Chapter 9
Role of Mitochondria in β-cell Function and Dysfunction

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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 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

9.1 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

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large majority of diabetic patients are classified as type 2 diabetes, or noninsulin-dependent diabetes mellitus. The patients display dysregulation of insulin secretion that may be associated with insulin resistance of 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 generators of metabolic signals. Finally, we will describe mitochondrial damages associated with β-cell dysfunction.

9.2 Overview of Metabolism–Secretion Coupling

Glucose entry within the β-cell initiates the cascade of metabolism–secretion coupling (Fig. 9.1). Glucose follows its concentration gradient by facilitative diffusion through specific transporters. Then, glucose is phosphorylated by glucokinase, thereby initiating glycolysis [1]. 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 [2]. This leads to Ca^{2+} influx through voltage-gated Ca^{2+} channels and a rise in cytosolic Ca^{2+} concentrations triggering insulin exocytosis [3].

Additional signals are necessary to reproduce the sustained secretion elicited by glucose. They participate in the amplifying pathway [4] 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+} [5]. The additional coupling factors amplifying the action of Ca^{2+} (Fig. 9.1) will be discussed in this chapter.

9.3 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. 9.2). In β-cells, according to low lactate dehydrogenase activity [6], high rates of glycolysis are maintained through the activity of mitochondrial NADH shuttles, thereby transferring glycolysis-derived electrons to mitochondria [7]. Early evidence for tight coupling between glycolysis and mitochondrial activation came from studies showing that anoxia inhibits glycolytic flux in pancreatic islets [8].

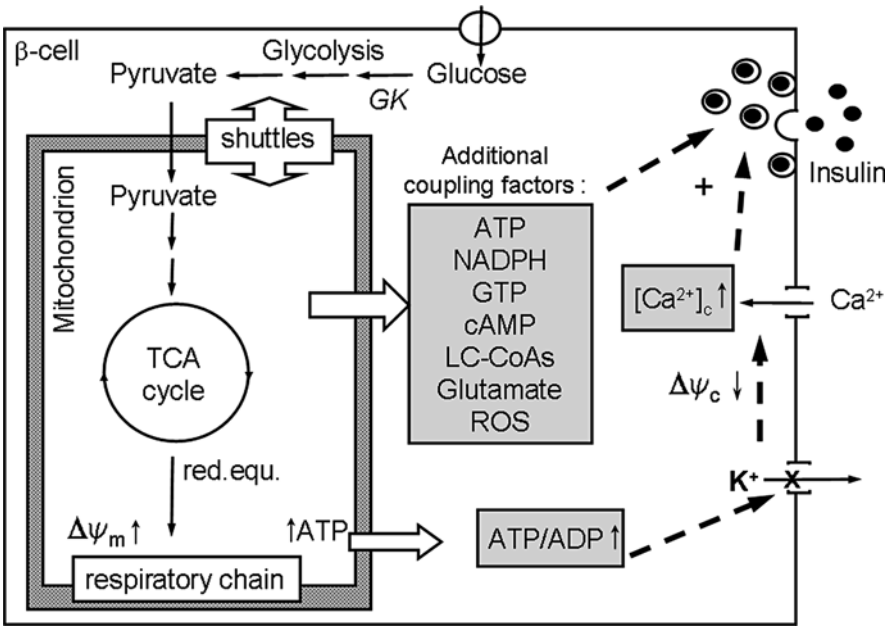

Fig. 9.1 Model for coupling of glucose metabolism to insulin secretion in the β-cell. Glucose equilibrates across the plasma membrane and is phosphorylated by glucokinase (GK). Further, glycolysis produces pyruvate, which preferentially enters the mitochondria and is metabolized by the TCA cycle. The 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

Therefore, NADH shuttle systems are necessary to couple glycolysis to activation of mitochondrial energy metabolism, leading to insulin secretion.

The NADH shuttle system is composed essentially of the glycerophosphate and the malate/aspartate shuttles [9], with its respective key members mitochondrial glycerol phosphate dehydrogenase and aspartate–glutamate carrier (AGC). Mice lacking mitochondrial glycerol phosphate dehydrogenase exhibit a normal phenotype [10], whereas general abrogation of AGC results in severe growth retardation, attributed to the observed impaired central nervous system function [11]. Islets isolated from mitochondrial glycerol phosphate dehydrogenase knockout mice respond normally to glucose regarding metabolic parameters and insulin secretion [10]. Additional inhibition of transaminases with aminooxyacetate, to nonspecifically inhibit the malate/aspartate shuttle in these islets, strongly impairs the secretory response to glucose [10]. The respective importance of these shuttles is indicated in islets of mice with abrogation of NADH shuttle activities, pointing

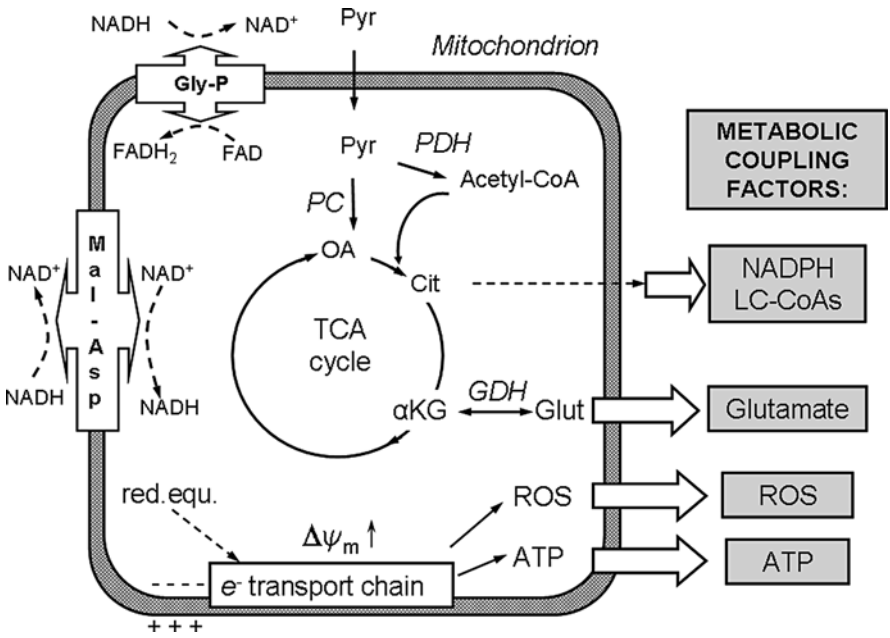

Fig. 9.2 In the mitochondria, pyruvate (Pyr) is a substrate both for pyruvate dehydrogenase (*PDH*) and pyruvate carboxylase (*PC*), forming, respectively, acetyl-CoA and oxaloacetate (OA). Condensation of acetyl-CoA with OA generates citrate (Cit) that is either processed by the TCA cycle or exported 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 (αKG) by glutamate dehydrogenase (GDH)

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 [12]. 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 [12, 13]. We showed that adenoviral-mediated overexpression of Aralar1/AGC1 in insulin-secreting cells increases glucose-induced mitochondrial activation and secretory response [14]. This is accompanied by enhanced glucose oxidation and reduced lactate production. 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.

9.4 Mitochondria as Metabolic Sensors

Downstream of the NADH shuttles, pyruvate produced by glycolysis is preferentially transferred to mitochondria. The pyruvate imported into mitochondrial matrix is associated with a futile cycle that transiently depolarizes the mitochondrial membrane [15]. After its entry into the mitochondria, the pyruvate is converted to acetyl-CoA by pyruvate dehydrogenase or to oxaloacetate by pyruvate carboxylase (Fig. 9.2). 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 [16-19]. Importance of this pathway is highlighted in a study showing that inhibition of the pyruvate carboxylase reduces glucose-stimulated insulin secretion in rat islets [20]. 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 [5].

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 has been shown to efficiently promote insulin secretion in pancreatic islets [21-23]. Succinate induces hyperpolarization of the mitochondrial membrane, resulting in elevation of mitochondrial Ca^{2+} and ATP generation, while its catabolism is Ca^{2+} dependent [21].

Beside of 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 [24]. In β-cells, approximately 50% of pyruvate is oxidized to acetyl-CoA by pyruvate dehydrogenase [17]. Pyruvate dehydrogenase is an important site of regulation as, among other effectors, the enzyme is activated by elevation of mitochondrial Ca^{2+} [25, 26] and, conversely, its activity is reduced upon exposures to either excess fatty acids [27] or chronic high glucose [28]. 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. 9.2). 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 co-factor 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 [24]. 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 [29]. 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 [30], 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 [16, 17], as confirmed by use of NMR spectroscopy [18, 19, 31].

9.5 A Focus on Glutamate Dehydrogenase

The enzyme glutamate dehydrogenase (GDH) has been proposed to participate in the development of the secretory response (Fig. 9.2). GDH is a homohexamer located in the mitochondrial matrix and catalyses the reversible reaction, α-ketoglutarate + NH_3 + NADH ↔ glutamate + NAD^+; inhibited by GTP and activated by ADP [32, 33]. Regarding β-cell, allosteric activation of GDH has triggered most of the attention over the last three decades [34]. Numerous studies have used the GDH allosteric activator L-leucine or its nonmetabolized analog beta-2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH) to question the role of GDH in the control of insulin secretion [34-37]. Alternatively, one can increase GDH activity by means of overexpression, an approach that we combined with allosteric activation of the enzyme [38]. 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 [39-41]. GDH is also an amino acid sensor triggering insulin release upon glutamine stimulation in conditions of GDH allosteric activation [35, 37, 42].

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 [43, 44]. 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 [45]. 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 ß*Glud1*$^{-/-}$) with conditional β-cell-specific deletion of GDH [46]. Data show that GDH accounts for about 40% of glucose-stimulated insulin secretion and that GDH pathway lacks redundant mechanisms. In ß*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 [46]. 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.

9.6 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. 9.2). 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+} [25], 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 [47]. Complex I is the only acceptor of electrons from NADH in the inner mitochondrial membrane and its blockade abolishes glucose-induced insulin secretion [30]. Complex II (succinate dehydrogenase) transfers electrons to coenzyme-Q from $FADH_2$, the latter being generated both by 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 [30].

Accordingly, potentiation of glucose-stimulated insulin secretion by enhanced mitochondrial NADH generation is accompanied by increased glucose metabolism and mitochondrial hyperpolarization [14].

Mitochondrial activity can be modulated according to nutrient nature, although glucose is the chief secretagogue as compared to amino acid catabolism [48] and fatty acid beta-oxidation [49]. Additional factors regulating ATP generation include mitochondrial Ca^{2+} levels [25, 50], mitochondrial protein tyrosine phosphatase [51], mitochondrial GTP [52], and matrix alkalinization [53].

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 [54]. Mitochondrial fission/fusion state was recently investigated in insulin-secreting cells. Altering fission by down regulation of fission-promoting Fis1 protein impairs respiratory function and glucose-stimulated insulin secretion [55]. The reverse experiment, consisting in overexpression of Fis1 causing mitochondrial fragmentation, results in a similar phenotype, i.e., reduced energy metabolism and secretory

defects [56]. Fragmented pattern obtained by dominant-negative expression of fusion-promoting Mfn1 protein does not affect metabolism–secretion coupling [56]. Therefore, mitochondrial fragmentation per se seems not to alter insulin-secreting cells at least in vitro.

9.7 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. 9.1). 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 [4], which is unmasked by glucose stimulation when cytosolic Ca^{2+} is clamped at permissive levels [57-59]. This suggests the existence of metabolic coupling factors generated by glucose.

9.8 Mitochondria Promote the Generation of Nucleotides Acting as Metabolic Coupling Factors

ATP is the primary metabolic factor implicated in K_{ATP}-channel regulation [60], secretory granule movement [61, 62], and the process of insulin exocytosis [63, 64].

Among other putative nucleotide messengers, NADH and NADPH are generated by glucose metabolism [65]. 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 [66, 67] and that the elevation in the cytosol is reached more rapidly than in the mitochondria [68]. Cytosolic NADPH is generated by glucose metabolism via the pentose phosphate shunt [69], although mitochondrial shuttles being the main contributors in β-cells [70]. 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 [70]. 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 [71, 72]. However, such effects have not been reproduced in primary cells in the form of rodent islets [73], 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 [74]. A direct effect of NADPH was reported on the release of insulin from isolated secretory granules [75], NADPH being possibly bound or taken up by granules [76]. 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 [77].

Glucose also promotes the elevation of GTP [78], which could trigger insulin exocytosis via GTPases [63, 79]. 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 [63]. An action of mitochondrial GTP as positive regulator of the TCA cycle has been mentioned above [52].

The universal second messenger cAMP, generated at the plasma membrane from ATP, potentiates glucose-stimulated insulin secretion [80]. 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 [81]. In human β-cells, activation of glucagon receptors synergistically amplifies the secretory response to glucose [82]. Glucose itself promotes cAMP elevation [83] and oscillations in cellular cAMP concentrations are related to the magnitude of pulsatile insulin secretion [84]. Moreover, GLP-1 might preserve β-cell mass, both by induction of cell proliferation and inhibition of apoptosis [85]. According to all these actions, GLP-1 and biologically active-related molecules are of interest for the treatment of diabetes [86].

9.9 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. 9.2) 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 [16]. In the cytosol, this process promotes the accumulation of long-chain acyl-CoAs such as palmitoyl-CoA [87, 88], which enhances Ca^{2+}-evoked insulin exocytosis [89].

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 beta-oxidation of fatty acids and to decrease insulin secretion at high glucose [49], 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 [90].

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 ways 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 [91]. 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 [92]. 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 [71], whereas another group concluded that metabolic flux through malonyl-CoA is not required for the secretory response to glucose [72].

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 [93]. Moreover, fatty acids stimulate the G-protein-coupled receptor GPR40/FFAR1 that is highly expressed in β-cells [94]. Activation of GPR40 receptor results in enhancement of glucose-induced elevation of cytosolic Ca^{2+} and consequently insulin secretion [95].

9.10 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 [96]. 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 [39, 40, 97]. Glutamate can be produced from the TCA cycle intermediate α-ketoglutarate or by transamination reactions [33, 48, 98]. 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 [18, 38, 39, 41, 99-101], whereas one study reported no change [102].

The finding that mitochondrial activation in permeabilized β-cells directly stimulates insulin exocytosis [5] initiated investigations that identified glutamate as a putative intracellular messenger [39, 40]. 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 [103]. The glutamate hypothesis was challenged by the overexpression of glutamate decarboxylase (GAD) in β-cells to reduce cytosolic glutamate levels [99]. 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 [99]. In contrast, it was reported by others that the glutamate changes may be dissociated from the amplification of insulin secretion elicited by glucose [100]. 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 [46]. 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 [104].

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 [39, 40]. 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 [105]. 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 [106]. 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 [107].

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 [108]. It has also been reported that glutamate, generated upon glucose stimulation, might sustain glucose-induced insulin secretion through inhibition of protein phosphatase enzymatic activities [101]. An alternative or additive mechanism of action would be the activation of acetyl-CoA carboxylase [96] 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 [109].

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 are metabolic stimulus-coupling factors for glucose-induced proinsulin biosynthesis [110]. 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 [111].

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 [70]. In the cytosol, citrate contributes to the formation of NADPH and malonyl-CoA, both proposed as metabolic coupling factors as discussed in this review.

9.11 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 [112]. 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 [113]. In insulin-secreting cells, it has been reported that ROS, and probably H_2O_2 in particular, is one of the metabolic coupling factor in glucose-induced insulin secretion [114]. 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 [115].

9.12 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 [116, 117]. The main sub-mitochondrial localization of ROS formation is the inner mitochondrial membrane, i.e., NADH dehydrogenase at complex I and the interface between ubiquinone and complex III [118]. 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 [119, 120]. This is consistent with the observation that inhibition of mitochondrial electron transport chain by mitochondrial complex blockers, antimycin A and rotenone, lead to increased ROS production in INS-1 β-cells [114].

9.13 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 [121, 122]. 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 [123, 124]. In the mitochondrial matrix, aconitase was also reported to be modified in an oxidative environment [125].

Furthermore, mitochondrial membrane lipids are highly susceptible to oxidants, in particular the long-chain poly-unsaturated fatty acids. ROS may directly lead to lipid peroxidation and the production of highly reactive aldehyde species exerts further detrimental effects [126]. The mitochondrion membrane-specific phospholipid cardiolipin is particularly vulnerable to oxidative damages, altering the activities of adenine nucleotide transporter and cytochrome c oxidase [127].

9.14 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 either by direct exposure to oxidants or secondary to gluco-lipotoxicity has been shown to impair β-cell functions [128–130]. In type 1 diabetes, ROS participate in β-cell dysfunction initiated by autoimmune reactions and inflammatory cytokines [131]. In type 2 diabetes, excessive ROS impair insulin synthesis [132–134] and activate β-cell apoptotic pathways [132, 135].

Hyperglycemia induces generation of superoxide at the mitochondrial level in endothelial cells and triggers of a vicious cycle of oxidative reactions implicated in the development of diabetic complications [118]. 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 [136].

Short transient exposure to oxidative stress is sufficient to impair glucose-stimulated insulin secretion in pancreatic islets [128]. Specifically, ROS attacks in insulin-secreting cells result in mitochondrial inactivation, thereby interrupting transduction of signals normally coupling glucose metabolism to insulin secretion [128]. 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 [137].

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 [138]. Other antioxidants like mitochondrial GPx, peroxiredoxin, vitamin E and Coenzymes Q, and various repair mechanisms contribute to maintain redox homeostasis in mitochondria [139, 140]. However, β-cells are characterized by relatively weak expression of free radical-quenching enzymes SOD, CAT, and GPx [141]. Overexpression of such enzymes in insulin-secreting cells inactivates ROS attacks [142]. Beside ROS inactivation, the uncoupling protein (UCP) 2 was shown to reduce cytokine-induced ROS production, an effect independent of mitochondrial uncoupling [143].

9.15 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 [47]. 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 [144]. Transgenic mice lacking expression of the mitochondrial genome specifically in the β-cells are diabetic and their islets exhibit impaired glucose-stimulated insulin secretion [145]. Moreover, mtDNA-deficient β-cell lines are glucose unresponsive and carry defective mitochondria, although they still exhibit secretory responses to Ca^{2+}-raising agents [146–148].

Mitochondrial inherited diabetes and deafness (MIDD) is often associated with mtDNA A3243G point mutation on the tRNA (Leu) gene [149, 150], 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 [151]. The aetiology of diabetes may not be primarily associated with β-cells, rendering the putative link between mtDNA mutations and β-cell dysfunction still hypothetical [152]. Moreover, pancreatic islets of such patients may carry low heteroplasmy percentage of the mutation [153] and, accordingly, the pathogenicity of this mutation is hardly detectable in the endocrine pancreas [153, 154].

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 [155–157]. 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 [155]. 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^o cells), resulting in cytosolic hybrids, namely cybrids.

Mitochondria derived from patients with mtDNA A3243G mutation were introduced into a human ρ^o osteosarcoma cell line. The resulting clonal cell lines contained either exclusively mutated mtDNA or wild-type mtDNA from the same patient [158]. The study shows that mitochondrial A3243G mutation is responsible for defective mitochondrial metabolism associated with impaired Ca^{2+} homeostasis [159]. The A3243G mutation induces a shift to dominantly glycolytic metabolism while glucose oxidation is reduced [159]. 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 [158]. As a metabolic consequence we observed a switch to anaerobic glucose utilization accompanied by increased lactate generation [159]. 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.

9.16 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.

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