157

Stagg J, Pommey S, Eliopoulos N, Galipeau J (2006) Interferon-γ-stimulated marrow stromal cells: a new type of nonhematopoietic antigen-presenting cell. Blood 107:2570–2577

Street CN, Sipione S, Helms L, Binette T, Rajotte RV, Bleackley RC, Korbutt GS (2004) Stem cell-based approaches to solving the problem of tissue supply for islet transplantation in type 1 diabetes. Int J Biochem Cell Biol 36:667–683

Suzuki A, Nakauchi H, Taniguchi H (2004) Prospective isolation of multipotent pancreatic progenitors using flow-cytometric cell sorting. Diabetes 53:2143–2152

Swenne I (1992) Pancreatic β-cell growth and diabetes mellitus. Diabetologia 35:193–201

Tang Q, Adams JY, Penaranda C, Melli K, Piaggio E, Sgouroudis E, Piccirillo CA, Salomon BL, Bluestone JA (2008) Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. Immunity 28:687–697

von Mach MA, Hengstler JG, Brulport M, Eberhardt M, Schormann W, Hermes M, Prawitt D, Zabel B, Grosche J, Reichenbach A, Muller B, Weilemann LS, Zulewski H (2004) İn vitro cultured islet-derived progenitor cells of human origin express human albumin in severe combined immunodeficiency mouse liver in vivo. Stem Cells 22:1134–1141

Wang RN, Kloppel G, Bouwens L (1995) Duct- to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats. Diabetologia 38:1405–1411

Wang J, Song LJ, Gerber DA, Fair JH, Rice L, LaPaglia M, Andreoni KA (2004) A model utilizing adult murine stem cells for creation of personalized islets for transplantation. Transplant Proc 36:1188–1190

Wang ZV, Mu J, Schraw TD, Gautron L, Elmquist JK, Zhang BB, Brownlee M, Scherer PE (2008) PANIC-ATTAC: a mouse model for inducible and reversible β-cell ablation. Diabetes 57:2137–2148

Watari N, Hotta Y, Mabuchi Y (1982) Morphological studies on a vitamin A-storing cell and its complex with macrophage observed in mouse pancreatic tissues following excess vitamin A administration. Okajimas Folia Anat Jpn 58:837–858

Weaver CV, Sorenson RL, Kaung HC (1985) Immunocytochemical localization of insulin-immunoreactive cells in the pancreatic ducts of rats treated with trypsin inhibitor. Diabetologia 28:781–785

Xu G, Stoffers DA, Habener JF, Bonner-Weir S (1999) Exendin-4 stimulates both β-cell replication and neogenesis, resulting in increased β-cell mass and improved glucose tolerance in diabetic rats. Diabetes 48:2270–2276

Xu X, D'Hoker J, Stangé G, Bonné S, De Leu N, Xiao X, Van de Casteele M, Mellitzer G, Ling Z, Pipeleers D, Bouwens L, Scharfmann R, Gradwohl G, Heimberg H (2008) β cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 132:197–207

Yamamoto K, Miyagawa J, Waguri M, Sasada R, Igarashi K, Li M, Nammo T, Moriwaki M, Imagawa A, Yamagata K, Nakajima H, Namba M, Tochino Y, Hanafusa T, Matsuzawa Y (2000) Recombinant human betacellulin promotes the neogenesis of β-cells and ameliorates glucose intolerance in mice with diabetes induced by selective alloxan perfusion. Diabetes 49:2021–2027

Yamaoka T, Yoshino K, Yamada T, Idehara C, Hoque MO, Moritani M, Yoshimoto K, Hata J, Itakura M (2000) Diabetes and tumor formation in transgenic mice expressing Reg I. Biochem Biophys Res Commun 278:368–376

Ye Z, Wang Y, Xie HY, Zheng SS (2008) Immunosuppressive effects of rat mesenchymal stem cells: involvement of $CD4^+$ $CD25^+$ regulatory T cells. Hepatobiliary Pancreat Dis Int 7:608–614

Zhang L, Hong TP, Hu J, Liu YN, Wu YH, Li LS (2005) Nestin-positive progenitor cells isolated from human fetal pancreas have phenotypic markers identical to mesenchymal stem cells. World J Gastroenterol 11:2906–2911

Zheng SG, Wang JH, Koss MN, Quismorio F Jr, Gray JD, Horwitz DA (2004) $CD4^+$ and $CD8^+$ regulatory T cells generated ex-vivo with IL-2 and TGF-β suppress a stimulatory graft-versus host disease with lupus-like syndrome. J Immunol 172:1531–1539

Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA (2008) In vivo reprogramming of adult pancreatic exocrine cells to β-cells. Nature 455:627–632

Zimmermann A, Gloor B, Kappeler A, Uhl W, Friess H, Buchler MW (2002) Pancreatic stellate cells contribute to regeneration early after acute necrotising pancreatitis in humans. Gut 51:574–578

Zulewski H, Abraham EJ, Gerlach MJ, Daniel PB, Moritz W, Muller B, Vallejo M, Thomas MK, Habener JF (2001) Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. Diabetes 50:521–533

Generating Pancreatic Endocrine Cells from Pluripotent Stem Cells

48

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0_49,

Abstract

Human islet transplantation shows considerable promise as a physiological means of insulin replacement for type 1 diabetes, although donor availability and progressive loss of graft function continues to hamper more widespread implementation. Pluripotent stem cells (PSCs) by definition have the ability to form all tissues of the body including insulin-secreting pancreatic β-cells. This potential has led many academic and industry groups to examine methods for efficient production of functional insulin-producing cells from PSCs. Engineered in vitro differentiation protocols generally mimic known pancreatic developmental cascades, which convert undifferentiated cells, through germ-layer specification, to restricted pancreatic endodermal progenitors. The continued development of these progenitors in vivo results in the formation of functional pancreatic endocrine cells capable of preventing and/or reversing diabetic hyperglycemia in rodent models. While the insulin-producing, antidiabetic capacity of differentiated PSCs is very promising, key questions remain about optimizing differentiation processes for functional in vitro maturation, as well as whether production of pancreatic endocrine tissue is reproducible on a clinical scale and sufficiently safe. With additional research and development in these areas, the induction of differentiation processes to yield pancreatic endocrine-like cells could yield a potentially limitless supply of functional β-cells capable of replacing current human islet transplantation therapies for diabetes.

Keywords

Pluripotency • Embryonic stem cells • Pancreatic development • Pancreatic progenitor • Cell differentiation • Cellular therapy • Regenerative medicine • Diabetes

Abbreviations

BMP	Bone morphogenic protein
DMSO	Dimethyl sulfoxide
EC	Embryonal carcinoma
FGF	Fibroblast growth factor
GLP1	Glucagon-like peptide 1
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hESC(s)	Human embryonic stem cell(s)
HGF	Hepatocyte growth factor
IGF	Insulin-like growth factor
iPSC(s)	Induced pluripotent stem cell(s)
LADA	Latent autoimmune diabetes of adults
mESC(s)	Mouse embryonic stem cell(s)
MODY	Maturity-onset diabetes of the young
PSC(s)	Pluripotent stem cell(s)
SCNT	Somatic cell nuclear transfer

Shh	Sonic hedgehog
TGF-β	Transforming growth factor β
VEGF	Vascular endothelial growth factor

Introduction

One of the many potential clinical applications of differentiated derivatives of pluripotent cells is a treatment for diabetes mellitus, a disease characterized by lack or insufficient activity of the glucose-lowering hormone insulin. Type 1 diabetes is an autoimmune disease in which insulin-producing pancreatic β-cells are destroyed by autoreactive $CD8^+$ T-cells (van Belle et al. 2011), while in type 2 diabetes, insulin's activity is insufficient due to progressive insulin resistance and eventual loss of pancreatic β-cells (Prentki and Nolan 2006). As β-cells are gradually lost, blood glucose levels rise due to insufficient levels of circulating insulin. While careful administration of insulin via injections can restore relatively normal blood glucose control in many patients temporarily, exogenous insulin administration does not fully match the sensitive and dynamic release kinetics achieved by endogenous β-cells, leading to numerous long-term complications including retinopathy, nephropathy, neuropathy, and cardiovascular disease (Melendez-Ramirez et al. 2010). Moreover, even when glucose levels are tightly controlled with insulin injections, patients are at an increased risk of potentially fatal hypoglycemic episodes due to an inability to mimic the normal decrease in insulin release in response to low blood glucose levels (The Diabetes Control and Complications Trial Research Group 1993; Fowler 2011).

Cadaveric islet transplantation, in conjunction with specific antirejection therapies, was developed as an alternative means of restoring physiological blood glucose control systems to patients with type 1 diabetes (Shapiro et al. 2000). The success of this trial where seven patients regained insulin independence and normal blood glucose control for up to 1 year following a relatively simple infusion of human islet cells into the hepatic circulation led to a great interest in islet transplantation as a curative approach for type 1 diabetes. Subsequent follow-up on this group of patients and others revealed that insulin independence upon islet transplantation was not sustainable for the majority of patients over a 5-year time period (Ryan et al. 2005). While insulin independence was maintained in only 10 % of these patients, 80 % had detectable C-peptide indicating that islet grafts were partially functional which may explain why these patients experienced fewer hypoglycemic events and had improved overall blood glucose control compared to patients treated by insulin injection alone (Ryan et al. 2005; Merani and Shapiro 2006; Shapiro 2011). Despite a poor rate of sustained insulin independence, the clinical benefits of islet transplant as well as the continuing goal of improving the durability of insulin independence has led to a number of islet transplantation programs being set up around the world (Shapiro et al. 2006). However, these programs continue to be constrained by a significant lack of cadaveric islet tissue

available for transplant, particularly considering most patients require multiple independent islet preparations to achieve initial insulin independence. (For more information on this topic, see the chapter entitled "► Successes and Disappointments with Clinical Islet Transplantation.")

In an effort to produce a defined and vast supply of cells for transplant into patients with diabetes, many groups have explored the production of functional pancreatic endocrine cells from human embryonic stem cells (hESCs) both in vitro (Gage et al. 2013; D'Amour et al. 2006; Jiang et al. 2007a, b; Nostro et al. 2011; Mfopou et al. 2010; Rezania et al. 2011; Shim et al. 2007; Xu et al. 2011; Zhang et al. 2009; Gutteridge et al. 2013; Bose et al. 2012; Micallef et al. 2012; Kunisada et al. 2012; Cheng et al. 2012; Seguin et al. 2008; Leon-Quinto et al. 2004) and in vivo (Kelly et al. 2011; Kroon et al. 2008; Rezania et al. 2011, 2012, 2013; Basford et al. 2012; Bruin et al. 2013; Shim et al. 2007; Gutteridge et al. 2013; Bose et al. 2012; Soria et al. 2000). As reviewed from a number of perspectives (Fryer et al. 2013; Bruin and Kieffer 2012; Guo and Hebrok 2009; Van Hoof et al. 2009; Pagliuca and Melton 2013; Yang and Wright 2009; Nostro and Keller 2012; Champeris Tsaniras and Jones 2010; Stanley and Elefanty 2008; Seymour and Sander 2011; Hebrok 2012; Hosoya 2012; Roche et al. 2006), the generation of functional pancreatic endocrine cells either in vitro or in vivo from hESCs could result in an unlimited supply of cells for insulin replacement therapy while theoretically enabling physiological blood glucose control without the concomitant risk of hypoglycemia associated with exogenous insulin.

However, while in vivo maturation of in vitro-derived pancreatic progenitors can produce pancreatic endocrine cells capable of controlling blood glucose, strictly in vitro protocols have been far less successful at producing functional endocrine cells. In this chapter, we will briefly review the early study of pluripotent cells and more recent research on their utility. We then focus on the generation of pancreatic cells through normal pancreas development and in vitro differentiation strategies. Finally, we address future challenges associated with the generation of pancreatic endocrine cells from progenitors, including reproducibility, scaling up, and protection against immune attack for clinical applications, and generation of new models for further research.

Origins and Utility of Pluripotent Stem Cells

The study of pluripotency began in the early 1950s and 1960s when the first mouse teratocarcinoma cells were observed, isolated, and found to be capable of forming derivatives of all three embryonic germ layers from a single originating embryonal carcinoma (EC) cell (Stevens and Little 1954; Kleinsmith and Pierce 1964). These early EC cell studies revealed that a single cell could form any cell type in the body, but unfortunately their murine origin, considerable genetic rearrangements, and a host of other concerns associated with the tumorigenic EC cells prevented their use in regenerative medicine applications. The first karyotypically normal mouse embryonic stem cells (mESCs) were isolated from the inner cell mass of cultured

blastocysts in 1981 (Evans and Kaufman 1981; Martin 1981). The development of these techniques allowed researchers to examine the factors which maintained the pluripotent state as well as signals which stimulate directed differentiation from the isolated cells.

Additionally, the isolation of mESCs capable of efficient chimera generation allowed for genetic targeting and the creation of modified mouse strains (Bradley et al. 1984). Seventeen years after the isolation of mESCs, the derivation of hESCs was first reported (Thomson et al. 1998), after considerable advances in non-human primate ESC derivation in the mid-1990s (Thomson et al. 1995, 1996). The lengthy delay in hESC isolation was presumably due to key differences between mESCs and hESCs. For example, mESCs are dependent on leukemia inhibitor factor for pluripotent growth, while hESCs rely on basic fibroblast growth factor to maintain a phenotype during extended in vitro expansion (Yu and Thomson 2008).

The generation of mESCs and hESCs has provided a benchmark definition of the properties that are required to define a cell as an embryonic stem cell and more importantly what experimental conditions must be met to define a cell as pluripotent. Associated with the pluripotent phenotype is the expression of key transcription factors such as OCT4, NANOG, and SOX2 as well as surface markers such as TRA-1–60, TRA-1–81, and SSEA3/4 in humans (SSEA1 in mice) (Adewumi et al. 2007; Smith et al. 2009). When combined with classical cell morphology of a high nuclear-to-cytoplasmic ratio and alkaline phosphatase activity, ESCs can be distinguished from other cell types in culture (O'Connor et al. 2008). Unfortunately, these assays are only correlative, and given the definition of a PSC is to be pluripotent, the standard assays of this trait are functional in nature, aiming to test the abilities of PSCs to develop into tissues of all three germ layers. This is most often tested through uncontrolled differentiation of PSCs in vitro in embryoid body differentiation assays and in vivo in teratoma formation assays. While the in vitro embryoid body assay provides some data regarding potency, the most widely accepted test for PSCs of all species remains the teratoma assay where undifferentiated PSCs are injected into immunocompromised mice and allowed to develop (Ungrin et al. 2007). A few months after transplant, the outgrowth is excised and examined for histological generation of differentiated derivatives of all three embryonic germ layers to demonstrate pluripotency. In the specific case of non-human PSCs, there is also the option of the generation of chimeras where the PSCs (most often of mouse origin) are injected into blastocysts to mix with the developing inner cell mass cells during subsequent in utero maturation. The resulting embryos are eventually examined to determine which tissues the PSCs were able to generate during in vivo development, and in the case of many genetically modified mouse strains, this process is continued to test for germline transmission to generate the entire embryo from a single PSC chimera-derived gamete (O'Connor et al. 2011; Ungrin et al. 2007).

The development of pluripotent stem cells (PSCs) took a dramatic step forward in 2006 with the report of induced pluripotent stem cells (iPSCs). In this work, combinatorial screening revealed that retrovirus-mediated expression of Oct4, Sox2, c-Myc, and Klf4 was sufficient to reprogram mouse fibroblasts into cells

resembling mESCs (Takahashi and Yamanaka 2006). This approach was quickly extended to the reprogramming of human fibroblasts (Takahashi et al. 2007) and independently validated in another screen identifying OCT4, SOX2, NANOG, and LIN28 as being capable of reprogramming human somatic cells to iPSCs (Yu et al. 2007). Further studies have recapitulated this reprogramming process with even fewer factors delivered using a variety of technologies including adenoviruses, plasmids, transposons, mRNAs, proteins, and even solely with small molecules (Gonzalez et al. 2011; Hou et al. 2013). Assuming that efficient differentiation protocols exist, the eventual iPSCs derived from these methods make it theoretically possible to generate a cellular therapy for a specific disease state from PSC-derived cells that are immunologically specific to an individual patient (Maehr 2011). Whether such an immunological patient match provides any advantages for autoimmune diseases, such as type 1 diabetes, remains to be determined. Nevertheless, their utility for studying disease mechanisms and treatments is clearly evident.

Interestingly, the type of cell used to generate the iPSC line seems to have an effect on the resulting final product, most notably when mature specialized cell types are used as a starting material for iPSC generation. Reprogramming mature human cell types has been observed in a number of systems including both mouse and human adult β-cells (Bar-Nur et al. 2011; Stadtfeld et al. 2008). While the reprogramming process was able to induce pluripotency, the conversion was incomplete in some aspects, and the resulting iPSCs seemed to retain some legacy of their origin. Upon differentiation back to β-cells, iPSCs derived from insulin-positive cells expressed higher amounts of insulin than did iPSCs derived from non-insulin-positive pancreatic cells. This predisposition toward the somatic cell type of origin was attributed to the similar genomic DNA methylation patterns observed in human β-cells and β-cell-derived iPSCs, which were not fully converted to undifferentiated PSC patterns during the reprogramming process (Bar-Nur et al. 2011).

One of the most recent advances in human PSC generation is the application of somatic cell nuclear transfer (SCNT). In this process, an unfertilized oocyte has its nucleus removed and replaced with the nucleus of a differentiated diploid somatic cell. Upon microinjection into the oocyte and parthenogenetic activation, the maternal contents of the cell elicit epigenetic changes in the somatic nucleus resulting in a diploid zygote free from the fertilization process. The SCNT method has allowed the cloning of domestic livestock such as the famed sheep "Dolly" (Wilmut et al. 1997). After 10 years of additional research, the first non-human primate-derived ESCs were produced, albeit at very low efficiencies (Byrne et al. 2007). This process was finally extended to human cells by Tachibana et al. who were able to achieve SCNT with human oocytes and subsequently generate hESC lines from the developing blastocysts (Tachibana et al. 2013). This study represents a key milestone in efforts to generate specific cell types from progenitors, as SCNT may offer more complete reprogramming of somatic cells without significant epigenetic legacy marks compared to iPSCs. If so, SCNT could allow efficient, patient-specific hESC generation with the reproducible differentiation capacity required for regenerative medicine applications.

Pancreatic Differentiation of PSCs

Inducing differentiation of human PSCs into pancreatic cells has been pursued to achieve two primary goals: (1) to generate functional insulin-secreting cells capable of restoring euglycemia from a diabetic state and (2) to provide a model system for exploring the processes underlying the development of glucagon, somatostatin, pancreatic polypeptide, ghrelin, and insulin-positive cell types in healthy and diseased humans. While both goals seek to understand and exploit natural human development processes, the limited availability of human fetal tissue and the inability to apply advanced genetic tools to such tissue continue to present hurdles. Consequently, the majority of our understanding of pancreatic development is based on data collected from model systems such as frogs, mice, and zebrafish. Zebrafish are appropriate for rapid combinatorial genetic studies and offer the advantage of a rapid life cycle in an animal that contains a minimal pancreatic endocrine islet structure (Tiso et al. 2009; Kinkel and Prince 2009). While comparatively slower to reproduce and mature, mouse models are also appropriate for a considerable number of genetic tools, while also enabling whole-body physiology analysis through which the consequences of altered pancreatic development can be evaluated. Together, these models have provided a basic framework of pancreatic development (see Fig. 1) which continues to be modified as new genes involved in pancreas development and maintenance are identified from animal models and confirmed in a wide variety of human-based approaches including tissue taken from human fetal samples and from patients with monogenic forms of diabetes (e.g., maturity-onset diabetes of the young (MODY), latent autoimmune diabetes of adults (LADA), and neonatal diabetes). Once identified in human systems, these genes often require previously described model organisms to better understand the complex roles the genes play in mammalian pancreatic development. Ultimately, this iterative process refines our pancreatic developmental model, which serves as a road map for many fields of study.

Pancreatic Development

Efforts to induce hESC differentiation into pancreatic endocrine cells typically attempt to recapitulate the current understanding of the normal pancreatic developmental cascade (see Fig. 1). Induction protocols are generally derived from empirical testing of various signalling molecules and culture conditions identified in developmental model systems such as frogs, fish, and mice to optimize the stage-specific differentiation conditions required to convert undifferentiated PSCs to either progenitors or hormone-positive cells. Pancreatic development begins with the specification of embryonic germ layers via gastrulation in a process requiring signalling from TGF-β family members such as Nodal (Brennan et al. 2001). TGF-β signalling induces formation of SOX17- and FOXA2-copositive definitive endoderm cells, which are capable of further developing into all endoderm-derived tissues including the pharynx, thyroid, lung, stomach, liver, pancreas, and intestine

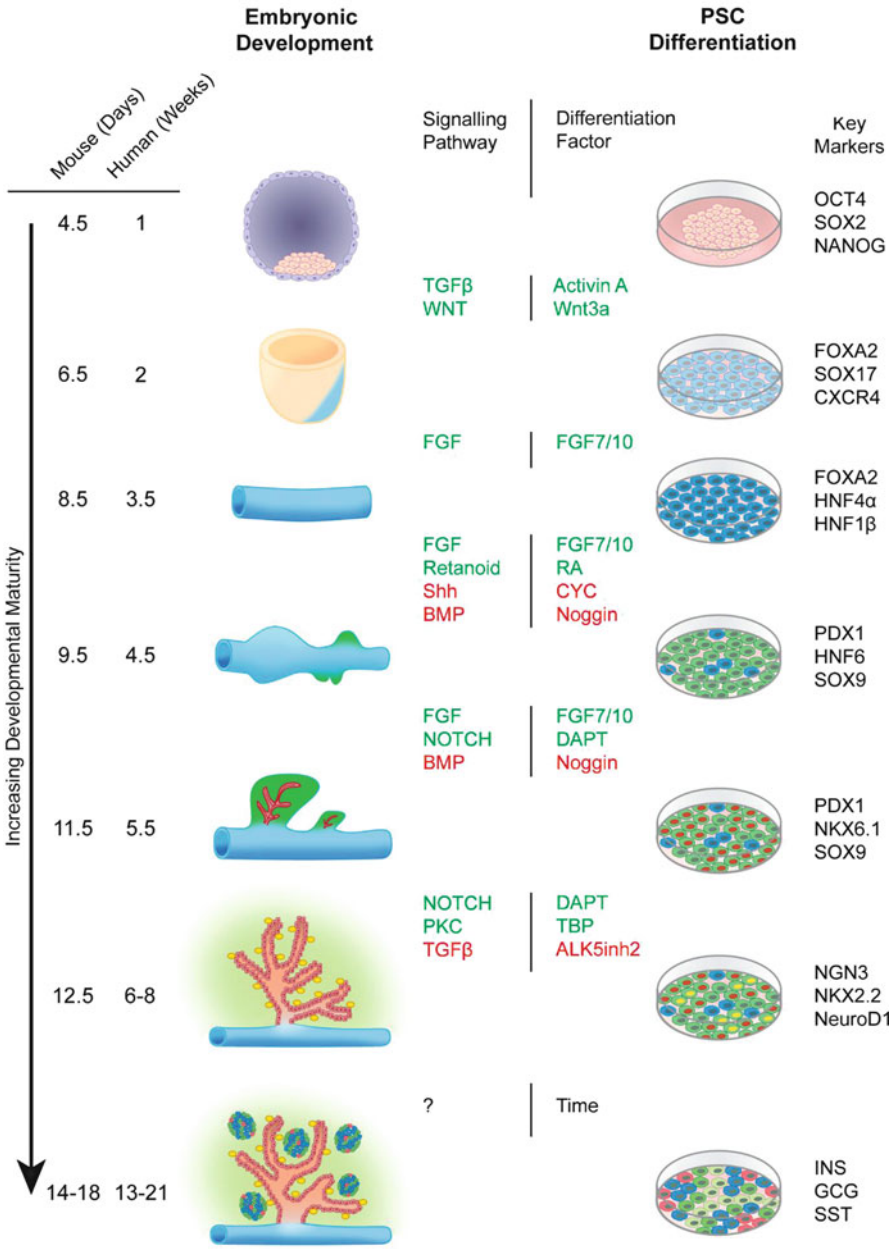

Fig. 1 Comparative pancreatic development in vivo and in vitro. Normal pancreatic development occurs through a complex series of morphogenic events that convert pluripotent cells into all potential cell types of the body. An approximate time line for mouse (days) and human (weeks) development is provided. Initially, pluripotent inner cell mass cells and their equivalent OCT4-positive hESCs (shown in *pink*) transition through a primitive streak intermediary stage to form

(Kanai-Azuma et al. 2002; Ang and Rossant 1994; Weinstein et al. 1994). The resulting sheet of endodermal cells invaginates into a tube formation in response to soluble factors including FGF4, which is released from the neighboring mesectodermal tissue (Wells and Melton 2000). This endoderm tube is next patterned along its length, making different portions permissive to the development of organ buds (Wells and Melton 1999). In mice, the earliest Pdx1-positive pancreas competent cells are derived from the transition zone between anterior Sox2-positive stomach progenitors and posterior Cdx2-positive intestinal progenitors (Jorgensen et al. 2007). The induction of Pdx1 positivity occurs in response to retinoic acid, BMP, and Shh signalling cascades (Stafford and Prince 2002; Tiso et al. 2002; Hebrok et al. 1998; Chen et al. 2004) and produces a cell population capable of generating the entire pancreatic organ in mice and humans (Jonsson et al. 1994; Stoffers et al. 1997). Similarly, available human developmental data suggest that these PDX1-positive cells subsequently gain expression of NKX6.1, identifying them as restricted pancreatic endodermal progenitors capable of differentiation to endocrine, exocrine, and ductal lineages (Jennings et al. 2013). In human development, it is this population which seems to form a complex tubular epithelial system which is regionalized based on location along the lengthening ductal tube into GATA4-positive "tip" progenitors which form PTF1a-positive exocrine cells and GATA4-negative "trunk" progenitors which can form both ductal and endocrine cells (Jennings et al. 2013). Commitment of PDX1-/NKX6.1-copositive, GATA4-negative "trunk" progenitors to the endocrine lineage is associated with transient expression of NGN3 to initiate endocrine fate specification (Jennings et al. 2013; Polak et al. 2000; Jeon et al. 2009; Slack 1995; Riedel et al. 2012; Piper et al. 2004). During this highly transcription factor-dependent cascade, within the pancreatic bud, some cells from the branched epithelial tree form early endocrine progenitor cells.

Fig. 1 (continued) cells committed to the endoderm lineage through TGF-β and WNT signalling pathways. Definitive endodermal cells (*light blue*) develop into endoderm-derived foregut cells (*dark blue*), which retain the ability to form any endoderm organ, in an FGF signalling-dependent process. Specification of the PDX1-positive dorsal and ventral pancreatic buds (shown in *green*) from the foregut tube occurs posterior to the developing stomach via high levels of FGF and retinoid signalling and inhibition of sonic hedgehog (*Shh*) and bone morphogenic protein (*BMP*) signals. Continued FGF and retinoid signalling specifies the NKX6.1-/PDX1-copositive pancreatic epithelial tree (*red* tree/nuclei within *green* bud/cells) within the expanding pancreatic buds, while the ventral bud rotates to fuse with the dorsal bud. NGN3-positive pancreatic endocrine precursor cells (shown as *yellow* buds/nuclei) form from pancreatic epithelial tree cells in a NOTCH signalling-dependent process enhanced by PKC activation and TGF-β inhibition. Over a considerable time frame and through processes that are incompletely understood, these endocrine precursor cells further mature through a number of fate specification stages (not described) into hormone-positive cells which coalesce into endocrine clusters (shown as clusters of *red*, *green*, and *blue* cells) within the surrounding pancreatic mesenchyme (*light green*). Signalling pathways are either activated (*green* text) or inhibited (*red* text) to drive development progression to the next stage. Differentiation factors (activators shown in *green* and inhibitors shown in *red*) utilized to modulate these signalling pathways are provided as examples and are not exhaustive. Key markers expressed at different developmental checkpoints are shown to the *right* of the figure

These cells continue to develop and eventually end up embedded in the surrounding pancreatic mesenchyme through incompletely understood mechanisms where they mature into functional endocrine cells (Pan and Wright 2011; Oliver-Krasinski and Stoffers 2008) (see Fig. 1).

Definitive Endoderm, Foregut, and Pancreatic Endodermal Progenitors from PSCs

The induced differentiation of PSCs into pancreatic cells generally follows known developmental stages as described above, but on an accelerated time line (see Fig. 1). While the formation of endocrine cells in humans takes approximately 7 weeks of development (Piper et al. 2004), early endocrine cells are first formed in differentiating hESCs by 2 weeks of culture (D'Amour et al. 2006). This considerably shortened culture time line of PSCs has been made possible by staging differentiating cells for efficient homogenous in vitro development without the need to enable morphogenesis of ectodermal and mesodermal lineages. To begin differentiation, pluripotent PSCs are seeded in cell culture plates. Once the cells have grown to a predetermined optimal density, which primes them to exit the replicative cell cycle (Chetty et al. 2013; Gage et al. 2013), the first inductive signals are provided via daily media changes which continue throughout the culture time line. Key marker genes and proteins are routinely monitored to ensure stage-specific differentiation and to identify cell homogeneity throughout the process. Primarily in response to activin A (a TGF-β family member) in low or no serum, the pluripotency program is repressed, and the formation of cells of the endoderm germ layer is stimulated (D'Amour et al. 2005; Kubo et al. 2004; Seguin et al. 2008; Brown et al. 2011). These signals induce the PSCs to move through an intermediate mesoderm/endoderm step that is developmentally similar to the primitive streak. Cells in this transient state can be identified via expression of the transcription factor Brachyury by 12–24 h after induction (D'Amour et al. 2005). Expression of FOXA2 and SOX17 follows approximately 48 h later and, together with the absence of extraembryonic primitive endoderm markers such as SOX7, demarks the formation of true definitive endoderm cells (D'Amour et al. 2005; Yasunaga et al. 2005; Seguin et al. 2008). The formation of this cell population was a key milestone in the pursuit of developing β-cells and other endoderm-derived tissues from PSCs. Remarkably, endodermal progenitors can also be isolated and expanded in culture producing a purified population free from pluripotent cells that maintains its ability to differentiate in an endoderm lineage-restricted manner (Cheng et al. 2012). Whether stimulated in a transient manner or from the endodermal progenitors which result from the in vitro expansion process, highly pure definitive endoderm cell populations are key to the success of later differentiation steps. Given the broad developmental potential of definitive endoderm progenitors, these cell populations are now being used to study the generation and continued differentiation of many tissue types including the lung (Mou et al. 2012), liver (Basma et al. 2009; Touboul et al. 2010;

Gouon-Evans et al. 2006; Hannan et al. 2013), intestine (Spence et al. 2011), as well as organs of the anterior foregut (Green et al. 2011; Kearns et al. 2013).

Following the generation of relatively pure definitive endoderm cells, the next challenge is to further pattern the sheet of cells to mimic the foregut stage of development. This is achieved with the addition of FGF signalling agonists, namely, FGF7 or FGF10, concomitant with prompt removal of the growth factors used to trigger previous differentiation stages. Expression of HNF4a and HNF1b marks the transition into foregut cells, which occurs over the next 72 h in culture (D'Amour et al. 2006; Kroon et al. 2008; Nostro et al. 2011; Schulz et al. 2012). This population of cells can form the gall bladder, hepatic, intestinal, and pancreatic cells, but requires further specification and repression of unwanted developmental programs. Retinoic acid plays a central role in the induction of pancreas formation from the foregut and has been found to be key in stimulating PDX1 expression in differentiating hESCs (D'Amour et al. 2006; Cai et al. 2010; Mfopou et al. 2010). At the same time, repression of hepatic and intestinal cell fates by the inhibition of BMP and Shh signalling is critical for proper specific pancreatic induction from hESCs (Spence et al. 2011; Green et al. 2011; Mfopou et al. 2007, 2010; Nostro et al. 2011; Sui et al. 2013; Gouon-Evans et al. 2006; Cho et al. 2012). Together, this mix of signalling cascades stimulates the formation of relatively homogenous PDX1-positive cell populations, which approach 95 % in purity in some reports, over the course of 3–5 days (Cai et al. 2010; Rezania et al. 2012). These PDX1-positive cells represent a key developmental step where the PSCs are partially restricted in cell fate but still retain the ability to form off-target tissues including extrahepatic biliary duct in humans depending on PDX1 expression levels (Jennings et al. 2013). Final maturation to PDX1-/NKX6.1-copositive pancreatic endodermal progenitors similar to those that predominate the pancreatic epithelium at 8–9 weeks of human fetal development (Riedel et al. 2012) and before the spatial regionalization associated with GATA4 expression to distinguish "tip" and "trunk" progenitors occurs over the next 72 h. The maturation of PDX1-positive cells to PDX1-/NKX6.1-copositive cells has been shown to occur in the absence of exogenous stimuli (Kroon et al. 2008) but can be enhanced by a mixture of BMP and ALK5 inhibition and PKC activation (Rezania et al. 2012, 2013). The state of the art in efficiency and homogeneity of PDX1-/NKX6.1-copositive progenitors is reported to be up to 86 % PDX1/NKX6.1 copositivity in 70 % of differentiation runs (Rezania et al. 2012). When transplanted into immunocompromised mouse models, these cells give rise to ductal cells and endocrine cells including functional insulin-positive cells, while PDX1-/NKX6.1-negative hormone-expressing cells appear to give rise predominantly to glucagon-positive α-cells (Rezania et al. 2011, 2012, 2013; Kelly et al. 2011; Kroon et al. 2008). The in vitro generation of a pancreatic progenitor pool from hESCs is an important checkpoint that has been achieved by many research groups and represents the second key milestone toward producing functional β-cells of sufficient quality and in quantities appropriate for future transplantation studies. However, these pancreatic endodermal progenitors have not yet specified which pancreatic cell type they will become.

Pancreatic Endocrine Development In Vitro

While the production of pancreatic endodermal progenitors has been relatively successful, the continued development of these cells in culture into fully functional endocrine cells remains poorly understood. To this end, two developmental routes are being explored. Both begin with the in vitro differentiation of PDX1-/NKX6.1-copositive progenitors over a 14–17-day culture period. Subsequently, these progenitors are either transplanted into immunocompromised mice to undergo relatively uncontrolled but highly successful development in vivo, toward functional endocrine cells, or alternatively the progenitors are cultured in vitro under more regulated conditions, again in an effort to elicit functional maturation of the cells.

The cumulative developmental literature on pancreatic endocrine induction, fate specification, and functional maturation suggests that temporally and spatially specific transcription factor expression is likely critical to efficient stimulation of β-cell formation. In particular, sequential expression of the endocrine restriction marker NGN3 followed by a number of fate-specifying factors (NKX2.2, PAX4, ARX, PAX6, and others) is thought to be critical for specification of pancreatic endodermal progenitors (Oliver-Krasinski and Stoffers 2008). After fate specification, endocrine cells begin to express maturation factors and eventually hormones, with MafA-driven insulin production in the β-cell being perhaps the most well-studied example (Oliver-Krasinski and Stoffers 2008; Zhang et al. 2005). During hESC differentiation, the induction of the endocrine cascade remains largely stochastic for many of the early differentiation protocols (D'Amour et al. 2006). This suggests that the process could be cell autonomous or more likely that the cultures themselves produce the signalling molecules required to activate endocrine development within the culture system.

In an effort to accelerate this endocrine induction process and improve its efficiency, a wide range of signalling molecules have been used, including but not limited to nicotinamide, exendin-4, IGF-1, HGF, noggin, bFGF, BMP4, VEGF, WNT, and various inhibitors of BMP, Shh, TGF-β, and Notch signalling pathways (Nostro et al. 2011; D'Amour et al. 2006; Rezania et al. 2011, 2012, 2013; Kelly et al. 2011; Kroon et al. 2008; Schulz et al. 2012; Jiang et al. 2007a, b; Xu et al. 2011; Sui et al. 2013). Some of these factors have a rational basis for testing as agents driving endocrine maturation. As one example, exendin-4 is a mimetic of the natural gut-derived hormone glucagon-like peptide 1 (GLP-1), which stimulates β-cell proliferation, decreases β-cell apoptosis, and renders β-cells glucose competent (Wideman and Kieffer 2009). In addition to rational factors, recently even seemingly innocuous factors have been found to have dramatic effects on differentiating hESCs. The organic solvent dimethyl sulfoxide (DMSO) decreased cell proliferation to a similar extent as high-density cell culture conditions, dramatically enhancing differentiation to definitive endoderm, PDX1-positive cells, and C-peptide-positive cells in more than 25 hESC and iPSC lines (Chetty et al. 2013). Even the buffering component HEPES was found to have significant inhibitory effects on endocrine maturation from pancreatic endodermal progenitors,

with HEPES stimulating intestinal commitment (elevated CDX2 expression) at the expense of the pancreatic endocrine lineage (decreased NKX6.1, NGN3, NEUROD1, PDX1, and PTF1a expression) (Rezania et al. 2012).

Beyond these unexpected results, the modulation of TGF-β, WNT, BMP, and protein kinase C signalling has also resulted in considerable improvements in the efficiency of conversion to an endocrine fate. The inhibition of endogenous WNT signalling from foregut-stage hESC cultures impaired the eventual expression of insulin, and when optimally agonized by addition of WNT3a, a 15-fold increase in insulin expression was observed (Nostro et al. 2011). During the generation of pancreatic progenitors, TGF-β agonists were found to have a positive effect on hESC differentiation by increasing the number of PDX1-positive cells (Guo et al. 2013). Remarkably tight temporal regulation of this signalling pathway is required for further maturation toward endocrine cells as continued administration of TGF-β agonists repressed insulin expression (Guo et al. 2013). This idea was solidified as TGF-β inhibition with ALK5 inhibitor II caused a dose-dependent increase in NGN3-positive cells from progenitor cultures (Rezania et al. 2011). This effect continued down the cascade, promoting increased expression of NKX2.2, NEUROD1, and eventually insulin and glucagon without appreciably decreasing PDX1 expression (Rezania et al. 2011). Similarly, protein kinase C (PKC) activation has been identified as a potentially key pathway required to maintain PDX1 expression in pancreatic progenitors (Chen et al. 2009a). Indeed, when the PKC activator TPB was added to ALK5 inhibition and continued BMP inhibition by noggin, this three-factor mixture in the absence of HEPES buffering stimulated increased expression of NGN3, NEUROD1, and NKX6.1 without loss of PDX1 expression in pancreatic progenitors or induction of off-target differentiation which would be characterized by expression of albumin (liver) or CDX2 (intestine) (Rezania et al. 2012). Recently, one report suggested that BMP signalling is key to maintaining a proliferative PDX1-positive progenitor pool and that BMP antagonism is subsequently required to induce further pancreatic endocrine maturation (Sui et al. 2013).

The formation of pancreatic progenitors from hESCs that can generate functional endocrine cells including β-cells in vivo has been convincingly demonstrated (Rezania et al. 2012; Kroon et al. 2008). While this suggests that in vitro-derived pancreatic progenitors should have the capacity to produce functional endocrine cells, presently the majority of the pancreatic endocrine cells produced in vitro by various groups are still immature in function and typically express multiple hormones including insulin, glucagon, and somatostatin, with a bias toward glucagon-positive lineages (Rezania et al. 2011; Nostro et al. 2011; Jiang et al. 2007a; D'Amour et al. 2006; Segev et al. 2004). Of particular note, one of the highest in vitro endocrine differentiation efficiencies reported to date yielded up to 75 % endocrine cells (synaptophysin positive) (Rezania et al. 2011). While these cells were initially polyhormonal (insulin and glucagon positive), during extended culture or transplantation, they developed into functional endocrine cells expressing only glucagon and not other endocrine hormones. High expression of the key α-cell transcription factor ARX, along with low expression of PAX4, PDX1, and NKX6.1, may have caused this biased maturation to α-cells (Rezania et al. 2011).

One of the key objectives of in vitro differentiation of hESCs is the development of functional insulin-secreting cells. With this goal in mind, D'amour et al. (2006) examined the capacity of their differentiated endocrine cultures to responsively release insulin/C-peptide into the culture media. In this study, differentiated hESCs clusters contained approximately one third the amount of C-peptide per μg DNA found in human islets, with a high proportion of proinsulin remaining unprocessed. hESC clusters released C-peptide (two to sevenfold over basal) in response to depolarizing stimuli such as KCl, K_{ATP} channel blockade by tolbutamide, increased cAMP levels by IBMX addition, and nutrient supplementation by methyl-pyruvate α-ketoisocaproic acid, L-leucine, and L-glutamine (D'Amour et al. 2006). Importantly, these clusters were unable to reproducibly release C-peptide in response to glucose, with many experiments recording stable or even decreased C-peptide release in response to increased extracellular glucose concentrations. While some groups have shown modest insulin secretion (~2-fold) from differentiated mESCs in response to elevated glucose levels (Jiang et al. 2008), the majority of reports suggest that this key attribute is lacking under current in vitro culture systems which employ human cells. These immature hESC-derived endocrine cells share some characteristics with neonatal β-cells which have significantly elevated insulin release in low glucose conditions and blunted release in high glucose conditions (Martens et al. 2013). This poor glucose responsiveness in neonatal β-cells has been attributed to a deficit in mitochondrial energy shuttling associated with poor glucose-stimulated NAD(P)H generation (Martens et al. 2013; Jermendy et al. 2011). One notable exception to the unresponsive nature of in vitro PSC-derived insulin-positive cells is the differentiated progeny of in vitro purified endodermal progenitors (Cheng et al. 2012). These cells, despite being differentiated with protocols that typically generate polyhormonal cells lacking robust glucose responsiveness with other ESC lines, were found to express C-peptide without glucagon or somatostatin and release C-peptide in response to elevated glucose levels similarly to adult human islets (Cheng et al. 2012). Given that the endodermal progenitors used are lineage restricted, can rapidly self-renew yet are nontumorigenic, and could be effectively differentiated into glucose-responsive insulin-secreting cells, the authors revealed an alternative differentiation method which pauses at the definitive endoderm stage to improve directed differentiation purity and potential safety of the final product. Nevertheless, the generally limited responsiveness of most hESCs differentiated exclusively in vitro has led many groups to examine the development of pancreatic endodermal progenitors in vivo as an alternate strategy to yield functional endocrine cells with more reasonable efficiency.

Pancreatic Endocrine Development In Vivo

Given that current protocols for in vitro differentiation of pancreatic progenitors into endocrine cells tends to produce immature polyhormonal cells with poor glucose responsiveness, research efforts have turned to in vivo maturation strategies to elicit functional maturation of progenitor cells. This strategy is based on the success of

functional maturation of human fetal pancreatic tissue upon transplantation in mice (Hayek and Beattie 1997; Castaing et al. 2001) and on the notion that in vivo maturation might enable exploitation of the full complexity of cellular interactions that drive normal pancreas development. Since our knowledge of pancreatic endocrine cell development, particularly the signals governing late endocrine maturation processes, remains incomplete, rational, literature-driven in vitro maturation is likely to remain challenging in the short term. However, if in vivo cell maturation is possible, it may provide key insights into the required signals that govern this process which are presumably deficient in the current in vitro culture systems. Moreover, in considering an eventual cell therapy product, the shorter time line associated with differentiation just to pancreatic progenitors is attractive, assuming adequate performance and safety following completion of maturation in vivo.

As previously reviewed, in vivo maturation protocols tend to begin from PDX1-/NKX6.1-copositive pancreatic endodermal progenitors generated through in vitro processes (see Figs. 1 and 2). These progenitor cells if differentiated as an adherent monolayer culture must be detached and prepared for transplantation in the form of a suspension of cell clusters (Kroon et al. 2008; Kelly et al. 2011; Rezania et al. 2012) or alternatively differentiated entirely in suspension prior to transplantation (Schulz et al. 2012). The composition and purity reported for these clusters vary among different groups, but they tend to be comprised predominantly of PDX1-/NKX6.1-copositive progenitors with lower numbers of pre-committed pancreatic endocrine cells (Rezania et al. 2012). Following harvest, progenitor cells are typically transplanted under the kidney capsule (Rezania et al. 2011, 2012; Kroon et al. 2008) or as part of a gel-foam disk transplanted into the epididymal fat pad (Kelly et al. 2011; Kroon et al. 2008; Schulz et al. 2012) of immunocompromised rodents. Initial engraftment of the progenitor mixture occurs over the next few weeks as blood vessels from the transplant recipient grow toward the transplanted tissue, likely in a VEGF-A-dependent process similar to islet engraftment and vascularization in mice (Vasir et al. 2001; Jansson and Carlsson 2002; Zhang et al. 2004; Brissova et al. 2006). The subsequent development of hormone-positive cells within the engrafted hESC origin tissue is initially rapid and results in the production of polyhormonal cells around 1 month posttransplantation (Rezania et al. 2012) (see Fig. 2). Over the next 2–3 months, the immature polyhormonal cell population decreases in number, and more mature cell types expressing a single major islet hormone predominate in the transplant tissue (see Fig. 2). This transition is also marked by the reorganization of endocrine cells within the grafts into endocrine clusters resembling islets and a significant increase in secretion of C-peptide from the graft (Rezania et al. 2012; Kroon et al. 2008). With extended in vivo maturation, glucose and/or meal-responsive C-peptide release continues to increase in grafts, along with nuclear NKX6.1 and MafA expression in insulin-positive cells (Kroon et al. 2008; Rezania et al. 2012). NKX6.1 has been shown to be both necessary and sufficient to maintain and specify the β-cell phenotype primarily due to repression of α-cell biasing factors such as ARX and PAX6 (Schaffer et al. 2013; Gauthier et al. 2007). MafA expression, which is known to mark maturation of insulin-producing cells into a glucose-responsive state

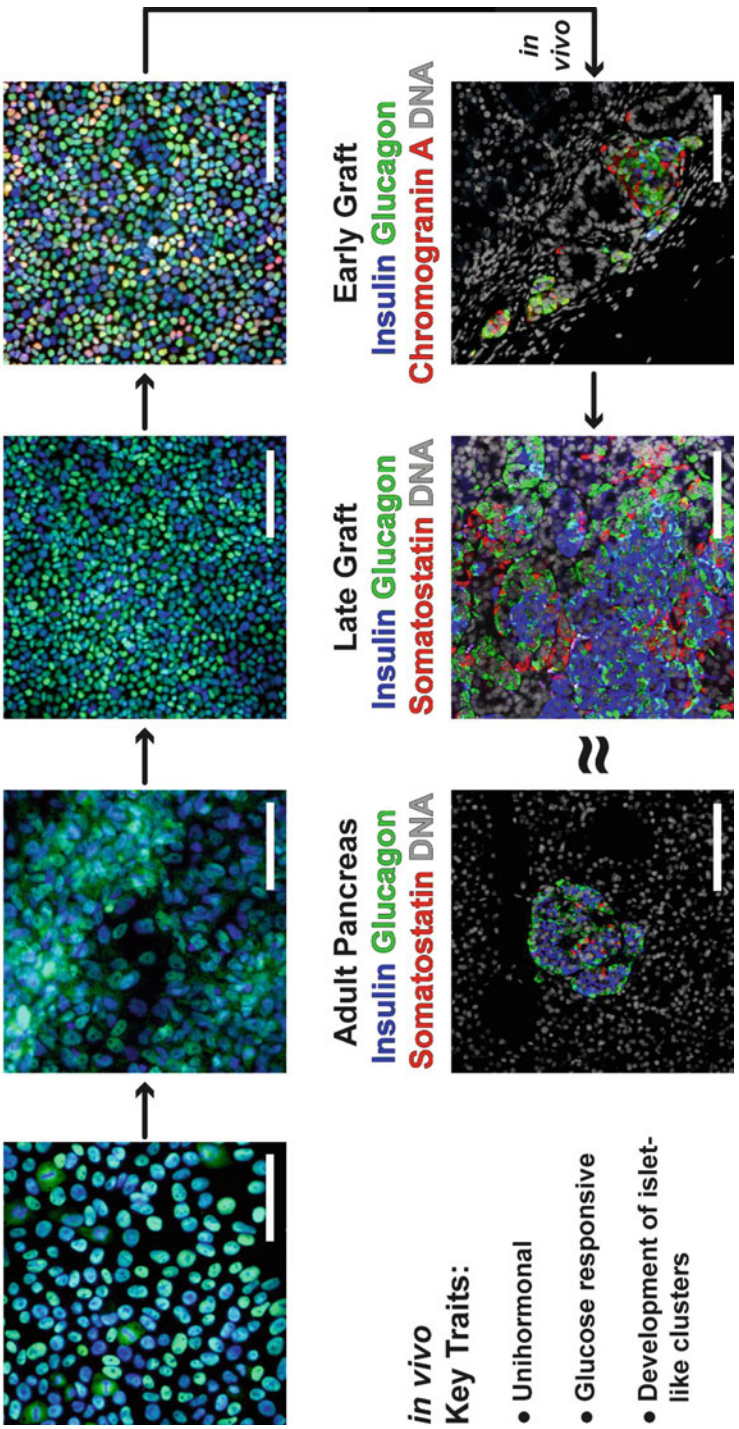

Fig. 2 **Current in vitro and in vivo hESC differentiation mimicking pancreatic development.** Representative immunohistochemical images are shown for various stages of in vitro generation of pancreatic progenitor cells and subsequent in vivo maturation to functional endocrine grafts. Pancreatic differentiation of hESCs converts OCT4-positive pluripotent cells into SOX17-positive definitive endoderm cells. These cells further develop into PDX1-positive foregut

(Zhang et al. 2005), is also associated with the point at which the hESC-derived grafts were able to restore normoglycemia in diabetic transplant recipients suggesting that a key functional transition had occurred within the graft (Rezania et al. 2012). Marking a key milestone in the field, this in vivo maturation process to yield glucose-responsive, insulin-producing cells has been independently shown to occur in normoglycemic (Kroon et al. 2008) and diabetic (Rezania et al. 2012) environments. However, the possibility that in vivo cell maturation in the host environment may be variable due to differing exposure to a variety of factors such as hormones and drugs remains a potential limitation of this approach.

The functional success of in vivo maturation strategies has led to intriguing questions about how to mimic the functional maturation of insulin-positive cells in culture and what cell population forms the final insulin-positive cell compartment in glucose-responsive grafts. Kelly et al. elegantly examined this question using a cell separation and transplantation strategy (Kelly et al. 2011). The authors followed an established in vitro differentiation protocol to produce a heterogeneous pancreatic cell population that was the basis for a flow-cytometry-based assay of 217 commercially available antibodies aimed at distinguishing endocrine cells from progenitors. Ultimately, CD142 was found to label a population of predominantly hormone-negative, NKX6.1-positive endodermal progenitors, while CD200 and CD318 preferentially labeled hormone-positive cells. The authors separated CD142-positive endodermal progenitor fractions (82 % progenitors) and CD318-positive hormone-positive fractions (84 % endocrine) by immunomagnetic cell separation methods and transplanted the cells into immunocompromised mice. Nine weeks after transplantation, CD318-enriched endocrine cells had developed mostly into glucagon-positive cells, while 13-week-old transplants of CD142-enriched pancreatic endodermal progenitors contained large numbers of cells expressing insulin, glucagon, or somatostatin, arranged in islet-like structures and surrounded by cells expressing markers of exocrine and ductal pancreatic cells. Taken together, this work suggests that the CD142-positive, NKX6.1-positive, hormone-negative population is the common progenitor for ductal, exocrine, and endocrine pancreatic cells including insulin, somatostatin, and glucagon lineages. In contrast, the in vitro-generated hormone-positive cells expressing CD318 and CD200 seemed to be predestined to form glucagon-positive cells (Kelly et al. 2011). Interestingly, the most functional grafts were generated from mixed cell populations which contained both the hormone-negative and hormone-positive populations (Kelly et al. 2011), the reasons for which are unclear. We compared maturation of hESC-derived pancreatic progenitors that contained high (~80 %) or

Fig. 2 (continued) endoderm cells that mature into pancreatic endodermal progenitors coexpressing NKX6.1 and PDX1. This population of cells is the basis for in vivo maturation where maturation of progenitors is achieved by transplantation into immunocompromised recipients. Transplanted hESC-derived cells mature from immature polyhormonal endocrine populations expressing insulin, glucagon, and the pan-endocrine marker chromogranin A into functional islet-like clusters resembling adult pancreatic islets and comprised of unihormonal cells. The colours of each marker are as indicated above each image. All scale bars are 100 μm

low (~25 %) fractions of NKX6.1-positive cell populations (Rezania et al. 2013). Upon transplantation and in vivo maturation of these cells, the NKX6.1-high grafts were found to have robust C-peptide release in response to physiological stimuli including meals, arginine, and glucose, which was not observed from the NKX6.1-low grafts. After 5 months of development, both NKX6.1-high and NKX6.1-low grafts generated pancreatic endocrine cells at high efficiencies, but the NKX6.1-high grafts contained increased numbers of insulin- and somatostatin-positive cells, while the NKX6.1-low grafts contained predominantly glucagon-positive cells (Rezania et al. 2013). Both of these studies support previous in vitro extended culture and transplantation studies in which glucagon-positive cells at the end of in vitro and in vivo differentiation protocols were found to arise from the glucagon-/insulin-copositive cells seen in the earlier in vitro differentiation stages (Rezania et al. 2011; Basford et al. 2012). Analysis of human fetal pancreas samples also supports the notion that polyhormonal endocrine cells are present during development and may give rise to mature, single hormone-producing cells (Riedel et al. 2012; Polak et al. 2000). Thus, the in vivo maturation of hESC-derived precursor cells presents a useful model for exploring the developmental capacity of cells initially produced in vitro. Moreover, in vivo maturation studies may help to further define the optimal cell population to produce functional hESC-derived pancreatic endocrine cells.

Future Challenges

Despite the success of in vitro differentiation of pancreatic endodermal progenitors following a pancreatic development model and the in vivo maturation of these progenitors to functional endocrine islet-like structures capable of reversing diabetic hyperglycemia, the cues that govern developmental conversion of these progenitors to functional endocrine cells are still incompletely understood. Moreover, while studies to date do solidify the notion that hESC-derived cells are able to control blood glucose in a diabetic recipient, the field must resolve a significant number of challenges ranging from how to reproducibly generate large amounts of functional, stable cells to how to prevent the recipient's immune system from destroying these cells once transplanted.

Reproducibility

If hESCs, or more broadly PSCs, are going to become a realistic source material for generating cellular therapies for diabetes, the reproducibility of differentiation and development must be addressed. In the undifferentiated state, hESC lines are generally considered to be quite uniform in terms of expression of pluripotency markers (Adewumi et al. 2007). Unfortunately, this uniformity does not seem to extend to the differentiation potential of hESCs. One report suggested that even among similarly derived hESC lines, more than 100-fold differences in lineage

specification efficiency exist (Osafune et al. 2008), perhaps because of inherent DNA methylation patterns which predispose different hESC and iPSC lines toward certain lineages (Bock et al. 2011). This inherent variability between lines has contributed to the inability of investigators to repeat published protocols with different cells and obtain the same results. For example, some hESC lines are better able to generate pancreatic endocrine cells with the protocol developed by D'Amour et al. than others (D'Amour et al. 2006; Mfopou et al. 2010; Osafune et al. 2008). As a direct test of the pancreatic differentiation propensity of different hESC lines, Mfopou et al. applied the differentiation conditions optimized by D'Amour et al. for the CyT203 hESC line (D'Amour et al. 2006) to five in-house-generated hESC lines (VUB01, VUB02, VUB07, VUB14, and VUB17). In the VUB lines, the D'Amour protocol effectively generated definitive endoderm, gut tube, and foregut cells but eventually produced hepatocytes instead of pancreatic endocrine cells (Mfopou et al. 2010). Ultimately, cell line-specific alterations in the differentiation protocol, namely, adjustment of the timing and dosage of BMP and FGF signalling modulators, were required to restore pancreatic endocrine differentiation capacity to the VUB lines. These modifications were similar to those applied by other groups (Nostro et al. 2011). Line-to-line variation was also described by the ViaCyte, Inc. group (formerly Novocell, Inc.), which reported varying progenitor differentiation efficiencies among the CyT49, CyT203, and MEL1 hESC lines (Kelly et al. 2011). Administration of DMSO to hESC lines resistant to pancreatic lineages has recently been reported to significantly improve differentiation efficiency in more than 25 hESC and iPSC lines (Chetty et al. 2013). This effect of DMSO is likely mediated by cell cycle arrest in view of other studies demonstrating a requirement for transition from the G2/M cell cycle phases to G1/Go in order for hESCs to be capable of targeted differentiation (Calder et al. 2013; Sela et al. 2012; Pauklin and Vallier 2013; Gage et al. 2013) and can be mimicked by high cell seeding density (Gage et al. 2013). Taken together, these data suggest that while a single protocol is unlikely to be effective at inducing differentiation of multiple hESC lines, most lines are likely to be capable of efficient pancreatic development given the appropriate signals. However, it may be more effective to identify hESC (or PSC) lines which have inherently reproducible differentiation for widespread use than to develop multiple individually optimized protocols for each PSC line.

In addition to resolving the preferred PSC starting material(s) and the reproducibility of differentiation protocols, the methods and tools used to characterize and quantify nutrient-responsive hormone release from resulting cells should be harmonized to enable direct comparison among research groups. Previous efforts to repeat observations have revealed such things as the confounding effects of insulin uptake in differentiated mESCs (Lumelsky et al. 2001; Hansson et al. 2004; Rajagopal et al. 2003) and variation in hESC pancreatic differentiation propensity (Mfopou et al. 2010; Osafune et al. 2008). Rigorous cell characterization is also important since the standards for surrogate β-cells are very high, including the paramount importance of glucose-regulated insulin secretion (Halban et al. 2001). Within the field of pancreatic islet research, methods for assessing insulin release

are well established, including normalizing secreted insulin amounts to total DNA or insulin content of the cell sample. These practices have not been uniformly applied to the task of testing PSC-derived pancreatic endocrine populations. Many in vitro studies simply report relative fold secretion of insulin (often as C-peptide) under static high glucose conditions versus low glucose conditions with different cell samples. This makes it hard to compare results between studies, and unfortunately, this approach fails to account for the number of endocrine cells in each sample, the pre-culture conditions (often higher glucose differentiation media), and the kinetics of secretion. Absolute hormone secretion in response to standardized glucose concentrations ideally in a kinetic secretion system with comparison to isolated islets would be preferable. By similarly assessing the function of differentiated PSCs, labs would be able to better compare methods and resultant cell populations, which will ultimately facilitate further improvements of PSC differentiation protocols toward developing robustly functional β-cells in vitro.

Scaling Up

Assuming that reproducible hESC differentiation can be achieved, the next major hurdle will be the production of clinically relevant quantities of pancreatic progenitors or functional endocrine cells in an economically feasible manner (Wong et al. 2012). One key aspect of this scale-up is determining what population of pancreatic endocrine cells will provide the most effective and safe treatment of diabetes. The functional capacity of β-cells seems to be significantly improved when islet cells, including α-cells, are clustered together with β-cells (Maes and Pipeleers 1984; Pipeleers et al. 1982). This may reflect the highly conserved natural arrangement of endocrine cells within islets and established paracrine signals between these cells (Bosco et al. 2010; Steiner et al. 2010; Hauge-Evans et al. 2009; Brunicardi et al. 2001). (For more information on this topic, please see the chapter entitled "► The Comparative Anatomy of Islets."). However, King et al. compared the ability of enriched β-cells and reaggregated islet cells to recover glycemic control in diabetic mice (King et al. 2007). The authors concluded that non-β-cell endocrine cells are not essential for transplantation success suggesting that a pure a β-cell product may be as effective as mixed islet cells, should protocols be successfully developed to produce pure a β-cells. Given the success in making relatively pure α-cells (Rezania et al. 2011), it should be possible to make highly enriched functional β-cells in the near future. While estimates vary, it is possible that one billion hESC-derived β-cells could be required to treat a single patient with diabetes (Docherty et al. 2007). In order to achieve this scale of production, considerable expansion of hESCs will be required. This is likely a reasonable goal given that hESCs are highly proliferative, doubling every 20 h (Chen et al. 2010) to allow an up to sixfold expansion in just 4–7 days in the undifferentiated state in stirred bioreactor systems (Zweigerdt et al. 2011). Once expanded, cultures can be differentiated toward pancreatic progenitor and or endocrine cells following the loosely established conversion ratio of 1:1 (undifferentiated hESC/differentiated progeny) (D'Amour et al. 2006).

Recently, the ViaCyte group reported a scalable production strategy for pancreatic progenitors (Schulz et al. 2012) which was subsequently reproduced and enhanced by researchers at Pfizer (Gutteridge et al. 2013). A large bank of frozen vials of undifferentiated hESCs is maintained, whereby a sample can be thawed, expanded over 2 weeks of adherent culture, and formed into suspension cell aggregates by dynamic rotation of dissociated undifferentiated cells. In the ViaCyte report, these aggregates were then differentiated into pancreatic progenitors in a rotating suspension format and transplanted in the epididymal fat pad of immunocompromised mice for final maturation. Graft maturation occurred over the next few months and ultimately resulted in glucose-stimulated C-peptide release at approximately 11–15 weeks posttransplant. Maturation continued until 4–5 months posttransplant, when graft tissue could maintain normal glycemic control in animals in which mouse pancreatic β-cells had been destroyed by injection of streptozotocin after graft maturation. In addition to the scalability of the rotational culture adaptation, the in vitro temporal expression patterns and the in vivo matured endocrine compartment were remarkably similar to previous reports, which were based on a minimally scalable two-dimensional adherent system (Kroon et al. 2008). While this bodes well for increased production of pancreatic progenitors using this potentially scalable differentiation method, some challenges remain. While the suspension differentiation methods significantly reduced the formation of non-endodermal origin tissues, approximately half of the grafts were considered cystic and thus were incompletely pancreatic endocrine cells (Schulz et al. 2012). The cultures did not uniformly express key pancreatic transcription factors, and ~2 % of the cells were unidentified, thereby carrying the risk of unknown developmental potential (Schulz et al. 2012). Presuming that all uniformity and safety concerns are addressed, the generation of these therapeutic cells is going to be costly. Indeed, relative to cadaveric and xenogeneic islet sources, there has been some debate as to whether a therapeutic product of this nature is ultimately economically viable (Wong et al. 2012; Wong and Nierras 2010).

One of the key aspects of clinical scale production which will continue to require further research and is likely to have a significant impact on the cost of the final product is the conversion of current differentiation protocols to ones relying on small molecules with fully defined composition rather than protein growth factors derived from animal products. As an example of such efforts, Borowiak et al. used high-throughput small-molecule screening to form ESC-derived cell types along the pancreatic developmental cascade. Using a fluorescent reporter mESC line in which expression of SOX17 was tracked by red fluorescent DsRED expression, ~4,000 compounds were screened to reveal that IDE1 and IDE2 significantly enhanced definitive endoderm induction from undifferentiated human and mouse ESCs (Borowiak et al. 2009). These two compounds could replace activin A, the recombinant protein widely used to activate TGF-β signalling via canonical phosphorylation of Smad2 (Borowiak et al. 2009). Further down the differentiation cascade, another screen was employed to identify small molecules capable of improving the induction of PDX1-positive pancreatic progenitor cells. Using an antibody-based high-content screen with dissociated and replated foregut progenitor cells treated with one of ~5,000 compounds or DMSO, (-)-indolactam V (ILV) was found to increase numbers

of PDX1-positive cells (Chen et al. 2009b). When coadministered with FGF10, it also improved production of pancreatic progenitors capable of in vivo maturation to functional insulin-positive cells. Based on the inability of retinoic acid to synergize with ILV and the similar effects of protein kinase C activators with respect to PDX1-positive cell stimulation, ILV is believed to activate protein kinase C by direct binding, although this has yet to be proven explicitly (Chen et al. 2009b). Taken together, the high-efficiency formation of pancreatic progenitors under clinically amenable, defined, and scalable culture conditions seems feasible. While work is still required to merge these independent research efforts, the demand for transplantable tissue remains high, and progress in the field is expected to be swift.

Immunological Control and Encapsulation

With the efficient and scalable production of functional pancreatic progenitors getting closer to a reality, cellular therapy for diabetes nevertheless presents an immunological problem. Human PSC-derived grafts will face not only alloimmune attack but also the specific autoimmune-mediated attack of insulin-positive cells associated with the pathogenesis of type 1 diabetes. With just a few efficiently differentiating hESCs lines established, cells derived from an equally minimal number of human leukocyte antigen (HLA) types are expected to be available for immunological matching to patients. HLA matching is an important variable influencing the success of human islet transplantation (Mohanakumar et al. 2006), so the matching of hESC-derived pancreatic progenitors to recipient HLA types may dampen alloimmune graft rejection, as was observed in the early islet transplant experience (Scharp et al. 1990). With the adoption of glucocorticoid-free immunosuppressive regimens, survival and ongoing function of transplanted hESC-derived cells seems feasible, similar to the initial insulin independence observed in many islet recipients in the first year posttransplant (Shapiro et al. 2000). However, despite strong immunosuppressive regimes, graft function decreases over the next 4 years after transplant in islet recipients due to progressive immune rejection by allo and autoimmune mechanisms as well as apoptosis associated with high metabolic and insulin production demands on the engrafted islets (Shapiro 2011; Plesner and Verchere 2011). The immunosuppressive agents used to protect the grafts have also been found to be directly toxic to β-cells (Nir et al. 2007; Johnson et al. 2009). Over time, the loss of islet function requires that patients return to supplementation with exogenous insulin (Shapiro et al. 2006). While PSC-derived sources offer the potential to deliver larger amounts of pancreatic endocrine tissue, it remains to be seen whether such transplants will be able to sustain long-term insulin independence in recipients.

Upon transplantation, both PSC-derived grafts and cadaveric islet grafts will be faced with an activated recipient immune system. One way to prevent graft loss associated with host immune attack, without using immunosuppressant drugs, is by using a physical barrier to isolate the graft from the circulating immune system. This idea has taken form in a series of immunoisolation devices which range from

thin cellular coatings to microencapsulation with thick cell cluster/islet coatings or macroencapsulation with engineered transplantable devices, as reviewed elsewhere (O'Sullivan et al. 2011; Weir 2013; Scharp and Marchetti 2013). These approaches are being actively developed and may be amenable to protecting PSC-based cell therapies.

Alginate microencapsulation forms a coating around islets or hESC-derived clusters which protects the cells from direct contact with host immune cells. This separation is presumed to be essential to preventing cytotoxic death of the transplanted cells, but due to the porous nature of the alginate gel, the graft can still secrete insulin in response to rising interstitial glucose concentrations (O'Sullivan et al. 2011). Depending on the chemical nature of the gel, this method may also protect the graft from antibody-mediated attack, but this defense typically comes at the cost of increased hypoxia-related necrosis (De Vos et al. 1999). Despite these considerable challenges, simple extrusion alginate encapsulation is remarkably effective in some mouse models, even with minimal surface modifications to restrict cytokine entry (Duvivier-Kali et al. 2001). In immunodeficient mice, alginate-encapsulated human islet cells delivered to the intraperitoneal cavity functioned better than free implants under the kidney capsule (Jacobs-Tulleneers-Thevissen et al. 2013). A pilot study was conducted in a patient with type 1 diabetes who received a peritoneal implant of the same encapsulated islet cells while on immunosuppression. While the transplant was without metabolic effect, likely due to the marginal transplant mass, functional cells within intact microcapsules were recovered 3 months posttransplant (Jacobs-Tulleneers-Thevissen et al. 2013). Clinical trials utilizing alginate-encapsulated porcine islets have begun with Living Cell Technologies reporting long-term graft survival, albeit in a single type 1 diabetic patient (Elliott et al. 2007). One key limitation of the alginate encapsulation system is that standard methods are minimally scalable due to processing capacities of current extrusion technologies (Hoesli et al. 2011). While scalable emulsification methods can effectively encapsulate β-cell lines at efficiencies adequate to reverse diabetes in mouse models (Hoesli et al. 2011, 2012), these methods have not yet been applied to larger animal models or at clinical scales. (For more information on this topic, please see the chapter entitled "▶ Islet Encapsulation.")

Macroencapsulation methods offer an alternate approach to immunoisolation of a transplanted graft with ease of retrieving representing a key advantage over microencapsulation methods. The TheraCyte™ device offers one example of this approach, whereby cells are loaded into the multilayered cell impermeable thin pouch via an access port. The loaded device may then be surgically implanted in a variety of places within the body, most simply subcutaneously. After its exterior surface becomes vascularized by the host, the graft gains function, ideally enabling effective blood glucose control without direct physical contact between the graft and host, thus providing an immunological barrier to protect the graft from the host immune system. One caveat of such an approach for diabetes therapy is that mature islets have a high demand for oxygen and thus are traditionally challenging to maintain in a macroencapsulation device in the absence of substantial vascularization or oxygen supplementation (Ludwig et al. 2012). Indeed, mature islets do not

do particularly well within the TheraCyte™ unless the devices are preimplanted to allow some vascularization of the outer membranes before cells are loaded (Rafael et al. 2003). Notably, by directly oxygenating alginate-encapsulated human islet preparations within a multilayer transplantable device, functional glucose-responsive insulin secretion was maintained up to 10 months after implantation in one patient with long-standing type 1 diabetes (Ludwig et al. 2013). Remarkably, this functional islet mass was protected from attack by the recipient's immune system despite the absence of any immunosuppressive agents supporting the functionality of such a macroencapsulation device (Ludwig et al. 2013). More immature cells may have advantages surviving in a macroencapsulation device. For example, human fetal pancreas tissue appears better able to survive the transiently hypoxic transplantation environment (Rafael et al. 2003; Lee et al. 2009). Likewise, despite one report of inconsistent development of hESC-derived progenitor cells within this type of macroencapsulation device (Matveyenko et al. 2010), we recently showed that functional maturation of hESC-derived pancreatic progenitor populations is possible and efficient within the TheraCyte™ device including the ability to reverse diabetic hyperglycemia in mice (Bruin et al. 2013; Rezania et al. 2013). Thus, pancreatic precursor cells derived from hESCs may be more like fetal cells and more resilient to the hypoxic transplantation environment. If similar results are not obtained when more mature islet endocrine cells are developed from PSCs, progenitor cells may have a distinct advantage for macroencapsulation.

While a considerable amount of effort continues to be focused on the generation of a scalable transplantation product, some aspects of this strategy remain concerning. The continuing concern with transplanting hESC-derived cells is the notion that they might overgrow, enabling uncontrolled release of secreted products such as insulin, or that they might form teratomas. This risk is presumably greater with the transplant of progenitor cells than it would be with fully differentiated cells. Indeed, transplantation of progenitors has been associated with varying levels of teratoma or overgrowth formation, due to incomplete differentiation of cultures such that pluripotent or possibly multipotent cell types capable of ectodermal and mesodermal lineages remain at the time of transplantation (Kroon et al. 2008; Kelly et al. 2011; Rezania et al. 2012). The formation of teratomas and overgrowths seems considerably reduced following enrichment of the transplanted cell population with endodermal progenitors (Cheng et al. 2012) or CD142-positive cells (Kelly et al. 2011), high uniformity scaled-up progenitor differentiation (Schulz et al. 2012), and transplantation of an in vitro matured endocrine population (Rezania et al. 2011). These data suggest that further adjustments to the differentiation, selection, and/or transplantation protocols may reduce or eliminate the capacity for graft overgrowth and teratoma formation. Indeed, we have shown that simple modification of established differentiation protocols followed by macroencapsulation was able to nearly eliminate all off-target germ layer development to one device of 74 compared to 18 out of 40 devices with our previous protocol (Bruin et al. 2013). The physical constraints provided by transplantable macroencapsulation devices also serve an important risk reduction

role in containing the graft. Thus, delivery of cells within a defined, growth limiting, and ultimately retrievable physical space provides considerable protection to the transplant recipient. If the device proves to be a completely effective immunoisolation system, the absence or at least minimization of immunosuppression requirements could provide an additional advantage as any escaping graft cells would presumably be quickly targeted by the host immune system given their foreign nature. Taken together, this suggests that a number of strategies may alleviate the concerns of tumor formation. When uniformly differentiated cells are combined with a sturdy graft encapsulation method allowing immunocompetent recipients, the safety profile of hESC-derived progenitor transplants may even rival current islet transplantation methods where immunosuppression poses significant risks.

In Vitro Maturation and Models of Development

As noted previously, in vitro hormone-positive cells generated by many current protocols seem to be developmentally biased to become glucagon-secreting cells, while in vivo maturation of the same progenitors can yield the full complement of mature endocrine cells albeit in a very uncontrolled and poorly defined manner. This suggests that our current understanding of in vitro pancreatic differentiation is deficient in critical stimuli which are required for the complete maturation observed in vivo. If we can correct this deficiency, we will be better able to produce a well-defined functional human pancreatic endocrine cell population that can be used as a platform for drug discovery and as a transplantation source that has reduced risk for formation of off-target cell types. Such a well-defined product free from contaminating non-endocrine cell types will have the advantage of functioning to control blood glucose levels in patients immediately following transplant and may have an improved safety profile over current progenitor populations that might respond unpredictably during their maturation in the uncontrolled transplantation environment of human patients. While previous studies have suggested that only β-cells are critical for successful reversal of diabetes and that non-β-cell islet endocrine cells are not required to ameliorate hyperglycemia during transplantation in mice (King et al. 2007), it remains to be seen whether this is true in hESC-derived endocrine cell transplants, as pure β-cell grafts have yet to be generated under any maturation or purification process reported to date. Similarly, whether the normal islet architecture seen in both endogenous human islets and in vivo matured hESC-derived grafts (Rezania et al. 2012; Kroon et al. 2008) is required for optimal graft function in terms of glycemic control is unknown. This issue may be particularly relevant to encapsulation technologies which may disrupt the normal islet architecture. To address these questions, the production of uniform functional endocrine cells from hESCs in vitro remains a key challenge in the field and at the same time positions hESC-derived pancreatic progenitor maturation as an interesting model with which to study human pancreas development.

Transcription factors play a key role in pancreatic development (Oliver-Krasinski and Stoffers 2008), and recently, researchers are turning to genetic

modification of hESCs to allow targeted study of transcription factor-activated pathways and networks in an effort to understand and control pancreatic endocrine development from PSCs. Based on this concept, mouse and human ESC lines bearing forced overexpression of single or multiple transcription factors, including SOX17, FOXA2, NGN3, NKX2.2, NKX6.1, NEUROD1, PAX4, and PDX1 (Blyszczuk et al. 2003; Miyazaki et al. 2004; Lavon et al. 2006; Bernardo et al. 2009; Lin et al. 2007; Liew et al. 2008; Treff et al. 2006; Shiroi et al. 2005; Kubo et al. 2011; Raikwar and Zavazava 2012; Marchand et al. 2009; Raikwar and Zavazava 2011; Seguin et al. 2008), have been generated. The majority of these studies have expressed transgenes by random integration of plasmids or lentivirus. Such strategies suffer from transgene silencing and loss of expression, which does not seem to be the case when targeted homologous recombination approaches using safe harbor loci are employed (Hockemeyer et al. 2009; Smith et al. 2008; Liew et al. 2007). Despite these limitations and the difficulties involved with modifying ESCs, a considerable number of developmental insights have been gained by transcription factor overexpression studies. In almost all cases examined, the forced expression of (combinations of) these transcription factors stimulated transcription of endogenous genes, most notably insulin, glucagon, and somatostatin over the course of in vitro differentiation. However, studies with the transcription factor PDX1 highlight a key caveat of these types of studies. Constitutive overexpression of PDX1 in hESCs increased pancreatic endocrine and exocrine induction in an embryoid body model, although robust insulin expression was distinctly absent in vitro (Lavon et al. 2006). Given that Pdx1 expression is believed to be biphasic in nature over mouse embryonic development from E13.5 and out to adulthood (Jorgensen et al. 2007), further examinations of PDX1 expression in hESCs attempted to recreate this expression pattern. Using a tamoxifen-inducible PDX1-expressing hESC line, Bernardo et al. (2009) found that a specific expression pattern of one 5-day pulse after definitive endoderm followed by a 5-day delayed pulse most efficiently induced insulin expression while minimizing expression of exocrine (amylase) and the liver (AFP and albumin) lineages. This work suggests that increased understanding of pancreatic development and the dynamics of transcription factor expression may yet inform key improvements in hESC differentiation protocols.

Similar to the idea of using transcription factor expression to control hESC development, small 22-nucleotide microRNAs (miRNAs) may show similarly powerful effects in PSC differentiation given their dynamic expression patterns (Krichevsky et al. 2006; Ivey et al. 2008). The importance of miRNAs within pancreatic development was established with mice which are unable to generate miRNAs due to deletion of the Dicer gene within the Pdx1-positive-developing pancreas – these animals show dramatic defects in exocrine, endocrine, and ductal cell pancreatic development (Lynn et al. 2007). miRNAs have been found to be dynamically regulated during hESC differentiation in a similar manner to those seen in human fetal pancreatic development (Wei et al. 2013; Joglekar et al. 2009; Gutteridge et al. 2013; Chen et al. 2011; Liao et al. 2013). During pancreatic endocrine development, miRNA-7 has been implicated in controlling both β-cell proliferation as well as specification of hormone-positive cells (Wang et al. 2013;

Kredo-Russo et al. 2012). Following the murine work that revealed the key role of miRNA 375 to regulate α-cell and β-cell mass (Poy et al. 2009) and similar work in zebrafish (Kloosterman et al. 2007), overexpression of miRNAs in differentiating hESCs has been recently explored. Lentiviral gene delivery to hESCs which resulted in increased expression of miRNA 375 was associated with an increased commitment and maturation toward the pancreatic endocrine lineage including increased expression of insulin, PDX1, NKX6.1, and PAX6 (Lahmy et al. 2013). In a similar study, miRNA-375 overexpression resulted in reduced translation of the miRNA-375 targets SOX9 and HNF1β (Wei et al. 2013), further emphasizing the role of miRNAs to modify gene expression patterns and influence pancreatic endocrine development of hESCs. While the full impact of miRNAs on the many stages of hESC differentiation is not completely known, it seems clear that these small modifiers of gene expression are likely to have dramatic effects in the functional maturation of hESC and could serve as useful targets to control this process.

The idea of modeling human development in hESCs was recently applied to understanding the effects of mutations in the glucokinase gene (GCK), which are associated with MODY2. In an elegant study by Hua et al. (2013), skin biopsies were acquired from two patients with MODY2. Patient-specific iPSCs were generated from each biopsy and were found to be pluripotent while retaining the heterozygous deletion in GCK. Upon in vitro differentiation and in vivo maturation, GCK mutant grafts developed to contain insulin-producing cells which displayed an impaired functional response to elevated glucose levels similar to that commonly observed in individuals with MODY2. The authors then used homologous recombination to repair the genetic lesion in GCK in the undifferentiated iPSCs and found that this restored normal glucose responsiveness to insulin-producing cells upon in vitro and in vivo maturation (Hua et al. 2013). This work suggests that PSC differentiation can be used to recapitulate and understand the effects of human genetic phenotypes. Such approaches could ultimately allow for the generation of patient-specific cellular therapies to restore functionally normal cells in patients bearing genetic mutations in a particular cell type and for developing new drugs.

The strategy of retesting knowledge generated from mouse developmental and rare human models in hESCs has been relatively fruitful. However, discovery-based methods using hESC lines that allow live cell lineage tracing and prospective isolation are also enabling the identification of new factors that influence the development of human diabetes. One popular recent tool has been hESC cell lines which allow tracking of endogenous human insulin promoter activation in its native loci through expression of cytoplasmic eGFP (Nostro et al. 2011; Basford et al. 2012; Micallef et al. 2012). By using homologous recombination, this approach circumvents the problems associated with variable integration and expression of transgenes and the epigenetic silencing which has been observed with lentiviral and retroviral transgenesis (Pannell and Ellis 2001; Cockrell and Kafri 2007; Hockemeyer et al. 2009). The ability to illuminate and isolate the cell type of interest has allowed direct whole transcriptome analysis, as well as

visualization of single-cell cytosolic calcium mobilization and single-whole-cell K_{ATP}, Ca_v, Na^+, and KV currents in hESC-derived insulin/eGFP-positive cells (Basford et al. 2012). As this kind of live cell labeling strategy expands to multiple colors and even to subcellularly localized reporters, the ability to specifically examine the characteristics of rare populations such as NKX6.1, PDX1, MafA, and insulin quadruple-positive, ARX, glucagon, and somatostatin triple-negative cells will become possible. If it is possible to understand how a single cell functionally develops, then we may be able to translate this knowledge to reproducibly guide the generation of glucose-responsive cells in vitro.

Concluding Remarks

With the debilitating complications of diabetes continuing to present a tremendous socioeconomic burden for patients, physicians, and health systems, the drive to develop novel curative approaches is high. While insulin injections continue to save the lives of patients with diabetes, cellular therapies with functional insulin-secreting cell populations should offer more dynamic and finely tuned glycemic control resulting in better quality of life. Human islet transplantation highlighted the potential of a cellular, transplantable source of β-cells for type 1 diabetes treatment but also the critical need for a readily available supply. To push toward this lofty goal, the stem cell field has approached and surpassed a number of key milestones. Research into the myriad of pluripotent cell types has shown that PSCs are broadly capable of producing the cells required for diabetes therapy, and the study of targeted differentiation strategies for PSCs has laid the groundwork for a PSC-derived β-cell source for transplantation. The formation of definitive endoderm and eventual pancreatic progenitors has become a key checkpoint in PSC development and maturation. With demonstration that in vivo maturation of these progenitors can ameliorate diabetic symptoms in multiple models, the capacity of PSC-derived cells to eventually release insulin is no longer a concern. Nevertheless, important questions remain about how this maturation occurs, and how to replicate it consistently and safely on a clinically appropriate scale, and ideally in vitro to generate cells that are fully differentiated and functional immediately upon transplantation. Given the fast pace of the field, progress in terms of fully in vitro hESC-derived β-cells is expected to be swift. While most research in this area has focused on exploiting the potential of hESCs for type 1 diabetes treatment, there remains a significant opportunity to apply these findings to the setting of insulin-dependent type 2 diabetes as well. While much is left to explore, optimize, and implement, the rewards of cell-based physiological glucose regulation will continue to drive innovation and ingenuity in this field of regenerative medicine.

Acknowledgments We thank Dr. J. Bruin and Dr. S. Erener for providing images used in this chapter. We are also indebted to Miss S. White for technical assistance in the generation of Fig. 1. BKG has received PhD funding from the Natural Sciences and Engineering Research Council, the Michael Smith Foundation for Health Research, and the University of British Columbia.

Cross-References

- ▶ Islet Encapsulation
- ▶ Regulation of Pancreatic Islet Formation
- ▶ Stem Cells in Pancreatic Islets
- ▶ Successes and Disappointments with Clinical Islet Transplantation
- ▶ The Comparative Anatomy of Islets

References

Adewumi O, Aflatoonian B, Ahrlund-Richter L, Amit M, Andrews PW, Beighton G, Bello PA, Benvenisty N (2007) Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. Nat Biotechnol 25(7):803–816

Ang SL, Rossant J (1994) HNF-3 β is essential for node and notochord formation in mouse development. Cell 78(4):561–574

Bar-Nur O, Russ HA, Efrat S, Benvenisty N (2011) Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet β cells. Cell Stem Cell 9(1):17–23

Basford CL, Prentice KJ, Hardy AB, Sarangi F, Micallef SJ, Li X, Guo Q, Elefanty AG, Stanley EG, Keller G, Allister EM, Nostro MC, Wheeler MB (2012) The functional and molecular characterisation of human embryonic stem cell-derived insulin-positive cells compared with adult pancreatic β cells. Diabetologia 55(2):358–371

Basma H, Soto-Gutierrez A, Yannam GR, Liu L, Ito R, Yamamoto T, Ellis E, Carson SD, Sato S, Chen Y, Muirhead D, Navarro-Alvarez N, Wong RJ, Roy-Chowdhury J, Platt JL, Mercer DF, Miller JD, Strom SC, Kobayashi N, Fox IJ (2009) Differentiation and transplantation of human embryonic stem cell-derived hepatocytes. Gastroenterology 136(3):990–999

Bernardo AS, Cho CH, Mason S, Docherty HM, Pedersen RA, Vallier L, Docherty K (2009) Biphasic induction of Pdx1 in mouse and human embryonic stem cells can mimic development of pancreatic β-cells. Stem Cells 27(2):341–351

Blyszczuk P, Czyz J, Kania G, Wagner M, Roll U, St-Onge L, Wobus AM (2003) Expression of Pax4 in embryonic stem cells promotes differentiation of nestin-positive progenitor and insulin-producing cells. Proc Natl Acad Sci USA 100(3):998–1003

Bock C, Kiskinis E, Verstappen G, Gu H, Boulting G, Smith ZD, Ziller M, Croft GF, Amoroso MW, Oakley DH, Gnirke A, Eggan K, Meissner A (2011) Reference maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. Cell 144(3):439–452

Borowiak M, Maehr R, Chen S, Chen AE, Tang W, Fox JL, Schreiber SL, Melton DA (2009) Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. Cell Stem Cell 4(4):348–358

Bosco D, Armanet M, Morel P, Niclauss N, Sgroi A, Muller YD, Giovannoni L, Parnaud G, Berney T (2010) Unique arrangement of α- and β-cells in human islets of Langerhans. Diabetes 59(5):1202–1210

Bose B, Shenoy SP, Konda S, Wangikar P (2012) Human embryonic stem cell differentiation into insulin secreting β-cells for diabetes. Cell Biol Int 36(11):1013–1020

Bradley A, Evans M, Kaufman MH, Robertson E (1984) Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. Nature 309(5965):255–256

Brennan J, Lu CC, Norris DP, Rodriguez TA, Beddington RS, Robertson EJ (2001) Nodal signalling in the epiblast patterns the early mouse embryo. Nature 411(6840):965–969

Brissova M, Shostak A, Shiota M, Wiebe PO, Poffenberger G, Kantz J, Chen Z, Carr C, Jerome WG, Chen J, Baldwin HS, Nicholson W, Bader DM, Jetton T, Gannon M, Powers AC (2006)

Pancreatic islet production of vascular endothelial growth factor–a is essential for islet vascularization, revascularization, and function. Diabetes 55(11):2974–2985
Brown S, Teo A, Pauklin S, Hannan N, Cho CH, Lim B, Vardy L, Dunn NR, Trotter M, Pedersen R, Vallier L (2011) Activin/Nodal signaling controls divergent transcriptional networks in human embryonic stem cells and in endoderm progenitors. Stem Cells 29(8):1176–1185
Bruin J, Kieffer T (2012) Differentiation of human embryonic stem cells into pancreatic endocrine cells. In: Hayat MA (ed) Stem cells and cancer stem cells, vol 8. Springer, Dordrecht, pp 191–206
Bruin JE, Rezania A, Xu J, Narayan K, Fox JK, O'Neil JJ, Kieffer TJ (2013) Maturation and function of human embryonic stem cell-derived pancreatic progenitors in macroencapsulation devices following transplant into mice. Diabetologia 56:1987–1998
Brunicardi FC, Kleinman R, Moldovan S, Nguyen TH, Watt PC, Walsh J, Gingerich R (2001) Immunoneutralization of somatostatin, insulin, and glucagon causes alterations in islet cell secretion in the isolated perfused human pancreas. Pancreas 23(3):302–308
Byrne JA, Pedersen DA, Clepper LL, Nelson M, Sanger WG, Gokhale S, Wolf DP, Mitalipov SM (2007) Producing primate embryonic stem cells by somatic cell nuclear transfer. Nature 450(7169):497–502
Cai J, Yu C, Liu Y, Chen S, Guo Y, Yong J, Lu W, Ding M, Deng H (2010) Generation of homogeneous PDX1^{+} pancreatic progenitors from human ES cell-derived endoderm cells. J Mol Cell Biol 2(1):50–60
Calder A, Roth-Albin I, Bhatia S, Pilquil C, Lee JH, Bhatia M, Levadoux-Martin M, McNicol J, Russell J, Collins T, Draper JS (2013) Lengthened G1 phase indicates differentiation status in human embryonic stem cells. Stem Cells Dev 22(2):279–295
Castaing M, Peault B, Basmaciogullari A, Casal I, Czernichow P, Scharfmann R (2001) Blood glucose normalization upon transplantation of human embryonic pancreas into β-cell-deficient SCID mice. Diabetologia 44(11):2066–2076
Champeris Tsaniras S, Jones PM (2010) Generating pancreatic β-cells from embryonic stem cells by manipulating signaling pathways. J Endocrinol 206(1):13–26
Chen Y, Pan FC, Brandes N, Afelik S, Solter M, Pieler T (2004) Retinoic acid signaling is essential for pancreas development and promotes endocrine at the expense of exocrine cell differentiation in Xenopus. Dev Biol 271(1):144–160
Chen M, Song G, Wang C, Hu D, Ren J, Qu X (2009a) Small-molecule selectively recognizes human telomeric G-quadruplex DNA and regulates its conformational switch. Biophys J 97(7):2014–2023
Chen S, Borowiak M, Fox JL, Maehr R, Osafune K, Davidow L, Lam K, Peng LF, Schreiber SL, Rubin LL, Melton D (2009b) A small molecule that directs differentiation of human ESCs into the pancreatic lineage. Nat Chem Biol 5(4):258–265
Chen X, Chen A, Woo TL, Choo AB, Reuveny S, Oh SK (2010) Investigations into the metabolism of two-dimensional colony and suspended microcarrier cultures of human embryonic stem cells in serum-free media. Stem Cells Dev 19(11):1781–1792
Chen BZ, Yu SL, Singh S, Kao LP, Tsai ZY, Yang PC, Chen BH, Shoei-Lung Li S (2011) Identification of microRNAs expressed highly in pancreatic islet-like cell clusters differentiated from human embryonic stem cells. Cell Biol Int 35(1):29–37
Cheng X, Ying L, Lu L, Galvao AM, Mills JA, Lin HC, Kotton DN, Shen SS, Nostro MC, Choi JK, Weiss MJ, French DL, Gadue P (2012) Self-renewing endodermal progenitor lines generated from human pluripotent stem cells. Cell Stem Cell 10(4):371–384
Chetty S, Pagliuca FW, Honore C, Kweudjeu A, Rezania A, Melton DA (2013) A simple tool to improve pluripotent stem cell differentiation. Nat Methods 10(6):553–556
Cho CH, Hannan NR, Docherty FM, Docherty HM, Joao Lima M, Trotter MW, Docherty K, Vallier L (2012) Inhibition of activin/nodal signalling is necessary for pancreatic differentiation of human pluripotent stem cells. Diabetologia 55(12):3284–3295
Cockrell AS, Kafri T (2007) Gene delivery by lentivirus vectors. Mol Biotechnol 36(3):184–204, MB:36:3:184 [pii]

D'Amour KA, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE (2005) Efficient differentiation of human embryonic stem cells to definitive endoderm. Nat Biotechnol 23(12):1534–1541

D'Amour KA, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG, Moorman MA, Kroon E, Carpenter MK, Baetge EE (2006) Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotechnol 24(11):1392–1401

De Vos P, Van Straaten JF, Nieuwenhuizen AG, de Groot M, Ploeg RJ, De Haan BJ, Van Schilfgaarde R (1999) Why do microencapsulated islet grafts fail in the absence of fibrotic overgrowth? Diabetes 48(7):1381–1388

Docherty K, Bernardo AS, Vallier L (2007) Embryonic stem cell therapy for diabetes mellitus. Semin Cell Dev Biol 18(6):827–838

Duvivier-Kali VF, Omer A, Parent RJ, O'Neil JJ, Weir GC (2001) Complete protection of islets against allorejection and autoimmunity by a simple barium-alginate membrane. Diabetes 50(8):1698–1705

Elliott RB, Escobar L, Tan PL, Muzina M, Zwain S, Buchanan C (2007) Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. Xenotransplantation 14(2):157–161

Evans MJ, Kaufman MH (1981) Establishment in culture of pluripotential cells from mouse embryos. Nature 292(5819):154–156

Fowler MJ (2011) The diabetes treatment trap: hypoglycemia. Clin Diabetes 29(1):36–39

Fryer BH, Rezania A, Zimmerman MC (2013) Generating β-cells in vitro: progress towards a Holy Grail. Curr Opin Endocrinol Diabetes Obes 20(2):112–117

Gage BK, Webber TD, Kieffer TJ (2013) Initial cell seeding density influences pancreatic endocrine development during in vitro differentiation of human embryonic stem cells. PLoS One 8:e82076

Gauthier BR, Gosmain Y, Mamin A, Philippe J (2007) The β-cell specific transcription factor Nkx6.1 inhibits glucagon gene transcription by interfering with Pax6. Biochem J 403(3):593–601

Gonzalez F, Boue S, Izpisua Belmonte JC (2011) Methods for making induced pluripotent stem cells: reprogramming a la carte. Nat Rev Genet 12(4):231–242

Gouon-Evans V, Boussemart L, Gadue P, Nierhoff D, Koehler CI, Kubo A, Shafritz DA, Keller G (2006) BMP-4 is required for hepatic specification of mouse embryonic stem cell-derived definitive endoderm. Nat Biotechnol 24(11):1402–1411

Green MD, Chen A, Nostro MC, d'Souza SL, Schaniel C, Lemischka IR, Gouon-Evans V, Keller G, Snoeck HW (2011) Generation of anterior foregut endoderm from human embryonic and induced pluripotent stem cells. Nat Biotechnol 29(3):267–272

Guo T, Hebrok M (2009) Stem cells to pancreatic β-cells: new sources for diabetes cell therapy. Endocr Rev 30(3):214–227

Guo T, Landsman L, Li N, Hebrok M (2013) Factors expressed by murine embryonic pancreatic mesenchyme enhance generation of insulin-producing cells from hESCs. Diabetes 62(5):1581–1592

Gutteridge A, Rukstalis JM, Ziemek D, Tie M, Ji L, Ramos-Zayas R, Nardone NA, Norquay LD, Brenner MB, Tang K, McNeish JD, Rowntree RK (2013) Novel pancreatic endocrine maturation pathways identified by genomic profiling and causal reasoning. PLoS One 8(2):e56024

Halban PA, Kahn SE, Lernmark A, Rhodes CJ (2001) Gene and cell-replacement therapy in the treatment of type 1 diabetes: how high must the standards be set? Diabetes 50(10):2181–2191

Hannan NR, Segeritz CP, Touboul T, Vallier L (2013) Production of hepatocyte-like cells from human pluripotent stem cells. Nat Protoc 8(2):430–437

Hansson M, Tonning A, Frandsen U, Petri A, Rajagopal J, Englund MC, Heller RS, Hakansson J, Fleckner J, Skold HN, Melton D, Semb H, Serup P (2004) Artifactual insulin release from differentiated embryonic stem cells. Diabetes 53(10):2603–2609, 53/10/2603 [pii]

Hauge-Evans AC, King AJ, Carmignac D, Richardson CC, Robinson IC, Low MJ, Christie MR, Persaud SJ, Jones PM (2009) Somatostatin secreted by islet δ-cells fulfills multiple roles as a paracrine regulator of islet function. Diabetes 58(2):403–411

Hayek A, Beattie GM (1997) Experimental transplantation of human fetal and adult pancreatic islets. J Clin Endocrinol Metab 82(8):2471–2475

Hebrok M (2012) Generating β cells from stem cells-the story so far. Cold Spring Harb Perspect Med 2(6):a007674

Hebrok M, Kim SK, Melton DA (1998) Notochord repression of endodermal Sonic hedgehog permits pancreas development. Genes Dev 12(11):1705–1713

Hockemeyer D, Soldner F, Beard C, Gao Q, Mitalipova M, DeKelver RC, Katibah GE, Amora R, Boydston EA, Zeitler B, Meng X, Miller JC, Zhang L, Rebar EJ, Gregory PD, Urnov FD, Jaenisch R (2009) Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases. Nat Biotechnol 27(9):851–857

Hoesli CA, Raghuram K, Kiang RL, Mocinecova D, Hu X, Johnson JD, Lacik I, Kieffer TJ, Piret JM (2011) Pancreatic cell immobilization in alginate beads produced by emulsion and internal gelation. Biotechnol Bioeng 108(2):424–434

Hoesli CA, Kiang RL, Mocinecova D, Speck M, Moskova DJ, Donald-Hague C, Lacik I, Kieffer TJ, Piret JM (2012) Reversal of diabetes by βTC3 cells encapsulated in alginate beads generated by emulsion and internal gelation. J Biomed Mater Res Part B, Appl Biomater 100(4):1017–1028

Hosoya M (2012) Preparation of pancreatic β-cells from human iPS cells with small molecules. Islets 4(3):249–252

Hou P, Li Y, Zhang X, Liu C, Guan J, Li H, Zhao T, Ye J, Yang W, Liu K, Ge J, Xu J, Zhang Q, Zhao Y, Deng H (2013) Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science 341:651–654

Hua H, Shang L, Martinez H, Freeby M, Gallagher MP, Ludwig T, Deng L, Greenberg E, Leduc C, Chung WK, Goland R, Leibel RL, Egli D (2013) iPSC-derived β cells model diabetes due to glucokinase deficiency. J Clin Invest 123:3146–3153

Ivey KN, Muth A, Arnold J, King FW, Yeh RF, Fish JE, Hsiao EC, Schwartz RJ, Conklin BR, Bernstein HS, Srivastava D (2008) MicroRNA regulation of cell lineages in mouse and human embryonic stem cells. Cell Stem Cell 2(3):219–229

Jacobs-Tulleneers-Thevissen D, Chintinne M, Ling Z, Gillard P, Schoonjans L, Delvaux G, Strand BL, Gorus F, Keymeulen B, Pipeleers D, β Cell Therapy Consortium E-F (2013) Sustained function of alginate-encapsulated human islet cell implants in the peritoneal cavity of mice leading to a pilot study in a type 1 diabetic patient. Diabetologia 56(7):1605–1614

Jansson L, Carlsson PO (2002) Graft vascular function after transplantation of pancreatic islets. Diabetologia 45(6):749–763

Jennings RE, Berry AA, Kirkwood-Wilson R, Roberts NA, Hearn T, Salisbury RJ, Blaylock J, Hanley KP, Hanley NA (2013) Development of the human pancreas from foregut to endocrine commitment. Diabetes 62:3514–3522

Jeon J, Correa-Medina M, Ricordi C, Edlund H, Diez JA (2009) Endocrine cell clustering during human pancreas development. J Histochem Cytochem 57(9):811–824

Jermendy A, Toschi E, Aye T, Koh A, Aguayo-Mazzucato C, Sharma A, Weir GC, Sgroi D, Bonner-Weir S (2011) Rat neonatal β cells lack the specialised metabolic phenotype of mature β cells. Diabetologia 54(3):594–604

Jiang J, Au M, Lu K, Eshpeter A, Korbutt G, Fisk G, Majumdar AS (2007a) Generation of insulin-producing islet-like clusters from human embryonic stem cells. Stem Cells 25(8):1940–1953

Jiang W, Shi Y, Zhao D, Chen S, Yong J, Zhang J, Qing T, Sun X, Zhang P, Ding M, Li D, Deng H (2007b) In vitro derivation of functional insulin-producing cells from human embryonic stem cells. Cell Res 17(4):333–344

Jiang W, Bai Z, Zhang D, Shi Y, Yong J, Chen S, Ding M, Deng H (2008) Differentiation of mouse nuclear transfer embryonic stem cells into functional pancreatic β cells. Diabetologia 51(9):1671–1679

Joglekar MV, Joglekar VM, Hardikar AA (2009) Expression of islet-specific microRNAs during human pancreatic development. Gene Expr Patterns 9(2):109–113

Johnson JD, Ao Z, Ao P, Li H, Dai LJ, He Z, Tee M, Potter KJ, Klimek AM, Meloche RM, Thompson DM, Verchere CB, Warnock GL (2009) Different effects of FK506, rapamycin, and mycophenolate mofetil on glucose-stimulated insulin release and apoptosis in human islets. Cell Transplant 18(8):833–845

Jonsson J, Carlsson L, Edlund T, Edlund H (1994) Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 371(6498):606–609

Jorgensen MC, Ahnfelt-Ronne J, Hald J, Madsen OD, Serup P, Hecksher-Sorensen J (2007) An illustrated review of early pancreas development in the mouse. Endocr Rev 28(6):685–705

Kanai-Azuma M, Kanai Y, Gad JM, Tajima Y, Taya C, Kurohmaru M, Sanai Y, Yonekawa H, Yazaki K, Tam PP, Hayashi Y (2002) Depletion of definitive gut endoderm in Sox17-null mutant mice. Development 129(10):2367–2379

Kearns NA, Genga RM, Ziller M, Kapinas K, Peters H, Brehm MA, Meissner A, Maehr R (2013) Generation of organized anterior foregut epithelia from pluripotent stem cells using small molecules. Stem Cell Res 11(3):1003–1012

Kelly OG, Chan MY, Martinson LA, Kadoya K, Ostertag TM, Ross KG, Richardson M, Carpenter MK, D'Amour KA, Kroon E, Moorman M, Baetge EE, Bang AG (2011) Cell-surface markers for the isolation of pancreatic cell types derived from human embryonic stem cells. Nat Biotechnol 29(8):750–756

King AJ, Fernandes JR, Hollister-Lock J, Nienaber CE, Bonner-Weir S, Weir GC (2007) Normal relationship of β- and non-β-cells not needed for successful islet transplantation. Diabetes 56(9):2312–2318

Kinkel MD, Prince VE (2009) On the diabetic menu: zebrafish as a model for pancreas development and function. Bioessays 31(2):139–152

Kleinsmith LJ, Pierce GB Jr (1964) Multipotentiality of single embryonal carcinoma cells. Cancer Res 24:1544–1551

Kloosterman WP, Lagendijk AK, Ketting RF, Moulton JD, Plasterk RH (2007) Targeted inhibition of miRNA maturation with morpholinos reveals a role for miR-375 in pancreatic islet development. PLoS Biol 5(8):e203

Kredo-Russo S, Mandelbaum AD, Ness A, Alon I, Lennox KA, Behlke MA, Hornstein E (2012) Pancreas-enriched miRNA refines endocrine cell differentiation. Development 139(16):3021–3031

Krichevsky AM, Sonntag KC, Isacson O, Kosik KS (2006) Specific microRNAs modulate embryonic stem cell-derived neurogenesis. Stem Cells 24(4):857–864

Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE (2008) Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 26(4):443–452

Kubo A, Shinozaki K, Shannon JM, Kouskoff V, Kennedy M, Woo S, Fehling HJ, Keller G (2004) Development of definitive endoderm from embryonic stem cells in culture. Development 131(7):1651–1662

Kubo A, Stull R, Takeuchi M, Bonham K, Gouon-Evans V, Sho M, Iwano M, Saito Y, Keller G, Snodgrass R (2011) Pdx1 and Ngn3 overexpression enhances pancreatic differentiation of mouse ES cell-derived endoderm population. PLoS One 6(9):e24058

Kunisada Y, Tsubooka-Yamazoe N, Shoji M, Hosoya M (2012) Small molecules induce efficient differentiation into insulin-producing cells from human induced pluripotent stem cells. Stem Cell Res 8(2):274–284

Lahmy R, Soleimani M, Sanati MH, Behmanesh M, Kouhkan F, Mobarra N (2013) Pancreatic islet differentiation of human embryonic stem cells by microRNA overexpression. J Tissue Eng Regen Med. doi:10.1002/term.1787

Lavon N, Yanuka O, Benvenisty N (2006) The effect of overexpression of Pdx1 and Foxa2 on the differentiation of human embryonic stem cells into pancreatic cells. Stem Cells 24(8):1923–1930

Lee SH, Hao E, Savinov AY, Geron I, Strongin AY, Itkin-Ansari P (2009) Human β-cell precursors mature into functional insulin-producing cells in an immunoisolation device: implications for diabetes cell therapies. Transplantation 87(7):983–991

Leon-Quinto T, Jones J, Skoudy A, Burcin M, Soria B (2004) In vitro directed differentiation of mouse embryonic stem cells into insulin-producing cells. Diabetologia 47(8):1442–1451

Liao X, Xue H, Wang YC, Nazor KL, Guo S, Trivedi N, Peterson SE, Liu Y, Loring JF, Laurent LC (2013) Matched miRNA and mRNA signatures from a hESC-based in vitro model of pancreatic differentiation reveal novel regulatory interactions. J Cell Sci 126:3848–3861

Liew CG, Draper JS, Walsh J, Moore H, Andrews PW (2007) Transient and stable transgene expression in human embryonic stem cells. Stem Cells 25(6):1521–1528

Liew CG, Shah NN, Briston SJ, Shepherd RM, Khoo CP, Dunne MJ, Moore HD, Cosgrove KE, Andrews PW (2008) PAX4 enhances β-cell differentiation of human embryonic stem cells. PLoS One 3(3):e1783

Lin HT, Kao CL, Lee KH, Chang YL, Chiou SH, Tsai FT, Tsai TH, Sheu DC, Ho LL, Ku HH (2007) Enhancement of insulin-producing cell differentiation from embryonic stem cells using pax4-nucleofection method. World J Gastroenterol 13(11):1672–1679

Ludwig B, Rotem A, Schmid J, Weir GC, Colton CK, Brendel MD, Neufeld T, Block NL, Yavriyants K, Steffen A, Ludwig S, Chavakis T, Reichel A, Azarov D, Zimermann B, Maimon S, Balyura M, Rozenshtein T, Shabtay N, Vardi P, Bloch K, de Vos P, Schally AV, Bornstein SR, Barkai U (2012) Improvement of islet function in a bioartificial pancreas by enhanced oxygen supply and growth hormone releasing hormone agonist. Proc Natl Acad Sci USA 109(13):5022–5027

Ludwig B, Reichel A, Steffen A, Zimerman B, Schally AV, Block NL, Colton CK, Ludwig S, Kersting S, Bonifacio E, Solimena M, Gendler Z, Rotem A, Barkai U, Bornstein SR (2013) Transplantation of human islets without immunosuppression. Proc Natl Acad Sci USA 110(47):19054–19058

Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R (2001) Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science 292(5520):1389–1394

Lynn FC, Skewes-Cox P, Kosaka Y, McManus MT, Harfe BD, German MS (2007) MicroRNA expression is required for pancreatic islet cell genesis in the mouse. Diabetes 56(12):2938–2945

Maehr R (2011) iPS cells in type 1 diabetes research and treatment. Clin Pharmacol Ther 89(5):750–753

Maes E, Pipeleers D (1984) Effects of glucose and 3',5'-cyclic adenosine monophosphate upon reaggregation of single pancreatic β-cells. Endocrinology 114(6):2205–2209

Marchand M, Schroeder IS, Markossian S, Skoudy A, Negre D, Cosset FL, Real P, Kaiser C, Wobus AM, Savatier P (2009) Mouse ES cells over-expressing the transcription factor NeuroD1 show increased differentiation towards endocrine lineages and insulin-expressing cells. Int J Dev Biol 53(4):569–578

Martens GA, Motte E, Kramer G, Gaarn LW, Stange G, Hellemans K, Aerts J, Nielsen JH, Ling Z, Pipeleers D (2013) Functional characteristics of neonatal rat β cells with distinct markers. J Mol Endocrinol 52:11–28

Martin GR (1981) Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci USA 78(12): 7634–7638

Matveyenko AV, Georgia S, Bhushan A, Butler PC (2010) Inconsistent formation and nonfunction of insulin-positive cells from pancreatic endoderm derived from human embryonic stem cells in athymic nude rats. Am J Physiol Endocrinol Metab 299(5):E713–E720

Melendez-Ramirez LY, Richards RJ, Cefalu WT (2010) Complications of type 1 diabetes. Endocrinol Metab Clin North Am 39(3):625–640

Merani S, Shapiro AM (2006) Current status of pancreatic islet transplantation. Clin Sci 110(6):611–625

Mfopou JK, De Groote V, Xu X, Heimberg H, Bouwens L (2007) Sonic hedgehog and other soluble factors from differentiating embryoid bodies inhibit pancreas development. Stem Cells 25(5):1156–1165

Mfopou JK, Chen B, Mateizel I, Sermon K, Bouwens L (2010) Noggin, retinoids, and fibroblast growth factor regulate hepatic or pancreatic fate of human embryonic stem cells. Gastroenterology 138(7): 2233–2245, 2245 e2231–e2214

Micallef SJ, Li X, Schiesser JV, Hirst CE, Yu QC, Lim SM, Nostro MC, Elliott DA, Sarangi F, Harrison LC, Keller G, Elefanty AG, Stanley EG (2012) INS(GFP/w) human embryonic stem cells facilitate isolation of in vitro derived insulin-producing cells. Diabetologia 55(3):694–706

Miyazaki S, Yamato E, Miyazaki J (2004) Regulated expression of pdx-1 promotes in vitro differentiation of insulin-producing cells from embryonic stem cells. Diabetes 53(4):1030–1037

Mohanakumar T, Narayanan K, Desai N, Ramachandran S, Shenoy S, Jendrisak M, Susskind BM, Olack B, Benshoff N, Phelan DL, Brennan DC, Fernandez LA, Odorico JS, Polonsky KS (2006) A significant role for histocompatibility in human islet transplantation. Transplantation 82(2):180–187

Mou H, Zhao R, Sherwood R, Ahfeldt T, Lapey A, Wain J, Sicilian L, Izvolsky K, Musunuru K, Cowan C, Rajagopal J (2012) Generation of multipotent lung and airway progenitors from mouse ESCs and patient-specific cystic fibrosis iPSCs. Cell Stem Cell 10(4):385–397

Nir T, Melton DA, Dor Y (2007) Recovery from diabetes in mice by β cell regeneration. J Clin Invest 117(9):2553–2561

Nostro MC, Keller G (2012) Generation of β cells from human pluripotent stem cells: potential for regenerative medicine. Semin Cell Dev Biol 23(6):701–710

Nostro MC, Sarangi F, Ogawa S, Holtzinger A, Corneo B, Li X, Micallef SJ, Park IH, Basford C, Wheeler MB, Daley GQ, Elefanty AG, Stanley EG, Keller G (2011) Stage-specific signaling through TGFβ family members and WNT regulates patterning and pancreatic specification of human pluripotent stem cells. Development 138(5):861–871

O'Connor MD, Kardel MD, Iosfina I, Youssef D, Lu M, Li MM, Vercauteren S, Nagy A, Eaves CJ (2008) Alkaline phosphatase-positive colony formation is a sensitive, specific, and quantitative indicator of undifferentiated human embryonic stem cells. Stem Cells 26(5):1109–1116

O'Connor MD, Kardel MD, Eaves CJ (2011) Functional assays for human embryonic stem cell pluripotency. Methods Mol Biol 690:67–80

O'Sullivan ES, Vegas A, Anderson DG, Weir GC (2011) Islets transplanted in immunoisolation devices: a review of the progress and the challenges that remain. Endocr Rev 32(6):827–844

Oliver-Krasinski JM, Stoffers DA (2008) On the origin of the β cell. Genes Dev 22(15):1998–2021

Osafune K, Caron L, Borowiak M, Martinez RJ, Fitz-Gerald CS, Sato Y, Cowan CA, Chien KR, Melton DA (2008) Marked differences in differentiation propensity among human embryonic stem cell lines. Nat Biotechnol 26(3):313–315

Pagliuca FW, Melton DA (2013) How to make a functional β-cell. Development 140(12):2472–2483

Pan FC, Wright C (2011) Pancreas organogenesis: from bud to plexus to gland. Dev Dyn 240(3):530–565

Pannell D, Ellis J (2001) Silencing of gene expression: implications for design of retrovirus vectors. Rev Med Virol 11(4):205–217

Pauklin S, Vallier L (2013) The cell-cycle state of stem cells determines cell fate propensity. Cell 155(1):135–147

Pipeleers D, in't Veld PI, Maes E, Van De Winkel M (1982) Glucose-induced insulin release depends on functional cooperation between islet cells. Proc Natl Acad Sci USA 79(23):7322–7325

Piper K, Brickwood S, Turnpenny LW, Cameron IT, Ball SG, Wilson DI, Hanley NA (2004) β cell differentiation during early human pancreas development. J Endocrinol 181(1):11–23

Plesner A, Verchere CB (2011) Advances and challenges in islet transplantation: islet procurement rates and lessons learned from suboptimal islet transplantation. J Transplant 2011:979527

Polak M, Bouchareb-Banaei L, Scharfmann R, Czernichow P (2000) Early pattern of differentiation in the human pancreas. Diabetes 49(2):225–232

Poy MN, Hausser J, Trajkovski M, Braun M, Collins S, Rorsman P, Zavolan M, Stoffel M (2009) miR-375 maintains normal pancreatic α- and β-cell mass. Proc Natl Acad Sci USA 106(14):5813–5818

Prentki M, Nolan CJ (2006) Islet β cell failure in type 2 diabetes. J Clin Invest 116(7):1802–1812

Rafael E, Wu GS, Hultenby K, Tibell A, Wernerson A (2003) Improved survival of macroencapsulated islets of Langerhans by preimplantation of the immunoisolating device: a morphometric study. Cell Transplant 12(4):407–412

Raikwar SP, Zavazava N (2011) Spontaneous in vivo differentiation of embryonic stem cell-derived pancreatic endoderm-like cells corrects hyperglycemia in diabetic mice. Transplantation 91(1):11–20

Raikwar SP, Zavazava N (2012) PDX1-engineered embryonic stem cell-derived insulin producing cells regulate hyperglycemia in diabetic mice. Transplant Res 1(1):19

Rajagopal J, Anderson WJ, Kume S, Martinez OI, Melton DA (2003) Insulin staining of ES cell progeny from insulin uptake. Science 299(5605):363

Rezania A, Riedel MJ, Wideman RD, Karanu F, Ao Z, Warnock GL, Kieffer TJ (2011) Production of functional glucagon-secreting α-cells from human embryonic stem cells. Diabetes 60(1):239–247

Rezania A, Bruin JE, Riedel MJ, Mojibian M, Asadi A, Xu J, Gauvin R, Narayan K, Karanu F, O'Neil JJ, Ao Z, Warnock GL, Kieffer TJ (2012) Maturation of human embryonic stem cell-derived pancreatic progenitors into functional islets capable of treating pre-existing diabetes in mice. Diabetes 61(8):2016–2029

Rezania A, Bruin JE, Xu J, Narayan K, Fox JK, O'Neil JJ, Kieffer TJ (2013) Enrichment of human embryonic stem cell-derived NKX6.1-expressing pancreatic progenitor cells accelerates the maturation of insulin-secreting cells in vivo. Stem Cells 31:2432–2442

Riedel MJ, Asadi A, Wang R, Ao Z, Warnock GL, Kieffer TJ (2012) Immunohistochemical characterisation of cells co-producing insulin and glucagon in the developing human pancreas. Diabetologia 55(2):372–381

Roche E, Ensenat-Waser R, Reig JA, Jones J, Leon-Quinto T, Soria B (2006) Therapeutic potential of stem cells in diabetes. Handb Exp Pharmacol 174:147–167

Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, Shapiro AM (2005) Five-year follow-up after clinical islet transplantation. Diabetes 54(7):2060–2069

Schaffer AE, Taylor BL, Benthuysen JR, Liu J, Thorel F, Yuan W, Jiao Y, Kaestner KH, Herrera PL, Magnuson MA, May CL, Sander M (2013) Nkx6.1 controls a gene regulatory network required for establishing and maintaining pancreatic β cell identity. PLoS Genet 9(1):e1003274

Scharp DW, Marchetti P (2013) Encapsulated islets for diabetes therapy: history, current progress, and critical issues requiring solution. Adv Drug Deliv Rev. doi:10.1016/j.addr.2013.07.018

Scharp DW, Lacy PE, Santiago JV, McCullough CS, Weide LG, Falqui L, Marchetti P, Gingerich RL, Jaffe AS, Cryer PE et al (1990) Insulin independence after islet transplantation into type I diabetic patient. Diabetes 39(4):515–518

Schulz TC, Young HY, Agulnick AD, Babin MJ, Baetge EE, Bang AG, Bhoumik A, Cepa I, Cesario RM, Haakmeester C, Kadoya K, Kelly JR, Kerr J, Martinson LA, McLean AB, Moorman MA, Payne JK, Richardson M, Ross KG, Sherrer ES, Song X, Wilson AZ, Brandon EP, Green CE, Kroon EJ, Kelly OG, D'Amour KA, Robins AJ (2012) A scalable system for production of functional pancreatic progenitors from human embryonic stem cells. PLoS One 7(5):e37004

Segev H, Fishman B, Ziskind A, Shulman M, Itskovitz-Eldor J (2004) Differentiation of human embryonic stem cells into insulin-producing clusters. Stem Cells 22(3):265–274

Seguin CA, Draper JS, Nagy A, Rossant J (2008) Establishment of endoderm progenitors by SOX transcription factor expression in human embryonic stem cells. Cell Stem Cell 3(2):182–195

Sela Y, Molotski N, Golan S, Itskovitz-Eldor J, Soen Y (2012) Human embryonic stem cells exhibit increased propensity to differentiate during the G1 phase prior to phosphorylation of retinoblastoma protein. Stem Cells 30(6):1097–1108

Seymour PA, Sander M (2011) Historical perspective: beginnings of the β-cell: current perspectives in β-cell development. Diabetes 60(2):364–376
Shapiro AM (2011) Strategies toward single-donor islets of Langerhans transplantation. Curr Opin Organ Transplant 16(6):627–631
Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 343(4):230–238
Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, Cagliero E, Alejandro R, Ryan EA, DiMercurio B, Morel P, Polonsky KS, Reems JA, Bretzel RG, Bertuzzi F, Froud T, Kandaswamy R, Sutherland DE, Eisenbarth G, Segal M, Preiksaitis J, Korbutt GS, Barton FB, Viviano L, Seyfert-Margolis V, Bluestone J, Lakey JR (2006) International trial of the Edmonton protocol for islet transplantation. N Engl J Med 355(13):1318–1330
Shim JH, Kim SE, Woo DH, Kim SK, Oh CH, McKay R, Kim JH (2007) Directed differentiation of human embryonic stem cells towards a pancreatic cell fate. Diabetologia 50(6):1228–1238
Shiroi A, Ueda S, Ouji Y, Saito K, Moriya K, Sugie Y, Fukui H, Ishizaka S, Yoshikawa M (2005) Differentiation of embryonic stem cells into insulin-producing cells promoted by Nkx2.2 gene transfer. World J Gastroenterol 11(27):4161–4166
Slack JM (1995) Developmental biology of the pancreas. Development 121(6):1569–1580
Smith JR, Maguire S, Davis LA, Alexander M, Yang F, Chandran S, ffrench-Constant C, Pedersen RA (2008) Robust, persistent transgene expression in human embryonic stem cells is achieved with AAVS1-targeted integration. Stem Cells 26(2):496–504
Smith KP, Luong MX, Stein GS (2009) Pluripotency: toward a gold standard for human ES and iPS cells. J Cell Physiol 220(1):21–29
Soria B, Roche E, Berna G, Leon-Quinto T, Reig JA, Martin F (2000) Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. Diabetes 49(2):157–162
Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, Hoskins EE, Kalinichenko VV, Wells SI, Zorn AM, Shroyer NF, Wells JM (2011) Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature 470(7332):105–109
Stadtfeld M, Brennand K, Hochedlinger K (2008) Reprogramming of pancreatic β cells into induced pluripotent stem cells. Curr Biol 18(12):890–894
Stafford D, Prince VE (2002) Retinoic acid signaling is required for a critical early step in zebrafish pancreatic development. Curr Biol 12(14):1215–1220
Stanley EG, Elefanty AG (2008) Building better β cells. Cell Stem Cell 2(4):300–301
Steiner DJ, Kim A, Miller K, Hara M (2010) Pancreatic islet plasticity: interspecies comparison of islet architecture and composition. Islets 2(3):135–145
Stevens LC, Little CC (1954) Spontaneous testicular teratomas in an inbred strain of mice. Proc Natl Acad Sci USA 40(11):1080–1087
Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF (1997) Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. Nat Genet 15(1):106–110
Sui L, Geens M, Sermon K, Bouwens L, Mfopou JK (2013) Role of BMP signaling in pancreatic progenitor differentiation from human embryonic stem cells. Stem Cell Rev 9:569–577
Tachibana M, Amato P, Sparman M, Gutierrez NM, Tippner-Hedges R, Ma H, Kang E, Fulati A, Lee HS, Sritanaudomchai H, Masterson K, Larson J, Eaton D, Sadler-Fredd K, Battaglia D, Lee D, Wu D, Jensen J, Patton P, Gokhale S, Stouffer RL, Wolf D, Mitalipov S (2013) Human embryonic stem cells derived by somatic cell nuclear transfer. Cell 153:1228–1238
Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676
Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5):861–872

The Diabetes Control and Complications Trial Research Group D (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N Engl J Med 329(14):977–986

Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Becker RA, Hearn JP (1995) Isolation of a primate embryonic stem cell line. Proc Natl Acad Sci USA 92(17):7844–7848

Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Hearn JP (1996) Pluripotent cell lines derived from common marmoset (*Callithrix jacchus*) blastocysts. Biol Reprod 55(2):254–259

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science 282(5391):1145–1147

Tiso N, Filippi A, Pauls S, Bortolussi M, Argenton F (2002) BMP signalling regulates anteroposterior endoderm patterning in zebrafish. Mech Dev 118(1–2):29–37

Tiso N, Moro E, Argenton F (2009) Zebrafish pancreas development. Mol Cell Endocrinol 312(1–2):24–30

Touboul T, Hannan NR, Corbineau S, Martinez A, Martinet C, Branchereau S, Mainot S, Strick-Marchand H, Pedersen R, Di Santo J, Weber A, Vallier L (2010) Generation of functional hepatocytes from human embryonic stem cells under chemically defined conditions that recapitulate liver development. Hepatology 51(5):1754–1765

Treff NR, Vincent RK, Budde ML, Browning VL, Magliocca JF, Kapur V, Odorico JS (2006) Differentiation of embryonic stem cells conditionally expressing neurogenin 3. Stem Cells 24(11):2529–2537

Ungrin M, O'Connor M, Eaves C, Zandstra PW (2007) Phenotypic analysis of human embryonic stem cells. Curr Protoc Stem Cell Biol. Chapter 1:Unit 1B 3. doi:10.1002/9780470151808.sc01b03s2

van Belle TL, Coppieters KT, von Herrath MG (2011) Type 1 diabetes: etiology, immunology, and therapeutic strategies. Physiol Rev 91(1):79–118

Van Hoof D, D'Amour KA, German MS (2009) Derivation of insulin-producing cells from human embryonic stem cells. Stem Cell Res 3(2–3):73–87

Vasir B, Jonas JC, Steil GM, Hollister-Lock J, Hasenkamp W, Sharma A, Bonner-Weir S, Weir GC (2001) Gene expression of VEGF and its receptors Flk-1/KDR and Flt-1 in cultured and transplanted rat islets. Transplantation 71(7):924–935

Wang Y, Liu J, Liu C, Naji A, Stoffers DA (2013) MicroRNA-7 regulates the mTOR pathway and proliferation in adult pancreatic β-cells. Diabetes 62(3):887–895

Wei R, Yang J, Liu GQ, Gao MJ, Hou WF, Zhang L, Gao HW, Liu Y, Chen GA, Hong TP (2013) Dynamic expression of microRNAs during the differentiation of human embryonic stem cells into insulin-producing cells. Gene 518(2):246–255

Weinstein DC, Ruiz i Altaba A, Chen WS, Hoodless P, Prezioso VR, Jessell TM, Darnell JE Jr (1994) The winged-helix transcription factor HNF-3 β is required for notochord development in the mouse embryo. Cell 78(4):575–588

Weir GC (2013) Islet encapsulation: advances and obstacles. Diabetologia 56(7):1458–1461

Wells JM, Melton DA (1999) Vertebrate endoderm development. Annu Rev Cell Dev Biol 15:393–410

Wells JM, Melton DA (2000) Early mouse endoderm is patterned by soluble factors from adjacent germ layers. Development 127(8):1563–1572

Wideman RD, Kieffer TJ (2009) Mining incretin hormone pathways for novel therapies. Trends Endocrinol Metab 20(6):280–286

Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH (1997) Viable offspring derived from fetal and adult mammalian cells. Nature 385(6619):810–813

Wong AL, Nierras CR (2010) Do stem cell-derived islets represent a commercially viable treatment for Type 1 and 2 diabetes? Regen Med 5(6):839–842

Wong AL, Hwa A, Hellman D, Greenstein JL (2012) Surrogate insulin-producing cells. F1000 Med Rep 4:15

Xu X, Browning VL, Odorico JS (2011) Activin, BMP and FGF pathways cooperate to promote endoderm and pancreatic lineage cell differentiation from human embryonic stem cells. Mech Dev 128(7–10):412–427

Yang YP, Wright C (2009) Chemicals turn human embryonic stem cells towards β cells. Nat Chem Biol 5(4):195–196

Yasunaga M, Tada S, Torikai-Nishikawa S, Nakano Y, Okada M, Jakt LM, Nishikawa S, Chiba T, Era T, Nishikawa S (2005) Induction and monitoring of definitive and visceral endoderm differentiation of mouse ES cells. Nat Biotechnol 23(12):1542–1550

Yu J, Thomson JA (2008) Pluripotent stem cell lines. Genes Dev 22(15):1987–1997

Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318(5858):1917–1920

Zhang N, Richter A, Suriawinata J, Harbaran S, Altomonte J, Cong L, Zhang H, Song K, Meseck M, Bromberg J, Dong H (2004) Elevated vascular endothelial growth factor production in islets improves islet graft vascularization. Diabetes 53(4):963–970

Zhang C, Moriguchi T, Kajihara M, Esaki R, Harada A, Shimohata H, Oishi H, Hamada M, Morito N, Hasegawa K, Kudo T, Engel JD, Yamamoto M, Takahashi S (2005) MafA is a key regulator of glucose-stimulated insulin secretion. Mol Cell Biol 25(12):4969–4976

Zhang D, Jiang W, Liu M, Sui X, Yin X, Chen S, Shi Y, Deng H (2009) Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. Cell Res 19(4):429–438

Zweigerdt R, Olmer R, Singh H, Haverich A, Martin U (2011) Scalable expansion of human pluripotent stem cells in suspension culture. Nat Protoc 6(5):689–700

Pancreatic Neuroendocrine Tumors

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Abstract

Pancreatic neuroendocrine tumors (pNETs) are rare neoplasms, with a prevalence of 1–2 per 100,000 people. Based on the presence or absence of a specific hormone-related clinical syndrome, they are divided into functioning and nonfunctioning. Among the former tumors, insulinomas are the most common. pNETs may be sporadic or associated with hereditary syndromes. The histopathology evaluation should include immunostaining with general (chromogranin A and synaptophysin) and specific neuroendocrine markers, as well as with the proliferation index Ki67. Chromogranin A is currently the most useful neuroendocrine biomarker for diagnosis and follow-up of pNETs. Other specific hormones released by the neoplastic cells can also be included in the biochemical evaluation. For tumor localization both noninvasive and invasive techniques may be used. Debulking procedures and medical therapy are the possible treatment options for pNETs, but surgery is the only modality that offers the possibility of cure.

Keywords

Pancreatic neuroendocrine tumors (pNETs) • Functioning/nonfunctioning pNETs • Well-differentiated pNETs • Neuroendocrine carcinomas • Sporadic/hereditary pNETs • Chromogranin A • Synaptophysin • Ki67 • Noninvasive/invasive tumor localization techniques • Debulking procedures • Medical treatment

Introduction

Pancreatic neuroendocrine tumors (pNETs) are rare neoplasms with a wide spectrum of clinical presentation. Their rarity in combination to the syndromic clinical scenario that accompanies a fraction of them has intensified fascination among the physicians that deal with these tumors. In contradiction to their infrequency, the relevant long survival of many patients with pNETs compared to that of their adenocarcinoma counterpart suggests that the point prevalence is not inconsiderable. However, pNETs can be difficult to be diagnosed, especially in their early stages. Nowadays, there have been a number of recent advances in various aspects of pNETs including diagnosis, molecular pathology, as well as in their treatment. The present book chapter reviews a number of these advances and the management of the pNETs.

Epidemiology

pNETs have a prevalence of 1–2 per 100,000 of the population and comprise 1–2 % of all pancreatic tumors (Kaltsas et al. 2004b; Halfdanarson et al. 2008). According to the Surveillance, Epidemiology, and End Results (SEER) Program (Modlin et al. 2008; Yao et al. 2008a), an increase in the frequency of pNETs has been observed, which might also be attributed to the employment of modern imaging modalities and the incidental detection of otherwise silent tumors (Vagefi et al. 2007). This notion is supported by data from autopsy series where the reported incidence of small (<1 cm) pNETs ranges from 0.8 % to 10 % (Kimura et al. 1991). No gender predisposition is observed, and these tumors may be diagnosed at all ages, with a peak incidence between 30 and 60 years (Halfdanarson et al. 2008).

Classification and Staging

Traditionally, pNETs have been classified in functioning and nonfunctioning (NF) according to their ability to secrete bioactive substances in sufficient amounts to evoke a relevant clinical syndrome (Klimstra et al. 2010). It should be noted that many pNETs may be accompanied by increased circulating hormone concentrations and/or show hormone immunoreactivity; however they do not give rise to a clinical syndrome, and thus they cannot be characterized as functioning (Tsolakis and Janson 2008). The majority of pNETs are NF (~60 %); among functioning tumors insulinomas and gastrinomas are the most frequently encountered, while the rest of functioning pNETs are quite uncommon (Falconi et al. 2012). The latter group is named rare functioning pNETs and includes VIPomas, glucagonomas, and somatostatinomas (SSomas), as well as tumors that ectopically secrete substances like adrenocorticotropic hormone (ACTH), corticotropin-releasing hormone (CRH), growth hormone (GH), growth hormone-releasing hormone (GHRH), parathyroid hormone-related protein (PTHrP), and calcitonin (Jensen et al. 2012).

According to the most recent classification system introduced by WHO, neuroendocrine neoplasms in general (pNETs included) are divided in three categories based on mitotic count or Ki67 proliferation index (Bosman et al. 2010):

- G1 with a mitotic count <2 per 10 high-power fields (HPF) and/or Ki67 index ≤2 %
- G2 with a mitotic count 2–20 per 10 HPF and/or Ki67 index 3–20 %
- G3 with a mitotic count <20 per 10 HPF and/or Ki67 index >20 %

G1 and G2 tumors comprise a subgroup of well-differentiated neuroendocrine tumors (NETs), whereas G3 tumors are characterized as poorly differentiated neuroendocrine carcinomas (NECs) of small or large cell type.

A summary of pNETs' characteristics is presented in Table 1.

Recently, the European Neuroendocrine Tumors Society (ENETS) introduced a staging system for NETs (Table 2) in line with the UICC (Union for International Cancer Control) TNM system (Rindi et al. 2006). According to a recent study, the

Table 1 Summary of pancreatic neuroendocrine tumors' characteristics

Tumor type (hormone)	Clinical presentation	Pancreatic localization	Incidence (cases/100,000/year)	Malignancy (%)	MEN-1 associated (%)
Insulinoma (insulin)	Whipple's triad[a]	Equal incidence in all parts	0.4	<10	4–6
Gastrinoma (gastrin)	Zollinger–Ellison syndrome[b]	Head	0.05–0.4	>60	20–30
Glucagonoma (glucagon)	Glucagonoma syndrome[c]	Tail	0.005	50–80	15
VIPoma (VIP)	Verner–Morrison syndrome[d]	Tail	0.01	50–60	<3
SSoma (somatostatin)	SSoma syndrome[e]	Head	<0.01	>70	<1
ACTHoma (ACTH)	Ectopic Cushing	No preferential	<0.01	95	Rare
CRHoma (CRH)	Ectopic Cushing	No preferential	Unknown (very rare)	>80	Rare
Calcitonin-secreting pNET	Diarrhea/ hypocalcemic symptoms	No preferential	Unknown (very rare)	>80	10–20
GHRHoma	Acromegaly	Tail	Unknown (very rare)	>80	Unknown
PTHrPoma (PTHrP)	Hypercalcemic symptoms	No preferential	Unknown (very rare)	>80	Rare
Serotonin-secreting pNET	Typical carcinoid syndrome[f]	Head	Unknown (very rare)	>80	Unknown
NF-pNET (e.g., PP)	Mass effect	Head	1–3	60–80	10–20
NECs	Mass effect/ syndromes (rare)	Head	<0.01	100	Unknown

Data obtained from Ehehalt et al. (2009) and Tsolakis and Janson (2008)
ACTH adrenocorticotropic hormone, *CRH* corticotropin-releasing hormone, *GHRH* growth hormone-releasing hormone, *NECs* neuroendocrine carcinomas, *NF* nonfunctioning, *pNET* pancreatic neuroendocrine tumor, *PP* pancreatic polypeptide, *PTHrP* parathyroid hormone-related peptide, *SS* somatostatin, *VIP* vasoactive intestinal polypeptide
[a]Hypoglycemic symptoms, low blood glucose levels, reversible upon glucose intake
[b]Diarrhea, hypergastrinemia, gastric acid hypersecretion, peptic ulcer diathesis
[c]Glucagonoma syndrome: necrolytic migrating erythema, diabetes mellitus, deep vein thrombosis, anemia, weight loss, neuropsychiatric symptoms
[d]WDHA syndrome: watery diarrhea, hypokalemia, achlorhydria
[e]SSoma syndrome: diabetes, hypochlorhydria, cholelithiasis, steatorrhea, anemia, weight loss
[f]Flushing, diarrhea, cardiac valvular diseases, bronchospasm

corresponding frequencies for each stage were stage I (5 %), stage II (15 %), stage III (22 %), and stage IV (55 %) (Pape et al. 2008).

Furthermore, various prognostic factors suggested to correlate to prognosis have been proposed for pNETs (Table 3) (Tsolakis and Janson 2008).

Table 2 Proposal for a TNM classification and disease staging for endocrine tumors of the pancreas according to Rindi et al. (2006)

TNM			
T – primary tumor			
TX	Primary tumor cannot be assessed		
T0	No evidence of primary tumor		
T1	Tumor limited to the pancreas and size <2 cm		
T2	Tumor limited to the pancreas and size 2–4 cm		
T3	Tumor limited to the pancreas and size >4 cm or invading duodenum or bile duct		
T4	Tumor invading adjacent organs (stomach, spleen, colon, adrenal gland) or the wall of large vessels (celiac axis or superior mesenteric artery)		
	For any T, add (m) for multiple tumors		
N – regional lymph nodes			
NX	Regional lymph node cannot be assessed		
N0	No regional lymph node metastasis		
N1	Regional lymph node metastasis		
M – distant metastases			
MX	Distant metastasis cannot be assessed		
M0	No distant metastases		
M1[a]	Distant metastasis		
Stage			
Disease stages			
Stage I	T1	N0	M0
Stage IIa	T2	N0	M0
IIb	T3	N0	M0
Stage IIIa	T4	N0	M0
IIIb	Any T	N1	M0
Stage IV	Any T	Any N	M1

[a]M1-specific sites defined according to Sobin and Wittekind (2002)

Pathology

pNETs are predominantly solid tumors; however they can rarely be cystic, being larger in size than solid ones. Cystic pNETs show a higher prevalence among Multipe Endocrine Neoplasia (MEN)-1 patients (Ligneau et al. 2001). The size of pNETs is quite variable, ranging from less than 1 cm to more than 15 cm. The majority of tumors are 1–5 cm, with NF tumors being larger at the time of diagnosis than their functioning counterparts. This is probably the result of delayed diagnosis due to the indolent course of NF tumors, rather than of more aggressive behavior (Falconi et al. 2012).

Table 3 Prognostic factors suggested to correlate to prognosis

Favorable prognosis	Unfavorable prognosis
1 Size <2 cm	1 Size >2 cm
2 Ki-67/MIB-1 <2 %	2 Ki-67/MIB-1 >2 %
3 Mitoses <2 per 10 HPF	3 Mitoses >2 per 10 HPF
4 No sign of angioinvasion	4 Sign of angioinvasion
5 No sign of perineural invasion	5 Sign of perineural invasion
6 No sign of metastases	6 Sign of metastases
7 No penetration through tumor capsule	7 Penetration through tumor capsule
8 Macroscopically radical surgery	8 No macroscopically radical surgery
9 No tumor necrosis	9 Tumor necrosis
10 Insulinoma	10 Functioning tumors (except insulinoma)

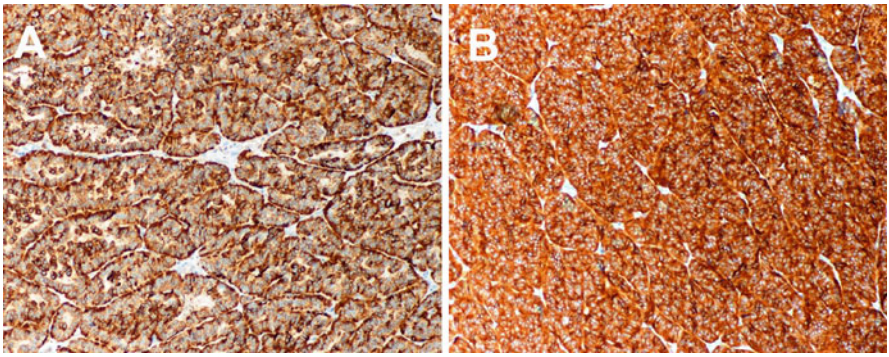

Fig. 1 A well-differentiated pancreatic neuroendocrine tumor immunostained with (**a**) chromogranin A and (**b**) synaptophysin; virtually all neoplastic cells are immunoreactive for both markers

The growth pattern of well-differentiated pNETs can vary (solid, trabecular, glandular, gyriform, tubuloacinar, pseudo rosette, and, rarely, rhabdoid), while different patterns can be observed in the same tumor (Perez-Montiel et al. 2003). Tumor cells are uniform with a centrally located nucleus, occasionally with oncocytic differentiation. In general, the histological growth pattern is not conclusive for the diagnosis, with the exception of amyloid deposits in insulinomas and psammoma bodies in SSomas (Kloppel et al. 2007b). NECs, on the other hand, display apparent atypia and high mitotic rate and/or Ki-67/MIB-l proliferation index, as well as areas with necroses. In general it is difficult to recognize their neuroendocrine differentiation in the absence of immunohistochemical analysis (Rindi and Kloppel 2004).

The neuroendocrine nature of tumor cells is confirmed immunohistochemically with general neuroendocrine markers such as chromogranin A (CgA) (Fig. 1a) that is expressed in the large dense-core vesicles; synaptophysin (Fig. 1b) which is associated to small vesicles; cell membrane markers, such as the neural cell

adhesion molecule (NCAM/CD56); and the cytosolic marker neuron-specific enolase (NSE) (Kloppel et al. 2007b). Further, in the case of functioning tumors, the relevant hormone might be sought by means of immunohistochemistry to confirm the diagnosis. However, this is not mandatory, as functioning pNETs may display weak (or even absent) immunoreactivity to the specific hormone because of its immediate release (Jensen et al. 2012).

According to the WHO guidelines, the diagnostic report should include (Bosman et al. 2010):

- The histological classification of the lesion (as NET or NEC, small or large cell type)
- The grade (G1, G2, or G3)
- The relevant TNM stage (according to ENETs and UICC 2009)
- Optionally, the expression of hormones, transcription factors, or somatostatin receptors

The observation in patients with MEN-1 syndrome that NETs less than 5 mm in diameter coexist with macro-tumors (>5 mm in diameter) has raised the hypothesis of precursor lesions and has been termed micro adenomatosis (Kloppel et al. 2007a). However, such precursor lesions have not been described as yet in sporadic pNETs.

Genetics

Sporadic Pancreatic Neuroendocrine Tumors

In the search of the genetic events in somatic cells that may lead to neoplasia, it is demonstrated that almost a third of pNET patients manifest allelic loss on chromosome 3p, a locus that harbors various tumor-suppressor genes (Chung et al. 1997). In addition, losses of chromosomes 1 and llq, as well as gains of 9q, occur in tumors less than 2 cm in size, suggesting that they are early events in the development of pNETs (Zhao et al. 2001). Further, loss of heterozygosity (LOH) at chromosome llq is encountered in functioning pNETs but not in NF ones. By contrast, LOH at chromosome 6q is associated with the development of NF tumors (Rigaud et al. 2001).

Hereditary Pancreatic Neuroendocrine Tumors

Germline mutations can give rise to genetic syndromes that manifest a predilection for NETs and pNETs in particular. Such syndromes are MEN-1, von Hippel–Lindau (vHL), neurofibromatosis (NF)-1, and tuberous sclerosis (TSCL). A summary of the hereditary pNETs is presented in Table 4.

MEN-1 is the result of a germline mutation in MENlN, a tumor-suppressor gene, located on chromosome llq13. Some 20–60 % of MEN-1 patients have pancreatic lesions when assessed by clinical screening and the incidence increases with

Table 4 Inherited disorders associated with pancreatic neuroendocrine tumors

Syndrome	Associated clinical features	Chromosomal location	Pancreatic neuroendocrine tumor type
Multiple endocrine neoplasia-1	Primary hyperparathyroidism	11q13	Nonfunctioning
	Pituitary tumor		Gastrinoma
	Less commonly		Insulinoma
	Adrenocortical tumor		Various
	Non-medullary thyroid tumor		
Von Hippel–Lindau disease	CNS hemangioblastoma	3p25	Nonfunctioning
	Retinal angioma		Various, including cystic tumors
	Pheochromocytoma (often bilateral)		
	Renal cell carcinoma		
Neurofibromatosis-1	Neurofibromas	17q11.2	Various
	Café au lait spots		
	Axillary or inguinal freckling		
	Bone defects		
	Optic glioma		
	Iris Lisch nodule		
	Duodenal somatostatinoma		
	Gastrointestinal stromal tumor		
Tuberous sclerosis	CNS involvement	9q34 and 16p13	Nonfunctioning
	Epilepsy		
	Subependymal nodule		
	Subependymal giant cell astrocytoma		Insulinoma
	Mental retardation		
	Retinal hamartoma		
	Renal angiomyolipoma		
	Renal cysts		
	Cardiac rhabdomyoma		
	Angiofibroma		
	Lymphangiomyomatosis		

advancing age (3 %, 34 %, and 53 % of patients at age 20, 50, and 80 years, respectively). This figure, however, approaches 100 % in autopsy materials. The majority of tumors are NF, whereas gastrinomas are the most common functioning ones. pNETs usually appear in MEN-1 at an earlier age than the sporadic tumors do, and they represent the major mortality factor among other neoplasias of this syndrome (Tonelli et al. 2011).

The vHL syndrome is a rare autosomal-dominant disease associated with the mutation of a gene located on the short arm of chromosome 3p25.5. The frequency of pancreatic lesions in vHL patients ranges from 17 % to 56 %; however the majority represent benign cysts (Hough et al. 1994).

NF-1 is an inherited autosomal-dominant disorder evoked by mutations of the tumor-suppressor gene NF-1 on chromosome 17, and pNETs are a rare manifestation of this syndrome. TSCL is another autosomal-dominant genetic disorder which results from mutations in the TSCl or TSC2 genes. In rare cases, NF-pNETs or insulinomas can occur (Verhoef et al. 1999).

Specific Tumor Types: Clinical Manifestations

Well-Differentiated Pancreatic Neuroendocrine Tumors

Nonfunctioning Pancreatic Neuroendocrine Tumors

These tumors are the most common pNETs accounting for approximately 60 % in recent studies (Falconi et al. 2012). NF-pNETs can be truly nonfunctional without any capability of secretion (null secreting cells); however, they might also consist of pancreatic polypeptide- (PP-) secreting cells (PPomas), without being related to a distinct clinical syndrome. Due to their silent nature, they are often diagnosed at advanced stages, and 46–73 % of them present liver metastases at the time of diagnosis, while only 14 % have localized disease (Garcia-Carbonero et al. 2010). As a consequence, the age at presentation is advanced and peaks in the sixth and seventh decade of life. Nevertheless, up to 38 % of patients remain asymptomatic and are diagnosed incidentally during abdominal surgery or imaging for an unrelated disease (Zerbi et al. 2011).

Whenever symptoms are present, the most common are abdominal pain (35–78 %), weight loss (20–35 %), and anorexia and nausea (45 %). Less frequent are intra-abdominal hemorrhage (4–20 %), jaundice (17–50 %), or a palpable mass (7–40 %). In rare cases, the activation of an NF tumor to become functioning has been reported (Madura et al. 1997; Phan et al. 1998; Matthews et al. 2000).

Functioning Pancreatic Neuroendocrine Tumors

Insulinoma

Insulinomas are the most common type of functioning pNETs; they have an annual incidence of around four cases per one million people (Tsolakis and Janson 2008). The median age at presentation is 47 years (range 8–82 years), and women seem to be slightly more frequently affected than men (1.5:1) (Service et al. 1991). These patients can have symptoms for several years and present a long history of seeking medical attention before the right diagnosis is set (mean duration varying between 26 and 50 months).

Insulinomas are located almost exclusively in the pancreas and affect all of its parts with equal frequency (Vagefi et al. 2007). The vast majority of insulinomas (90–95 %) is benign at presentation and has an excellent prognosis, with an overall

5-year survival rate of 97 %. The rate of malignancy is less than 10 %; however in such case, especially in the presence of liver metastases, the median survival is less than 2 years according to older data (Soga et al. 1998). In contrast to the sporadic cases that are in the vast majority (91 %), solitary tumors in MEN-1 patients are often multiple (59 %), and they present earlier in life (15–45 years of age) (Norton et al. 2006).

The clinical hallmark of insulinoma are episodes of hypoglycemia characterized by adrenergic symptoms (tachycardia, tremor, sweating) and as glucose levels decrease neurological symptoms (visual disturbances, confusion, comma). These episodes often occur several hours after the last meal (fasting, typically during the night or before breakfast) and can be triggered or aggravated by physical exercise or the omission of meals. Eventually, patients often overeat to compensate and develop obesity (de Herder et al. 2006).

Gastrinoma (Zollinger–Ellison Syndrome)

Gastrinoma is the most common type of malignant functioning pNETs and the second most common functioning pNET in general after insulinoma, with an overall annual incidence between 0.5 and 4.0 per million people. This figure rises to approximately 0.1 % among patients with duodenal ulcer disease. The male to female ratio is 3:2, and the mean age at diagnosis is 48 years (range 8–94 years), with a delay from onset of symptoms of 5.5 years (Anlauf et al. 2006).

Almost 90 % of gastrinomas develop in the anatomical region underlying the right upper abdominal quadrant, which includes the head of the pancreas, the superior and descending portion of the duodenum, and the associated lymph nodes (gastrinoma triangle) (Stabile et al. 1984). Sporadic tumors were thought till recently more common to develop in the pancreas; however advances in endoscopy have revealed an increased incidence of duodenal gastrinomas that now account for 50–88 % in sporadic Zollinger–Ellison syndrome (ZES) patients. At the time of diagnosis, over 60 % of gastrinomas are metastatic, but in general, these are slowly progressive tumors with 10-year survival rate estimated at 90–100 % of patients with radical resection, 46 % with lymph node metastasis, and 40 % in patients with hepatic metastases. Pancreatic gastrinomas are larger in size and present liver metastases (22–35 %) more frequently than their duodenal counterparts (Jensen et al. 2006). Among ZES patients, 20–30 % of the cases present in the context of MEN-1 syndrome, and in such case they develop at an earlier age than sporadic (mean 32–35 years). Consequently, the diagnosis of a gastrinoma should be followed by screening for other components of MEN-1 syndrome (Anlauf et al. 2006).

The clinical symptoms result from gastric acid hypersecretion (ZES) and consist of abdominal pain from developing peptic ulcer disease (PUD) or gastroesophageal reflux disease, as well as diarrhea and malabsorption due to inactivation of pancreatic enzymes (Roy et al. 2000). Atypical or multiple ulcers strongly suggest the diagnosis even though the use of proton pump inhibitors (PPIs) has made this manifestation less frequent than in the past. Moreover, successful therapy with PPIs may mask the diagnosis, which is often delayed (Corleto et al. 2001; Banasch

and Schmitz 2007). ZES should be suspected in cases of recurrent, severe, or familial PUD; *H. pylori*-negative PUD without other risk factors (gastrotoxic drugs); PUD and hypergastrinemia concomitantly with prominent gastric folds; or PUD with hypercalcemia. Patients with MEN-1 should be screened for the presence of ZES as gastrinomas are quite frequent in this group (20–30 %) (Gibril et al. 2004).

Rare Functioning Pancreatic Neuroendocrine Tumors

The majority of patients with rare functioning pNETs have advanced disease at the time of diagnosis, and their prognosis is determined mainly by the size of the primary tumor. Five-year survival for these patients is 29–45 % (O'Toole et al. 2006).

Glucagonoma

Glucagonomas represent approximately 5 % of all pNETs and 8–13 % of functioning tumors. A male to female ratio of 0.8:1 with a mean age of 52.5 years (range 11–88) at diagnosis has been reported, while 80 % are already metastatic. The 10-year survival in patients without metastases is 64 % and in those with metastatic disease does not exceed 51 % (Soga and Yakuwa 1998a). Approximately 15 % of patients with glucagonomas have MEN-1. The pancreatic tail is the site where these tumors are more frequently found (Levy-Bohbot et al. 2004).

Glucagon excess is associated with catabolic action, and patients with glucagonomas present a distinct clinical syndrome (glucagonoma syndrome) consisting of diabetes mellitus, normochromic and normocytic anemia, and a predilection to venous thrombosis. The most characteristic clinical finding is necrolytic migratory erythema (NME), described in 65–80 % of the patients (Fig. 2). NME is a pruritic skin rash that usually begins in the groin and perineum, subsequently spreading to the extremities and complicated with secondary infections of the skin by *Candida* and *Staphylococcus aureus*. The cause of NME is still unknown (Kindmark et al. 2007). Interestingly, clinical cases of hyperglucagonemia and pancreatic α-cell hyperplasia/islet cell tumors that lack the manifestations of the relevant clinical syndrome have been reported. This entity has been attributed to inactivating mutations of the human glucagon receptor (Zhou et al. 2009).

VIPoma

These rare pancreatic tumors secreting vasoactive intestinal polypeptide (VIP) represent 3–8 % of all pNETs (O'Toole et al. 2006). Men and women are equally affected and the overall median age at diagnosis is 42 years (range 1–82 years). VIPomas are located mainly in the tail, followed by the head and the body. Apart from pancreatic VIPomas (75–80 %), exopancreatic neurogenic tumors located in the retroperitoneum and in the mediastinum have been reported. Approximately half of all VIPomas are metastatic, the majority affecting the liver. The 5-year survival is estimated at 94.4 % for patients without metastatic disease and 59.6 % for patients with metastases (Soga and Yakuwa 1998b).

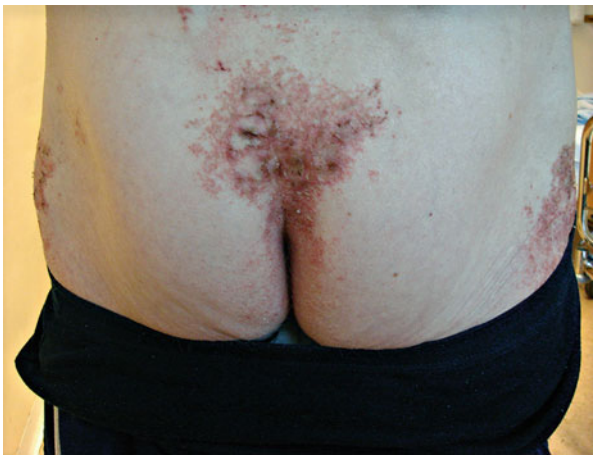

Fig. 2 Necrolytic migratory erythema in a patient with glucagonoma

VIP stimulates the secretion and inhibits the absorption of sodium, chloride, potassium, and water from the mucosa of the small bowel. It also stimulates bowel motility. These effects lead to the syndrome of diarrhea/hypokalemia/achlorhydria (watery diarrhea hypokalemia achlorhydria – WDHA) or Verner-Morrison syndrome. Diarrheas are devastating and can range from 0.5 to 15.0 l/day, with consequent dehydration.

Somatostatinoma

Pancreatic SSomas are usually large, with an average diameter of 5 cm, and show a predilection for the head of the pancreas. Duodenal SSomas have also been reported that tend to be smaller (O'Brien et al. 1993). The estimated annual incidence is less than 1 in 10 million people. Men and women are equally affected (Soga and Yakuwa 1999).

The clinical features of the SSoma syndrome are quite insidious and result from the inhibitory properties of somatostatin: hyperglycemia caused by insulin release inhibition, cholelithiasis as a result of decreased cholecystokinin release, and consequently diminished gallbladder contractility. Steatorrhea is also observed due to inhibition of pancreatic enzyme/bicarbonate secretion and diminished intestinal absorption. Finally, hypochlorhydria/achlorhydria is often caused by the inhibitory effect of SS on gastrin release and acid secretion (O'Toole et al. 2006).

Rare Ectopic Hormone Syndromes

Due to the pluripotent secretory capacity of neuroendocrine cells, pNETs may secrete substances not related to the endocrine pancreas (ectopic secretion), leading to distinct clinical syndromes (Kaltsas et al. 2010). The clinical syndrome may herald the presence of the neoplasm and lead to early diagnosis; however, it may sometimes dominate the clinical picture and confuse the clinician. About 10–20 % of total cases presenting with Cushing's syndrome (CS) are due to ectopic secretion of ACTH (Newell-Price et al. 2006). Moreover, CS occurs in 5 % of cases with

sporadic ZES (Maton et al. 1986). Similarly, acromegaly has been reported in patients with pNETs, being the second most frequent cause of paraneoplastic syndrome related to acromegaly after lung carcinoids (Gola et al. 2006). In this setting, the humoral syndrome is mainly related to GHRH hypersecretion rather than to GH itself (Biermasz et al. 2007). There are also case reports in the literature of humoral hypercalcemia of malignancy due to PTH-related peptide and syndrome of inappropriate antidiuretic hormone secretion (Kanakis et al. 2012).

Neuroendocrine Carcinomas

NECs are highly malignant epithelial neoplasms that constitute approximately 2–3 % of pNETs. By means of morphology, they can be divided in small- and large-cell variants based on the criteria used for the classification of the pulmonary counterparts (Travis et al. 2004). The tumors are already large at diagnosis, with an average diameter of 4 cm, and present frequently metastases involving the liver and regional lymph nodes, as well as distant organs. The prognosis is poor, with survival times ranging from 1 month to up to approximately 1 year (Nilsson et al. 2006).

Biomarkers

Several serum tumor markers are used for the diagnosis and management of pNETs, and they are divided in general (nonspecific that are found in all NETs) and tumor-specific markers (O'Toole et al. 2009; Oberg 2011).

General Biomarkers

The most widely used general markers of neuroendocrine differentiation are CgA, PP, and NSE. CgA is currently the most useful general biomarker available for the diagnosis of pNETs as it is co-secreted by the majority of neuroendocrine cells and persists after malignant transformation. The overall sensitivity of the assay ranges from 67 % to 93 % (Stridsberg et al. 2003). However, there are conditions in which CgA measurements could be misleading: the most common being those associated with achlorhydria. Chronic atrophic gastritis (type A), and treatment with antisecretory medications, especially PPIs cause falsely elevated CgA levels. Therefore, proper discontinuation of such agents should be applied prior to CgA assessment (Marotta et al. 2012). Apart from diagnosis, CgA serves as a marker for the evaluation of the response to therapy of patients with liver metastases as it correlates well with tumor burden (Massironi et al. 2010).

PP is a 36 amino acid linear oligopeptide mostly useful in the diagnosis of NF-pNETs. It is often elevated in metastatic disease and has an overall sensitivity of about 50–80 % (Lonovics et al. 1981). The diagnostic accuracy is further improved by combining serum CgA and PP levels offering an increase in sensitivity to 90 %

(Walter et al. 2012). In order to maximize the sensitivity of PP assay in the diagnosis of pNETs, a specific meal stimulatory test (mixed meal) has been developed; however its value has been challenged in recent guidelines (Langer et al. 2001; O'Toole et al. 2009). The assessment of PP may be particularly useful for early detection of pancreatic tumors in MEN-1 patients. NSE is elevated in 30–50 % of patients with pNETs, particularly those of poor differentiation. In such patients, NSE might be elevated despite normal CgA levels (Nobels et al. 1997).

Tumor-Specific Biomarkers

To establish the diagnosis of functioning pNETs, inappropriate elevation of the relevant, tumor-specific marker (i.e., gastrin in ZES) should be sought, as well as clinical/laboratory evidence of the relevant clinical syndrome (e.g., high gastric output).

Insulinoma

In order to diagnose insulinomas, the presence of endogenous hyperinsulinemia (EH) (e.g., inappropriately high levels of insulin) during hypoglycemia should be demonstrated (Jensen et al. 2012). This can be established using the following criteria:

- Blood glucose level $\leq$2.2 mmol/l ($\leq$40 mg/dl)
- Concomitant serum insulin level $\geq$6 μU/l ($\geq$36 pmol/l) or $\geq$3 U/l by ICMA
- Plasma/serum C-peptide level $\geq$200 pmol/l
- Serum proinsulin level of at least 5 pmol/l
- Plasma β-hydroxybutyrate levels $\leq$2.7 mmol/l
- Absence of sulfonylurea (metabolites) in patient's plasma and/or urine

These might be documented during a spontaneous episode, providing that the triad suggested by Whipple is fulfilled: (1) symptoms of hypoglycemia, (2) blood glucose $\leq$2.2 mmol/l ($\leq$40 mg/dl), and (3) relief of symptoms with administration of glucose (Service 1995). The diagnosis is supported by the absence of plasma/urine ketones during fasting as a consequence of suppression of β-oxidation by endogenous hyperinsulinemia. Furthermore, exogenous insulin administration or sulfonylurea abuse should be excluded. The work-up is complete with the measurement of proinsulin that might reveal insulinomas that secrete immature forms of insulin due to defective hormone processing (Kao et al. 1994).

In case of difficulty to document such episodes, the patient should undergo fasting for 72 h. This test is considered the gold standard in the diagnosis of insulinomas with sensitivity reaching 100 %. The test should be ended when the patient develops symptoms and plasma glucose levels are $\leq$2.2 mmol/l ($\leq$40 mg/dl) or when the 72 h are completed (Service and Natt 2000). Usually, insulinoma patients develop hypoglycemia early after fasting (one third of patients at 12 h and 80 % within 24 h). The assessment of the results relies on the criteria mentioned for spontaneous hypoglycemia. If the results are still equivocal, 1 mg of glucagon is administered immediately after the 72-h fasting. In healthy patients

glucose levels after glucagon administration should not rise more than 25 mg/dl above basal due to depletion of hepatic glycogen stores (Hoff and Vassilopoulou-Sellin 1998). An alternative diagnostic test is the calcium infusion test, during which hypoglycemia is achieved 2 h after intravenous calcium infusion.

Gastrinoma

The diagnosis of gastrinoma is set by elevated fasting gastrin levels in the setting of high gastric acid output (Arnold 2007). The criteria used to establish the diagnosis are:

- Gastric juice pH <2.0
- Fasting serum gastrin (FSG) > tenfold the upper limit of normal (ULN)

In cases of marginal FSG values (two to tenfold ULN), performing a secretin test and assessment of basal acid output (BAO) is suggested. During the test, 2 U/kg secretin is given as an intravenous bolus after overnight fasting, and serum gastrin is measured before and 2, 5, 10, 15, 20, and 30 min after infusion. A delta gastrin >120 pg/ml any time during the test is indicative of the presence of a gastrinoma with a sensitivity of 94 %. If the secretin test is negative but the suspicion for the presence of ZES remains high, the calcium provocation test may be helpful as it may be positive in 5–10 % of such cases (Frucht et al. 1989). An alternative diagnostic test is the glucagon provocative test, during which glucagon (20 mg/kg) is given intravenously, followed by 20 mg/kg/h for the next 30 min. A gastrinoma is suggested if plasma gastrin levels peak within 10 min after glucagon administration, with an increase of greater than 200 pg/ml and greater than 35 % of the basal value (Shibata et al. 2012). Regarding BAO, a rate of more than 15 mmol/h is highly suggestive of ZES (O'Toole et al. 2009).

Elevated FSG is not specific for ZES, as it can be encountered in other conditions with hyperchlorhydria as in *H. pylori*-positive gastritis, gastric outlet obstruction, renal insufficiency, and antral G-cell syndromes. Moreover, elevated FSG can also be found in states of achlorhydria, such as atrophic gastritis, and chronic PPI use (Rehfeld et al. 2012). Consequently, PPIs should be interrupted at least 1 week prior to FSG testing and replaced for this period by histamine receptor 2 (H2) blockers, which in turn have to be discontinued 48 h before the test (Banasch and Schmitz 2007).

Rare Functioning Tumors

In patients with rare functioning tumors, the diagnosis can be established by demonstrating elevated levels of the relevant hormone combined with general markers of neuroendocrine differentiation (O'Toole et al. 2006). In glucagonomas, fasting glucagon levels are usually increased 10– 20-fold. In the rare case of equivocal values, tolbutamide or arginine stimulation test may be used to establish the diagnosis. In VIPomas, levels of VIP exceeding 60 pmol/L are diagnostic. Similarly, diagnosis of SSomas should be based on high SS levels; however, SS assays are cumbersome and not routinely available. The diagnosis of ectopic Cushing's syndrome comprises initially the documentation of hypercortisolemia and afterward the detection of its source. Once the diagnosis is established, ACTH or CRH may serve as follow-up markers (O'Toole et al. 2009).

Localization

The morphology and imaging properties of different pNETs vary according to the specific tumor type. Thus, there is no general consensus regarding the most appropriate imaging modality for the detection of pNETs. ENETS has recently proposed an algorithm for the localization and staging of NF-pNETs implicating abdominal ultrasound (US), abdominal computerized tomography (CT), magnetic resonance imaging (MRI), somatostatin receptor scintigraphy (SRS), positron emission tomography (PET), and endoscopic/intraoperative US (EUS/IOUS) (Sundin et al. 2009; Falconi et al. 2012). The technique which proves to depict better the individual tumor should be preferred for follow-up.

Ultrasonography

Abdominal US (B-mode) is a widely available, low-cost modality, which however has a low detection rate for pNETs that do not exceed 40 %. Besides, it is an operator-sensitive modality. Several techniques have been developed to improve the diagnostic accuracy of US (Sundin et al. 2009):

Contrast-enhanced ultrasonography (CEUS) allows the depiction of the micro-/macrovasculature and enhancement patterns of pNETs. A correlation between CEUS enhancement pattern and the Ki67 index has been reported (D'Onofrio et al. 2004; Malago et al. 2009).

EUS has enhanced sensitivity (overall >90 %), especially for small pancreatic lesions (even smaller than 0.5 cm), due to the proximity between the transducer and the pancreas (Fig. 3). pNETs are generally depicted as round lesions with well-defined margins that tend to be hypoechoic, but they may also be isoechoic or anechoic. The vascular nature of these tumors can be appreciated using the color Doppler. In addition, EUS can estimate the distance between the tumor and the pancreatic duct, which is essential when deciding if tumor enucleation is feasible.

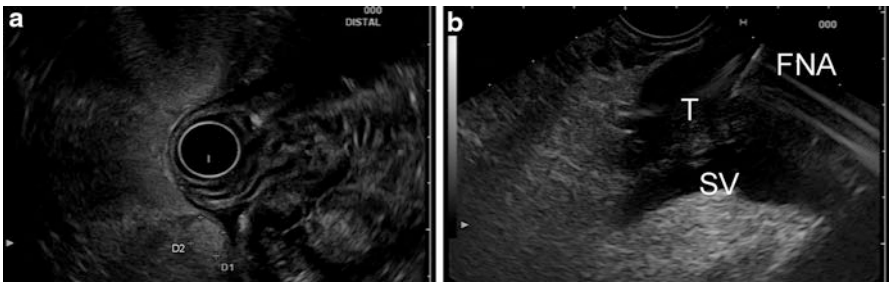

Fig. 3 (**a**) Endoscopic ultrasound of an insulinoma located at the tail of the pancreas. The tumor is isoechoic and measures D1:9 × D2:7 mm. (**b**) Fine needle aspiration (FNA) under endoscopic ultrasound guidance of a nonfunctioning pancreatic neuroendocrine tumor (T). The tumor is anechoic and in close proximity to the splenic vein (SV)

Thus, EUS is the modality of choice for small insulinomas that cannot be detected otherwise.

Furthermore, preoperative EUS-guided injection of India ink has been shown to aid in intraoperative localization of insulinomas. Nevertheless, EUS's accuracy is decreased for tumors located at the pancreatic tail (McLean and Fairclough 2005).

IOUS, when combined with palpation of the pancreas, has sensitivity rates that reach 98 %. This technique is essential in MEN-1 patients as it allows the detection of small multiple pNETs or duodenal tumors and depending on the findings may alter the surgical procedure (Norton et al. 1988; Jensen et al. 2008).

Computerized Tomography and Magnetic Resonance Imaging

Abdominal CT usually depicts pNETs as lesions isodense to the surrounding pancreatic parenchyma and can be distinguished by the strong enhancement they show after the infusion of contrast medium (Nino-Murcia et al. 2003). This is a result of the increased vascularity that characterizes such lesions and is better observed during the arterial phase images. The presence of calcification, invasion of adjacent organs, and central necrosis all suggest malignancy. In order to reveal possible liver metastases, the combination of arterial phase with portal venous phase is recommended (Ichikawa et al. 2000). The sensitivity and specificity of CT scan in the diagnosis of pNETs are 73 % and 96 %, respectively (Sundin et al. 2009).

MRI has similar sensitivity rates (85–94 %). pNETs show low signal intensity on T1-weighted images and high signal on T2-weighted images and also exhibit strong enhancement after infusion of paramagnetic contrasts. Fat-suppressed T1-weighted images are preferred for detecting pancreatic lesions, whereas T2-weighted images are useful in distinguishing pNETs from adenocarcinomas that have low signal (Owen et al. 2001).

Radionuclear Imaging

SRS is a functional imaging technique, using the ability of somatostatin analogs (SSAs) to bind to somatostatin receptor (SSTR) subtypes. Given the fact that 80–90 % of NETs express SSTRs (Zamora et al. 2010), the sensitivity and specificity rates of SRS for pNETs are similarly high (90 % and 80 %, respectively) (Lamberts et al. 1992). An exception is benign insulinomas that do not sufficiently express SSTR2, the main target of SRS, and the corresponding sensitivity rates fall to 50–60 % (Portela-Gomes et al. 2000). Interestingly, insulinomas have been shown to overexpress glucagon-like peptide (GLP) receptors; therefore, radiolabeled GLP-1 analogs could serve for depicting such tumors (Christ et al. 2009). Eventually, SRS is the modality of choice for staging of pNETs, especially for the detection of extrahepatic metastases, since it allows whole-body imaging. Routine

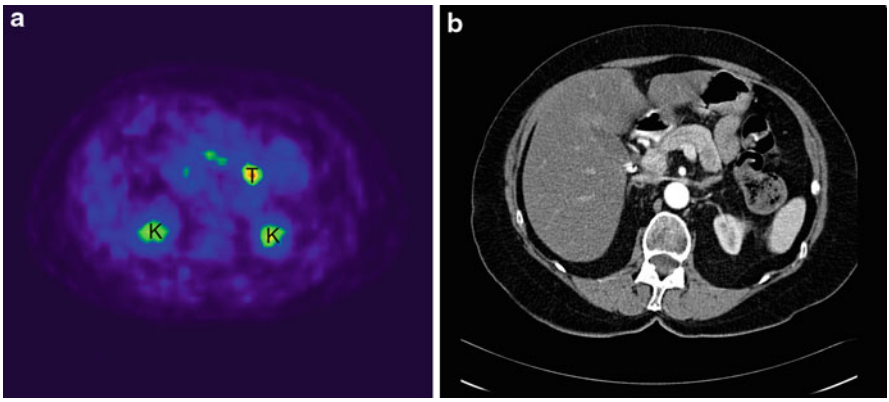

Fig. 4 (**a**) Positron emission tomography using ^{11}C-5-hydroxy-L-tryptophan as a tracer (transaxial view). Distinct uptake by the tumor (T) localized in cauda pancreatis and normal uptake by the kidneys (K) are evident. (**b**) The corresponding computerized tomography scan (arterial phase) did not depict the tumor

SRS should not be employed if other imaging studies for NETs are negative. The most commonly used tracer is 111In-DTPA-octreotide.

PET is a more recent technique with better spatial resolution than scintigraphy that allows the quantification of tracer uptake. Several specific tracers have been used for the detection of pNETs, among which the 68Ga-labeled SSA DOTA-D-Phe1-Tyr3-octreotide (DOTATOC) is the most widely employed (Buchmann et al. 2007). ^{11}C-5-hydroxy-L-tryptophan (5-HTP) (Fig. 4) seems to have the optimal properties for staging pNETs; however it is not widely available (Orlefors et al. 2005). Standard PET with ^{18}F-labeled deoxyglucose (FDG) depicts tumors with rapid proliferation; thus it may not be accurate in detecting well-differentiated pNETs. However, it is useful in the detection of aggressive poorly differentiated tumors with a proliferation index around or greater than 15 % (Pasquali et al. 1998).

Angiography

This is an invasive technique that allows visualization of pNETs due to their increased vascularity, especially during the early arterial phase (Jackson 2005). However, it has a detection rate of approximately 60 %, being superseded by noninvasive cross-sectional imaging techniques (MRI/CT). Thus, the main utility of this technique is to determine the localization of multiple gastrinomas/insulinomas in MEN-1 patients by combining angiography with hepatic venous sampling for the relevant hormone after intra-arterial secretin/calcium administration. In such case, the diagnostic accuracy reaches 88–100 % (Doppman et al. 1995).

Diagnostic Procedures

Tissue diagnosis is the cornerstone of designing the clinical management strategy and predicting the outcome of an underlying malignancy. Several procedures of tissue sampling have been developed and applied in pancreatic tumors. In general, if the pancreatic lesion is clearly resectable and the patient is fit for surgery, it is suggested not to perform an attempt to obtain neoplastic tissue preoperatively. In that way the possible risk of needle tract seeding is avoided. Imaging-guided fine needle aspiration and core needle biopsy CT and abdominal US may be used to guide percutaneous pancreatic fine needle aspiration (FNA). FNA or core needle biopsy (CNB) reaches a sensitivity and accuracy of 93.9 % and 94.4 %, respectively (Paulsen et al. 2006). CNB may have an advantage over FNA, in which false-positive results have been reported; however it has been associated with higher complication rates. The initial enthusiasm over the use of percutaneous FNA has been decreased as a result of needle tract seeding and the development of EUS FNA.

EUS-Guided Fine Needle Aspiration and Core Needle Biopsy

With the guidance of EUS, it is possible to obtain cytologic material from pancreatic or liver lesions via FNA (Fig. 3b), and the results have been shown to be superior to conventional computerized tomography-guided FNA (Horwhat et al. 2006; Chatzipantelis et al. 2008). EUS FNA is performed using a linear array echoendoscope, while the patient is under conscious sedation and cardiorespiratory monitoring. Apart from diagnosis, FNA can provide prognostic information by the estimation of the Ki-67 proliferation index; however an experienced cytopathologist may be needed and the results are still equivocal (Piani et al. 2008; Kaklamanos et al. 2011). The diagnostic yield of EUS may be increased by employing CNB instead of FNA; however the safety profile of CNB is yet to be established (Thomas et al. 2009).

Treatment

Surgery

Complete surgical resection represents the only potential curative therapy for pNETs, providing that the diagnosis has been set at early stages (Hill et al. 2009). The extent of the surgery depends on the biological behavior (grading), the metastatic extent (staging), the secretory status (functioning or not), and the possible presence of the tumor in the context of a hereditary syndrome.

The type of resection of the primary pNETs depends largely on the location of the tumor, the tumor size, as well as the histological type. For any tumor larger than

2 cm, a standard pancreatic resection should be preferred: pancreaticoduodenectomy for tumors located at the head and distal pancreatectomy with or without spleen preservation for lesions of the body and tail (Falconi et al. 2010). For smaller well-differentiated tumors with clear margins, parenchyma-sparing procedures may be an option such as middle pancreatectomy for tumors of the body. For small tumors located no closer than 2–3 mm from the pancreatic duct, especially insulinomas, enucleation might be considered.

Laparoscopic removal of pNETs, either by resection or enucleation, is a surgical option that is associated with less morbidity and mortality than standard resections. The overall surgical morbidity rate ranges from 31 % to 42 %, with pancreatic fistula being the most common complication (Fernández-Cruz et al. 2008). Although laparoscopic tumor enucleation is a parenchyma-sparing procedure, it is associated with a higher rate of development of pancreatic fistulas. It is thus recommended that laparoscopic tumor enucleation may be reserved for tumors of the pancreatic head that are considered noninvasive, whereas tumors of the pancreatic body and/or tail should be treated with laparoscopic resection (Haugvik et al. 2013). The results between G1 and G2 tumors are comparable; however it should be noted that in case of malignant tumors, laparoscopic techniques cannot ensure complete lymphadenectomy and negative surgical margins (Al-Taan et al. 2010).

Gastrinomas should undergo resection even in the case of metastatic tumors as debulking is helpful for the control of hormone secretion (Lorenz and Dralle 2007). The general properties mentioned above for pNET surgery should be followed. Additionally, duodenotomy with transillumination should be routinely performed to detect small duodenal tumors as liver exploration and regional lymphadenectomy to exclude abdominal metastases (Norton et al. 2004). Consequently, laparoscopic surgery is not indicated for this type of tumors.

Insulinomas, on the other hand, due to their excellent prognosis are treated laparoscopically with parenchyma-sparing techniques (Richards et al. 2011). No standard lymphadenectomy is recommended. In case the tumor cannot be localized preoperatively, a laparotomy is indicated as well as pancreas exploration by both palpation and IOUS (Espana-Gomez et al. 2009). If this is also negative, intraoperative insulin sampling may be helpful (Gimm et al. 2007).

MEN-1 patients represent a distinct subgroup of patients with pNETs whose pancreas may harbor multiple tumors; therefore, careful intraoperative exploration of the pancreas is mandatory (Anlauf et al. 2006). Regarding NF-pNETs, all tumors accompanied by metastases, larger than 2 cm, and presenting a yearly increased size $>$0.5 cm should be resected (Triponez et al. 2006). When multiple tumors are found throughout the pancreas, a reasonable approach is distal subtotal pancreatectomy and enucleation of the tumors located in the head. Tumors smaller than 2 cm show a malignant potential less than 6 %, and their excision is debated; an intensive follow-up is recommended instead (Kouvaraki et al. 2006). Regarding gastrinomas, pancreaticoduodenectomy is the treatment of choice; otherwise the risk of recurrence is high (Fendrich et al. 2007). If insulin hypersecretion is detected in association with multiple pancreatic tumors, it is mandatory to define which of

the tumors are insulinomas (Norton et al. 2006). This can be obtained preoperatively with venous sampling following arterial calcium stimulation or with intraoperative insulin sampling. Tumor resection follows the gradient indicated by these procedures (Doppman et al. 1995; Gimm et al. 2007).

In the setting of locally advanced or metastatic disease, the minimal requirements of the surgery include the resection of the primary tumor, the regional lymph nodes, and/or regional and distant metastases, providing that at least 90 % of the disease is resectable (Joseph et al. 2011). This is particularly important in the case of functioning tumors since reducing the tumor burden ameliorates the hormonal syndrome (Jensen et al. 2012). On the contrary, debulking is debated in the case of NF tumors, unless surgery is indicated for compassionate reasons to treat acute bleeding, obstruction, or perforation of the adjacent organs. In all cases a standard pancreatectomy is performed. Conditions that may exclude a patient from surgery are the invasion of portal vein and the entrapment of superior mesenteric artery (Boudreaux 2011).

Particularly for metastatic liver disease, ENETS has proposed guidelines based on the magnitude of liver involvement (Steinmuller et al. 2008): for localized disease involving one or two contiguous segments, an anatomic resection is adequate, whereas for "complex" disease where the contralateral lobe is involved, a multistep approach is recommended. Initially, the primary tumor is resected along with part of liver metastases, and a second operation is performed after adequate restoration of the liver parenchyma has occurred (Kianmanesh et al. 2008). Patients with well-differentiated (G1 or G2) tumors when treated with hepatic resection exhibit 5-year survival rates of 47–76 % compared to 30–40 % of untreated patients, providing that no extra-abdominal metastases are present (Sarmiento et al. 2003). The most advanced stage of liver involvement is the "diffuse" pattern, where more than 75 % of the liver, including both lobes, is affected. In such case surgery is not recommended and other cytoreductive therapies should be sought (Steinmuller et al. 2008).

Cytoreductive Therapies of Liver Disease

Radiofrequency Ablation

In patients with unresectable liver disease, radiofrequency ablation (RFA) is an option, providing that the lesions do not exceed 5 cm in diameter (Gillams et al. 2005). The heat produced locally by high-frequency alternative current destroys malignant cells and the transducer can be inserted percutaneously or laparoscopically, depending on the lesions' location. Furthermore, RFA may serve as neoadjuvant therapy, in order to convert an unresectable disease into a resectable one (Ahlman et al. 2000).

Embolization, Chemoembolization, and Radioembolization

Selective embolization of branches of the hepatic arteries with particles (microspheres) is an alternative ablation modality for patients with unresectable liver

disease, particularly if the lesions are not susceptible to RFA. This technique is based on the property that most of the tumor vasculature originates from the hepatic arteries, whereas the normal parenchyma is irrigated mainly from the portal vein system (Gupta et al. 2005). Nevertheless, liver function enzymes should be monitored closely after the embolization, as necrosis of great areas of the liver may occur.

Chemoembolization (transarterial chemoembolization/TACE) is a variation of this technique where chemotherapeutic drugs (5-FU, doxorubicin, and mitomycin C) are used instead of particles (Drougas et al. 1998). There are no data comparing neither the efficacy of transarterial embolization and TACE in pNETs nor guidelines regarding the regiment of drugs and dosages administered. The most recent achievement in this type of therapy is radioembolization with the use of radioactive yttrium-90 microspheres, a β-emitter with long-range tissue penetration of up to 11 mm which is as effective as and potentially less toxic than TACE (Kalinowski et al. 2009).

Liver Transplantation

An alternative option in patients with diffuse liver involvement may be orthotopic liver transplantation (OLT); however strict criteria should be set as high recurrence rates (up to 76 %) have been reported (Lehnert 1998). Moreover, the use of immunosuppressive drugs required after OLT may even accelerate the development of new metastases at the transplanted organ or elsewhere. Thus, patients younger than 50 year without extra-abdominal disease are generally preferred, while some centers may not accept patients with liver involvement exceeding 50 % (Olausson et al. 2002). Additionally, patients with poorly differentiated tumors are not good candidates due to their dismal prognosis. A proliferation index of Ki-67 less than 5 % is suggested (Rosenau et al. 2002).

Medical Treatment

Biotherapy

SSAs and interferon α (IFNα) have been both used successfully in the control of symptoms in functioning pNETs (Oberg et al. 2010).

Currently available SSAs (octreotide and lanreotide) target SSTRs 2, 3, and 5. Octreotide controls diarrhea in over 75 % of patients with VIPomas and reduces VIP concentrations in 80 % of the patients. Similarly it cures NME in 30 % of cases and reduces circulating glucagon in 80 % (Maton 1993). The corresponding rates for biochemical response in insulinomas are somewhat lower, since these tumors do not express SSTR2 avidly (Vezzosi et al. 2005). Interestingly, the symptomatic relief caused by SSAs is not always accompanied by a reduction in circulating hormone levels, implying a direct effect of these compounds on the peripheral target organ. However, in some cases, an escape from the initial response occurs that is attributed to tachyphylaxis and managed by increasing the therapeutic dose (Oberg et al. 2010).

Regarding metastatic NF-pNETs, a single center phase 4 study using octreotide acetate LAR as a first-line treatment resulted in stable disease in 38 % of the patients after a median follow-up of 49.5 months. Sixty-two percent of the patients developed disease progression after a median follow-up of 18 months (Butturini et al. 2006). The authors concluded that a Ki67 index >5 % may justify more aggressive therapy without waiting for radiological progression of the disease. Apart from their antisecretory action, SSAs were also considered to have an antiproliferative effect; however hitherto this has been demonstrated only for well-differentiated small intestine NETs (PROMID study) (Rinke et al. 2009). This benefit was mostly evident in patients who had less than 10 % tumor liver disease and in those in whom the primary tumor was removed. Moreover, a novel multivalent SSA has been developed, SOM 230 (pasireotide) that has high affinity for all SSTRs except SSTR4 and has been shown to control the symptoms of carcinoid syndrome in 25 % of patients resistant to octreotide (Schmid and Schoeffter 2004). A trial assessing the antiproliferative effects of SOM-230 in gastroenteropancreatic NETs is currently conducted. Furthermore, an ongoing phase 3 study of lanreotide (NCT00353496) in patients with NF-NETs is enrolling patients with pNETs and may provide more information about the antitumor efficacy of SSAs in pNETs.

IFNa is an alternative therapeutic choice that has been shown to elicit an objective biochemical response in 77 %, symptomatic response in 40–60 %, and a reduction of tumor size in 10–15 % of patients with advanced pNETs (Fazio et al. 2007). It can also be added to chemotherapy regiments; however, it seems to be less effective than SSAs and with more side effects.

Chemotherapy

Patients with inoperable metastatic disease and G2 tumors are candidates for systemic chemotherapy. Till recently, the only licensed chemotherapy for well-differentiated pNETs was streptozotocin (STZ) in combination with doxorubicin (DOX) and/or 5-fluorouracil (5-FU), based on the results reported by Moertel et al. (1980). In more recent studies, the combination of STZ and 5-FU proved to be more effective than STZ and DOX with less serious adverse effects (Moertel et al. 1992). The response rates of the above combinations range from 30 % to 40 %. Regarding G3 tumors, the therapy is similar to that used in small-cell lung cancer, based on the combination of etoposide (VpI6) with cisplatin (CDDP) (Moertel et al. 1991).

In addition to registered therapies, several other compounds have been used in the treatment of pNETs either as monotherapy or as combinations (Eriksson 2010). Temozolomide (TMZ), the active metabolite of dacarbazine taken orally, was reported to elicit a partial response of 8 % and stabilize the disease in 67 % of patients with pNETs resistant to other chemotherapeutic regimens (Ekeblad et al. 2007). In this study the expression of O-6-methylguanine DNA methyltransferase (MGMT) was a negative predictive factor for the response to therapy. In another study of TMZ in patients with progressive pNETs, responses up to 53 % were reported; however, response was not related to MGMT expression

(Kulke et al. 2006). Based on in vitro data, TMZ has been used in combinations with capecitabine and thalidomide, achieving impressive response rates as high as 45 % and 74 %, respectively (Strosberg et al. 2010).

Novel Molecular Therapies

Bevacizumab is a monoclonal antibody that targets vascular endothelial growth factor and is used in combination with other chemotherapeutic agents in the treatment of pNETs (Yao 2007). Combined with TMZ partial response up to 24 % has been reported, whereas a randomized phase III trial comparing octreotide plus IFNα or bevacizumab in advanced gastroenteropancreatic NETs is ongoing (Yao et al. 2008b).

Sunitinib is a tyrosine kinase inhibitor showing antiproliferative and anti-angiogenic properties. It was recently licensed for the treatment of advanced pNETs based on the results of a phase III study (sunitinib 37.5 mg/d vs. placebo) that demonstrated a significantly higher progression-free survival for the patients treated with sunitinib (Raymond et al. 2011). Regarding the safety profile of this drug, grade 3 and 4 neutropenia, hypertension, hand-foot syndrome, abdominal pain, diarrhea, and fatigue have been reported. Nevertheless, as yet sunitinib should not be considered as a first-line therapy, but only in selected cases.

An alternative treatment option for pNETs progressing on conventional regimens is everolimus, an inhibitor of the mammalian target of rapamycin (mTOR) pathway. The RADIANT-3 study, a phase III randomized trial of octreotide plus everolimus 10 mg or placebo in patient with metastatic pNETs, showed promising results with increased progression-free survival and relatively minor side effects (Yao et al. 2011). These results were equally positive in treatment naïve, as well as in patients already treated with other agents. Thus everolimus could be used as first-line therapy in selected cases.

Radiolabeled Somatostatin Analogs and Peptide Receptor Radionuclide Therapy

Radiolabeled SSAs use high-energy β-emitters including indium 111, yttrium 90, and lutetium 177 (a combined β and γ emitter) in order to stop the growth of SSTR2 baring tumor cells (Kaltsas et al. 2004a; Kwekkeboom et al. 2005; Kalinowski et al. 2009). Thus, the expression of SSTR2 should be sought by means of SRS, before the use of PPRT. PRRT is applicable for both functioning and NF-pNETs, and the most promising results refer to 177Lu-DOTA0,Tyr3 for which partial remission rates up to 33 % have been reported. The most important side effects are related to hematological and renal toxicity (Kwekkeboom et al. 2008). Nevertheless, PRRT should not be used as first-line therapy, but after failure of medical therapy.

External-Beam Radiotherapy

External-beam radiotherapy treatment has been considered ineffective for pNETs, except for patients with bone and brain metastases (Alexandraki and Kaltsas 2012).

Supportive Therapy

Occasionally, patients with advanced disease, particularly with functioning tumors, become refractory to the effect of previously mentioned treatment alternatives. In such cases the administration of supportive therapy is mandatory.

In patients with insulinomas, frequent small meals can be used for small periods (e.g., preoperatively) to prevent prolonged fasting. If hypoglycemia symptoms are more frequent, diazoxide that inhibits the release of insulin may be helpful. In refractory cases glucocorticoids and intravenous infusion of glucose may support to ameliorate hypoglycemia (Service 1995). In case of malignant insulinomas, mTOR inhibitors (everolimus) may be an option as besides their antitumoral action they have been shown to impede insulin secretion and thus control hypoglycemia (Kulke et al. 2009).

Regarding gastrinomas, the employment of PPIs in the therapy of ZES has almost changed the natural history of the disease, as they are very effective in the treatment of PUD, as yet no resistance or tachyphylaxis has been reported and the safety profile is quite favorable (Banasch and Schmitz 2007). In MEN1/ZES patients, the correction of primary hyperparathyroidism can ameliorate gastrin secretion and facilitate the control of ZES.

In patients with VIPomas, fluid and electrolytes should be monitored and replaced appropriately due to gross intestinal losses. Medical agents reducing intestinal motility such as loperamide and in advanced stages morphine may also be helpful. Glucagonoma patients may suffer from hyperglycemia and antidiabetic drugs should be then employed (Jensen et al. 2012). Patients with CS should promptly receive adrenostatic (ketoconazole, metyrapone) or adrenolytic agents (mitotane). Newer agents, such as the novel SSA pasireotide and glucocorticoid receptor antagonists, like mifepristone, have shown promising results. In refractory metastatic disease, bilateral adrenalectomy should be considered (Feelders et al. 2010).

Treatment Algorithm

Since the primary treatment goal in pNETs is the eradication of the neoplastic cells, surgery is still the first-line therapy. In patients who present metastases and when curative surgery is not feasible, debulking surgery with additional measures is suggested, in order to achieve symptom relief and suppress further tumor progression. If the disease is confined locoregionally and involves a limited part of the liver, cytoreductive therapies like RFA and TACE are usually sufficient to reduce tumor burden. By contrast, in patients presenting disseminated disease or when patients are unfit for surgery, medical management with systematically administered agents is required. SSAs or chemotherapy represents the first-line medical treatment. In cases of metastatic well-differentiated pNETs (particularly those with a Ki67 proliferation index <5 %), the tumors may respond to biotherapy (usually disease stabilization) with SSAs or IFNα. Biotherapy can also improve the symptoms of secretory syndromes. Using the same criteria as for SSA treatment, systemic chemotherapy is used, which may be combined with biotherapy if needed

(e.g., functioning tumors). If the above regimens are not effective, a treatment among everolimus, receptor tyrosine kinase inhibitors, other anti-angiogenic factors, or PRRT should be sought. In patients with poorly differentiated tumors, surgical intervention is usually obsolete and cytotoxic chemotherapy should be the initial choice. The above algorithm is usually adjusted depending on the expertise and possible available treatment in the respective NET center.

Cross-References

- ▶ Basement Membrane in Pancreatic Islet Function
- ▶ Microscopic Anatomy of the Human Islet of Langerhans
- ▶ Regulation of Pancreatic Islet Formation
- ▶ Stem Cells in Pancreatic Islets

References

Ahlman H, Wangberg B et al (2000) Interventional treatment of gastrointestinal neuroendocrine tumours. Digestion 62(Suppl 1):59–68

Alexandraki KI, Kaltsas G (2012) Gastroenteropancreatic neuroendocrine tumors: new insights in the diagnosis and therapy. Endocrine 41(1):40–52

Al-Taan OS, Stephenson JA et al (2010) Laparoscopic pancreatic surgery: a review of present results and future prospects. HPB (Oxford) 12(4):239–243

Anlauf M, Garbrecht N et al (2006) Sporadic versus hereditary gastrinomas of the duodenum and pancreas: distinct clinico-pathological and epidemiological features. World J Gastroenterol 12 (34):5440–5446

Arnold R (2007) Diagnosis and differential diagnosis of hypergastrinemia. Wien Klin Wochenschr 119(19–20):564–569

Banasch M, Schmitz F (2007) Diagnosis and treatment of gastrinoma in the era of proton pump inhibitors. Wien Klin Wochenschr 119(19–20):573–578

Biermasz NR, Smit JW et al (2007) Acromegaly caused by growth hormone-releasing hormone-producing tumors: long-term observational studies in three patients. Pituitary 10(3):237–249

Bosman F, Carneiro F et al (eds) (2010) WHO classification of tumours of the digestive system. IARC Press, Lyon

Boudreaux JP (2011) Surgery for gastroenteropancreatic neuroendocrine tumors (GEPNETS). Endocrinol Metab Clin North Am 40(1):163–171, ix

Buchmann I, Henze M et al (2007) Comparison of 68Ga-DOTATOC PET and 111In-DTPAOC (Octreoscan) SPECT in patients with neuroendocrine tumours. Eur J Nucl Med Mol Imaging 34(10):1617–1626

Butturini G, Bettini R et al (2006) Predictive factors of efficacy of the somatostatin analogue octreotide as first line therapy for advanced pancreatic endocrine carcinoma. Endocr Relat Cancer 13(4):1213–1221

Chatzipantelis P, Salla C et al (2008) Endoscopic ultrasound-guided fine-needle aspiration cytology of pancreatic neuroendocrine tumors: a study of 48 cases. Cancer 114(4):255–262

Christ E, Wild D et al (2009) Glucagon-like peptide-1 receptor imaging for localization of insulinomas. J Clin Endocrinol Metab 94(11):4398–4405

Chung DC, Smith AP et al (1997) A novel pancreatic endocrine tumor suppressor gene locus on chromosome 3p with clinical prognostic implications. J Clin Invest 100(2):404–410

Corleto VD, Annibale B et al (2001) Does the widespread use of proton pump inhibitors mask, complicate and/or delay the diagnosis of Zollinger-Ellison syndrome? Aliment Pharmacol Ther 15(10):1555–1561

D'Onofrio M, Mansueto G et al (2004) Neuroendocrine pancreatic tumor: value of contrast enhanced ultrasonography. Abdom Imaging 29(2):246–258

de Herder WW, Niederle B et al (2006) Well-differentiated pancreatic tumor/carcinoma: insulinoma. Neuroendocrinology 84(3):183–188

Doppman JL, Chang R et al (1995) Localization of insulinomas to regions of the pancreas by intra-arterial stimulation with calcium. Ann Intern Med 123(4):269–273

Drougas JG, Anthony LB et al (1998) Hepatic artery chemoembolization for management of patients with advanced metastatic carcinoid tumors. Am J Surg 175(5):408–412

Ehehalt F, Saeger HD et al (2009) Neuroendocrine tumors of the pancreas. Oncologist 14:456–467

Ekeblad S, Sundin A et al (2007) Temozolomide as monotherapy is effective in treatment of advanced malignant neuroendocrine tumors. Clin Cancer Res 13(10):2986–2991

Eriksson B (2010) New drugs in neuroendocrine tumors: rising of new therapeutic philosophies? Curr Opin Oncol 22(4):381–386

Espana-Gomez MN, Velazquez-Fernandez D et al (2009) Pancreatic insulinoma: a surgical experience. World J Surg 33:1966–1970

Falconi M, Zerbi A et al (2010) Parenchyma-preserving resections for small nonfunctioning pancreatic endocrine tumors. Ann Surg Oncol 17(6):1621–1627

Falconi M, Bartsch DK et al (2012) ENETS Consensus Guidelines for the management of patients with digestive neuroendocrine neoplasms of the digestive system: well-differentiated pancreatic non-functioning tumors. Neuroendocrinology 95(2):120–134

Fazio N, de Braud F et al (2007) Interferon-α and somatostatin analog in patients with gastroenteropancreatic neuroendocrine carcinoma: single agent or combination? Ann Oncol 18 (1):13–19

Feelders RA, Hofland LJ et al (2010) Medical treatment of cushing's syndrome: adrenal-blocking drugs and ketoconazole. Neuroendocrinology 92(Suppl 1):111–115

Fendrich V, Langer P et al (2007) Management of sporadic and multiple endocrine neoplasia type 1 gastrinomas. Br J Surg 94(11):1331–1341

Fernández-Cruz L, Blanco L, Cosa R, Rendón H. (2008) Is laparoscopic resection adequate in patients with neuroendocrine pancreatic tumors? World J Surg 32(5):904–17.

Frucht H, Howard JM et al (1989) Secretin and calcium provocative tests in the Zollinger-Ellison syndrome. A prospective study. Ann Intern Med 111(9):713–722

Garcia-Carbonero R, Capdevila J et al (2010) Incidence, patterns of care and prognostic factors for outcome of gastroenteropancreatic neuroendocrine tumors (GEP-NETs): results from the National Cancer Registry of Spain (RGETNE). Ann Oncol 21(9):1794–1803

Gibril F, Schumann M et al (2004) Multiple endocrine neoplasia type 1 and Zollinger-Ellison syndrome: a prospective study of 107 cases and comparison with 1009 cases from the literature. Medicine (Baltimore) 83(1):43–83

Gillams A, Cassoni A et al (2005) Radiofrequency ablation of neuroendocrine liver metastases: the Middlesex experience. Abdom Imaging 30(4):435–441

Gimm O, Konig E et al (2007) Intra-operative quick insulin assay to confirm complete resection of insulinomas guided by selective arterial calcium injection (SACI). Langenbecks Arch Surg 392(6):679–684

Gola M, Doga M et al (2006) Neuroendocrine tumors secreting growth hormone-releasing hormone: pathophysiological and clinical aspects. Pituitary 9(3):221–229

Gupta S, Johnson MM et al (2005) Hepatic arterial embolization and chemoembolization for the treatment of patients with metastatic neuroendocrine tumors: variables affecting response rates and survival. Cancer 104(8):1590–1602

Halfdanarson TR, Rabe KG et al (2008) Pancreatic neuroendocrine tumors (PNETs): incidence, prognosis and recent trend toward improved survival. Ann Oncol 19(10):1727–1733

Haugvik SP, Marangos IP et al (2013) Long-term outcome of laparoscopic surgery for pancreatic neuroendocrine tumors. World J Surg 37(3):582–590
Hill JS, McPhee JT et al (2009) Pancreatic neuroendocrine tumors: the impact of surgical resection on survival. Cancer 115(4):741–751
Hoff AO, Vassilopoulou-Sellin R (1998) The role of glucagon administration in the diagnosis and treatment of patients with tumor hypoglycemia. Cancer 82(8):1585–1592
Horwhat JD, Paulson EK et al (2006) A randomized comparison of EUS-guided FNA versus CT or US-guided FNA for the evaluation of pancreatic mass lesions. Gastrointest Endosc 63(7):966–975
Hough DM, Stephens DH et al (1994) Pancreatic lesions in von Hippel-Lindau disease: prevalence, clinical significance, and CT findings. AJR Am J Roentgenol 162(5):1091–1094
Ichikawa T, Peterson MS et al (2000) Islet cell tumor of the pancreas: biphasic CT versus MR imaging in tumor detection. Radiology 216(1):163–171
Jackson JE (2005) Angiography and arterial stimulation venous sampling in the localization of pancreatic neuroendocrine tumours. Best Pract Res Clin Endocrinol Metab 19(2):229–239
Jensen RT, Niederle B et al (2006) Gastrinoma (duodenal and pancreatic). Neuroendocrinology 84(3):173–182
Jensen RT, Berna MJ et al (2008) Inherited pancreatic endocrine tumor syndromes: advances in molecular pathogenesis, diagnosis, management, and controversies. Cancer 113(7 Suppl):1807–1843
Jensen RT, Cadiot G et al (2012) ENETS Consensus Guidelines for the management of patients with digestive neuroendocrine neoplasms: functional pancreatic endocrine tumor syndromes. Neuroendocrinology 95(2):98–119
Joseph S, Wang YZ et al (2011) Neuroendocrine tumors: current recommendations for diagnosis and surgical management. Endocrinol Metab Clin North Am 40(1):205–231, x
Kaklamanos M, Karoumpalis I et al (2011) Diagnostic accuracy and clinical significance of the fine needle aspiration Ki-67 labelling index in pancreatic endocrine tumours. Endocr Relat Cancer 18(6):L1–L3
Kalinowski M, Dressler M et al (2009) Selective internal radiotherapy with Yttrium-90 microspheres for hepatic metastatic neuroendocrine tumors: a prospective single center study. Digestion 79(3):137–142
Kaltsas G, Rockall A et al (2004a) Recent advances in radiological and radionuclide imaging and therapy of neuroendocrine tumours. Eur J Endocrinol 151(1):15–27
Kaltsas GA, Besser GM et al (2004b) The diagnosis and medical management of advanced neuroendocrine tumors. Endocr Rev 25(3):458–511
Kaltsas G, Androulakis II et al (2010) Paraneoplastic syndromes secondary to neuroendocrine tumours. Endocr Relat Cancer 17(3):R173–R193
Kanakis G, Kaltsas G et al (2012) Unusual complication of a pancreatic neuroendocrine tumor presenting with malignant hypercalcemia. J Clin Endocrinol Metab 97(4):E627–E631
Kao PC, Taylor RL et al (1994) Proinsulin by immunochemiluminometric assay for the diagnosis of insulinoma. J Clin Endocrinol Metab 78(5):1048–1051
Kianmanesh R, Sauvanet A et al (2008) Two-step surgery for synchronous bilobar liver metastases from digestive endocrine tumors: a safe approach for radical resection. Ann Surg 247(4):659–665
Kimura W, Kuroda A et al (1991) Clinical pathology of endocrine tumors of the pancreas. Analysis of autopsy cases. Dig Dis Sci 36(7):933–942
Kindmark H, Sundin A et al (2007) Endocrine pancreatic tumors with glucagon hypersecretion: a retrospective study of 23 cases during 20 years. Med Oncol 24(3):330–337
Klimstra DS, Modlin IR et al (2010) The pathologic classification of neuroendocrine tumors: a review of nomenclature, grading, and staging systems. Pancreas 39(6):707–712
Kloppel G, Anlauf M et al (2007a) Endocrine precursor lesions of gastroenteropancreatic neuroendocrine tumors. Endocr Pathol 18(3):150–155

Kloppel G, Rindi G et al (2007b) Site-specific biology and pathology of gastroenteropancreatic neuroendocrine tumors. Virchows Arch 451(Suppl 1):S9–S27
Kouvaraki MA, Shapiro SE et al (2006) Management of pancreatic endocrine tumors in multiple endocrine neoplasia type 1. World J Surg 30(5):643–653
Kulke MH, Stuart K et al (2006) Phase II study of temozolomide and thalidomide in patients with metastatic neuroendocrine tumors. J Clin Oncol 24(3):401–406
Kulke MH, Bergsland EK et al (2009) Glycemic control in patients with insulinoma treated with everolimus. N Engl J Med 360(2):195–197
Kwekkeboom DJ, Mueller-Brand J et al (2005) Overview of results of peptide receptor radionuclide therapy with 3 radiolabeled somatostatin analogs. J Nucl Med 46(Suppl 1):62S–66S
Kwekkeboom DJ, de Herder WW et al (2008) Treatment with the radiolabeled somatostatin analog [177 Lu-DOTA 0, Tyr3]octreotate: toxicity, efficacy, and survival. J Clin Oncol 26(13):2124–2130
Lamberts SW, Reubi JC et al (1992) Somatostatin receptor imaging in the diagnosis and treatment of neuroendocrine tumors. J Steroid Biochem Mol Biol 43(1–3):185–188
Langer P, Wild A et al (2001) Prospective controlled trial of a standardized meal stimulation test in the detection of pancreaticoduodenal endocrine tumours in patients with multiple endocrine neoplasia type 1. Br J Surg 88(10):1403–1407
Lehnert T (1998) Liver transplantation for metastatic neuroendocrine carcinoma: an analysis of 103 patients. Transplantation 66(10):1307–1312
Levy-Bohbot N, Merle C et al (2004) Prevalence, characteristics and prognosis of MEN 1-associated glucagonomas, VIPomas, and somatostatinomas: study from the GTE (Groupe des Tumeurs Endocrines) registry. Gastroenterol Clin Biol 28(11):1075–1081
Ligneau B, Lombard-Bohas C et al (2001) Cystic endocrine tumors of the pancreas: clinical, radiologic, and histopathologic features in 13 cases. Am J Surg Pathol 25(6):752–760
Lonovics J, Devitt P et al (1981) Pancreatic polypeptide. A review. Arch Surg 116(10):1256–1264
Lorenz K, Dralle H (2007) Surgical treatment of sporadic gastrinoma. Wien Klin Wochenschr 119:597–601
Madura JA, Cummings OW et al (1997) Nonfunctioning islet cell tumors of the pancreas: a difficult diagnosis but one worth the effort. Am Surg 63(7):573–577, discussion 577–578
Malago R, D'Onofrio M et al (2009) Contrast-enhanced sonography of nonfunctioning pancreatic neuroendocrine tumors. AJR Am J Roentgenol 192(2):424–430
Marotta V, Nuzzo V et al (2012) Limitations of Chromogranin A in clinical practice. Biomarkers 17(2):186–191
Massironi S, Conte D et al (2010) Plasma chromogranin A response to octreotide test: prognostic value for clinical outcome in endocrine digestive tumors. Am J Gastroenterol 105(9):2072–2078
Maton PN (1993) Use of octreotide acetate for control of symptoms in patients with islet cell tumors. World J Surg 17(4):504–510
Maton PN, Gardner JD et al (1986) Cushing's syndrome in patients with the Zollinger-Ellison syndrome. N Engl J Med 315(1):1–5
Matthews BD, Heniford BT et al (2000) Surgical experience with nonfunctioning neuroendocrine tumors of the pancreas. Am Surg 66(12):1116–1122, discussion 1122–1113
McLean AM, Fairclough PD (2005) Endoscopic ultrasound in the localisation of pancreatic islet cell tumours. Best Pract Res Clin Endocrinol Metab 19(2):177–193
Modlin IM, Oberg K et al (2008) Gastroenteropancreatic neuroendocrine tumours. Lancet Oncol 9(1):61–72
Moertel CG, Hanley JA et al (1980) Streptozocin alone compared with streptozocin plus fluorouracil in the treatment of advanced islet-cell carcinoma. N Engl J Med 303 (21):1189–1194
Moertel CG, Kvols LK et al (1991) Treatment of neuroendocrine carcinomas with combined etoposide and cisplatin. Evidence of major therapeutic activity in the anaplastic variants of these neoplasms. Cancer 68(2):227–232

Moertel CG, Lefkopoulo M et al (1992) Streptozocin-doxorubicin, streptozocin-fluorouracil or chlorozotocin in the treatment of advanced islet-cell carcinoma. N Engl J Med 326(8):519–523

Newell-Price J, Bertagna X et al (2006) Cushing's syndrome. Lancet 367(9522):1605–1617

Nilsson O, Van Cutsem E et al (2006) Poorly differentiated carcinomas of the foregut (gastric, duodenal and pancreatic). Neuroendocrinology 84(3):212–215

Nino-Murcia M, Tamm EP et al (2003) Multidetector-row helical CT and advanced postprocessing techniques for the evaluation of pancreatic neoplasms. Abdom Imaging 28(3):366–377

Nobels FR, Kwekkeboom DJ et al (1997) Chromogranin A as serum marker for neuroendocrine neoplasia: comparison with neuron-specific enolase and the α-subunit of glycoprotein hormones. J Clin Endocrinol Metab 82(8):2622–2628

Norton JA, Cromack DT et al (1988) Intraoperative ultrasonographic localization of islet cell tumors. A prospective comparison to palpation. Ann Surg 207(2):160–168

Norton JA, Alexander HR et al (2004) Does the use of routine duodenotomy (DUODX) affect rate of cure, development of liver metastases or survival in patients with Zollinger-Ellison syndrome? Ann Surg 239:617–626

Norton JA, Fang TD et al (2006) Surgery for gastrinoma and insulinoma in multiple endocrine neoplasia type 1. J Natl Compr Canc Netw 4(2):148–153

O'Brien TD, Chejfec G et al (1993) Clinical features of duodenal somatostatinomas. Surgery 114(6):1144–1147

O'Toole D, Salazar R et al (2006) Rare functioning pancreatic endocrine tumors. Neuroendocrinology 84(3):189–195

O'Toole D, Grossman A et al (2009) ENETS Consensus Guidelines for the standards of care in neuroendocrine tumors: biochemical markers. Neuroendocrinology 90(2):194–202

Oberg K (2011) Circulating biomarkers in gastroenteropancreatic neuroendocrine tumours. Endocr Relat Cancer 18(Suppl 1):S17–S25

Oberg KE, Reubi JC et al (2010) Role of somatostatins in gastroenteropancreatic neuroendocrine tumor development and therapy. Gastroenterology 139(3):742–753, 753 e741

Olausson M, Friman S et al (2002) Indications and results of liver transplantation in patients with neuroendocrine tumors. World J Surg 26(8):998–1004

Orlefors H, Sundin A et al (2005) Whole-body (11)C-5-hydroxytryptophan positron emission tomography as a universal imaging technique for neuroendocrine tumors: comparison with somatostatin receptor scintigraphy and computed tomography. J Clin Endocrinol Metab 90(6):3392–3400

Owen NJ, Sohaib SA et al (2001) MRI of pancreatic neuroendocrine tumours. Br J Radiol 74(886):968–973

Pape UF, Jann H et al (2008) Prognostic relevance of a novel TNM classification system for upper gastroenteropancreatic neuroendocrine tumors. Cancer 113(2):256–265

Pasquali C, Rubello D et al (1998) Neuroendocrine tumor imaging: can ^{18}F-fluorodeoxyglucose positron emission tomography detect tumors with poor prognosis and aggressive behavior? World J Surg 22(6):588–592

Paulsen SD, Nghiem HV et al (2006) Evaluation of imaging-guided core biopsy of pancreatic masses. AJR Am J Roentgenol 187(3):769–772

Perez-Montiel MD, Frankel WL et al (2003) Neuroendocrine carcinomas of the pancreas with 'Rhabdoid' features. Am J Surg Pathol 27(5):642–649

Phan GQ, Yeo CJ et al (1998) Surgical experience with pancreatic and peripancreatic neuroendocrine tumors: review of 125 patients. J Gastrointest Surg 2(5):472–482

Piani C, Franchi GM et al (2008) Cytological Ki-67 in pancreatic endocrine tumours: an opportunity for pre-operative grading. Endocr Relat Cancer 15(1):175–181

Portela-Gomes GM, Stridsberg M et al (2000) Expression of the five different somatostatin receptor subtypes in endocrine cells of the pancreas. Appl Immunohistochem Mol Morphol 8(2):126–132

Raymond E, Dahan L et al (2011) Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. N Engl J Med 364(6):501–513
Rehfeld JF, Bardram L et al (2012) Pitfalls in diagnostic gastrin measurements. Clin Chem 58(5):831–836
Richards ML, Thompson GB et al (2011) Setting the bar for laparoscopic resection of sporadic insulinoma. World J Surg 35:785–789
Rigaud G, Missiaglia E et al (2001) High resolution allelotype of nonfunctional pancreatic endocrine tumors: identification of two molecular subgroups with clinical implications. Cancer Res 61(1):285–292
Rindi G, Kloppel G (2004) Endocrine tumors of the gut and pancreas tumor biology and classification. Neuroendocrinology 80(Suppl 1):12–15
Rindi G, Kloppel G et al (2006) TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. Virchows Arch 449(4):395–401
Rinke A, Muller HH et al (2009) Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: a report from the PROMID Study Group. J Clin Oncol 27(28):4656–4663
Rosenau J, Bahr MJ et al (2002) Ki67, E-cadherin, and p53 as prognostic indicators of long-term outcome after liver transplantation for metastatic neuroendocrine tumors. Transplantation 73(3):386–394
Roy PK, Venzon DJ et al (2000) Zollinger-Ellison syndrome. Clinical presentation in 261 patients. Medicine (Baltimore) 79(6):379–411
Sarmiento JM, Heywood G et al (2003) Surgical treatment of neuroendocrine metastases to the liver: a plea for resection to increase survival. J Am Coll Surg 197(1):29–37
Schmid HA, Schoeffter P (2004) Functional activity of the multiligand analog SOM230 at human recombinant somatostatin receptor subtypes supports its usefulness in neuroendocrine tumors. Neuroendocrinology 80(Suppl 1):47–50
Service FJ (1995) Hypoglycemic disorders. N Engl J Med 332(17):1144–1152
Service FJ, Natt N (2000) The prolonged fast. J Clin Endocrinol Metab 85(11):3973–3974
Service FJ, McMahon MM et al (1991) Functioning insulinoma–incidence, recurrence, and long-term survival of patients: a 60-year study. Mayo Clin Proc 66(7):711–719
Shibata C, Kakyo M, et al (2012) Criteria for the glucagon provocative test in the diagnosis of gastrinoma. Surg Today 43(11):1281–5
Sobin LH, Wittekind C (2002) TNM classification of malignant tumours. Wiley-Liss, New York
Soga J, Yakuwa Y (1998a) Glucagonomas/diabetico-dermatogenic syndrome (DDS): a statistical evaluation of 407 reported cases. J Hepatobiliary Pancreat Surg 5(3):312–319
Soga J, Yakuwa Y (1998b) Vipoma/diarrheogenic syndrome: a statistical evaluation of 241 reported cases. J Exp Clin Cancer Res 17(4):389–400
Soga J, Yakuwa Y (1999) Somatostatinoma/inhibitory syndrome: a statistical evaluation of 173 reported cases as compared to other pancreatic endocrinomas. J Exp Clin Cancer Res 18(1):13–22
Soga J, Yakuwa Y et al (1998) Insulinoma/hypoglycemic syndrome: a statistical evaluation of 1085 reported cases of a Japanese series. J Exp Clin Cancer Res 17(4):379–388
Stabile BE, Morrow DJ et al (1984) The gastrinoma triangle: operative implications. Am J Surg 147(1):25–31
Steinmuller T, Kianmanesh R et al (2008) Consensus guidelines for the management of patients with liver metastases from digestive (neuro)endocrine tumors: foregut, midgut, hindgut, and unknown primary. Neuroendocrinology 87(1):47–62
Stridsberg M, Eriksson B et al (2003) A comparison between three commercial kits for chromogranin A measurements. J Endocrinol 177(2):337–341
Strosberg JR, Fine RL et al (2010) First-line chemotherapy with capecitabine and temozolomide in patients with metastatic pancreatic endocrine carcinomas. Cancer 117(2):268–275

Sundin A, Vullierme MP et al (2009) ENETS Consensus Guidelines for the standards of care in neuroendocrine tumors: radiological examinations. Neuroendocrinology 90(2):167–183

Thomas T, Kaye PV et al (2009) Efficacy, safety, and predictive factors for a positive yield of EUS-guided Trucut biopsy: a large tertiary referral center experience. Am J Gastroenterol 104(3):584–591

Tonelli F, Giudici F et al (2011) Pancreatic endocrine tumors in multiple endocrine neoplasia type 1 syndrome: review of literature. Endocr Pract 17(Suppl 3):33–40

Travis WD, World Health Organization et al (2004) Pathology and genetics of tumours of the lung, pleura, thymus and heart. Oxford University Press, Oxford (distributor)

Triponez F, Goudet P et al (2006) Is surgery beneficial for MEN1 patients with small ($< \text{or} = 2$ cm), nonfunctioning pancreaticoduodenal endocrine tumor? An analysis of 65 patients from the GTE. World J Surg 30(5):654–662, discussion 663–654

Tsolakis AV, Janson ET (2008) Endocrine pancreatic tumors: diagnosis and treatment. Expert Rev Endocrinol Metab 3(2):187–205

Vagefi PA, Razo O et al (2007) Evolving patterns in the detection and outcomes of pancreatic neuroendocrine neoplasms: the Massachusetts General Hospital experience from 1977 to 2005. Arch Surg 142(4):347–354

Verhoef S, van Diemen-Steenvoorde R et al (1999) Malignant pancreatic tumour within the spectrum of tuberous sclerosis complex in childhood. Eur J Pediatr 158(4):284–287

Vezzosi D, Bennet A et al (2005) Octreotide in insulinoma patients: efficacy on hypoglycemia, relationships with Octreoscan scintigraphy and immunostaining with anti-sst2A and anti-sst5 antibodies. Eur J Endocrinol 152(5):757–767

Walter T, Chardon L et al (2012) Is the combination of chromogranin A and pancreatic polypeptide serum determinations of interest in the diagnosis and follow-up of gastro-entero-pancreatic neuroendocrine tumours? Eur J Cancer 48(12):1766–1773

Yao JC (2007) Neuroendocrine tumors. Molecular targeted therapy for carcinoid and islet-cell carcinoma. Best Pract Res Clin Endocrinol Metab 21(1):163–172

Yao JC, Hassan M et al (2008a) One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. J Clin Oncol 26(18):3063–3072

Yao JC, Phan A et al (2008b) Targeting vascular endothelial growth factor in advanced carcinoid tumor: a random assignment phase II study of depot octreotide with bevacizumab and pegylated interferon α-2b. J Clin Oncol 26(8):1316–1323

Yao JC, Shah MH et al (2011) Everolimus for advanced pancreatic neuroendocrine tumors. N Engl J Med 364(6):514–523

Zamora V, Cabanne A et al (2010) Immunohistochemical expression of somatostatin receptors in digestive endocrine tumours. Dig Liver Dis 42(3):220–225

Zerbi A, Capitanio V et al (2011) Surgical treatment of pancreatic endocrine tumours in Italy: results of a prospective multicentre study of 262 cases. Langenbecks Arch Surg 396(3):313–321

Zhao J, Moch H et al (2001) Genomic imbalances in the progression of endocrine pancreatic tumors. Genes Chromosomes Cancer 32(4):364–372

Zhou C, Dhall D et al (2009) Homozygous P86S mutation of the human glucagon receptor is associated with hyperglucagonemia, α cell hyperplasia, and islet cell tumor. Pancreas 38(8):941–946

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M.S. Islam (ed.), *Islets of Langerhans*, DOI 10.1007/978-94-007-6686-0