Pancreatic β Cell Ca_V Channels in Health and Disease

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Abstract

Multiple types of voltage-gated calcium (Ca_V) channels including $Ca_V1.2$, $Ca_V1.3$, $Ca_V2.1$, $Ca_V2.2$, $Ca_V2.3$, $Ca_V3.1$, and $Ca_V3.2$ operate in electrically excitable β cells under both physiological and pathophysiological conditions. Physiologically functioning β cell Ca_V channels mediate spatiotemporally distinct Ca^{2+} entries to trigger exquisite glucose-stimulated insulin secretion (GSIS) and to sustain β cell maturity, viability, and physiological expansion, thereby maintaining blood glucose homeostasis. In addition, these channels also interconnect with exocytotic proteins, $InsP_3$ receptors, and PKC to form signalosomes for fine-tuning GSIS. Pathologically affected β cell Ca_V channels, resulting from their phenotypic switch, mutation, altered expression and dysregulation, impair GSIS, and compensatory β cell expansion, induce β cell dedifferentiation, and even drive β cell death due to insufficient or excessive Ca^{2+} influx. In fact, abnormal upregulation of β cell Ca_V channels couples diabetogenic serum components, like apolipoprotein CIII and IgGs in sera from patients with amyotrophic lateral sclerosis and type 2 diabetes, to Ca^{2+}-dependent β cell demise. Consequently, these detrimental Ca^{2+}-dependent events lead to insulin deficiency, hyperglycemia, and diabetes. The efficacy of curative treatments for diabetes critically relies on molecular target- and mechanism-based therapies. β Cell Ca_V channels per se, their regulation mechanisms and signaling pathways most likely serve as druggable targets for medical intervention of Ca^{2+}-dependent β cell dysfunction, abnormal expansion, dedifferentiation, and death in association with diabetes.

Keywords

β cell · Calcium channel · Cell death · Cell dedifferentiation · Cell expansion · Cell maturity · Cell viability · Glucose-stimulated insulin secretion

Abbreviations

$[Ca^{2+}]_i$	cytoplasmic-free Ca^{2+} concentration
ALS	amyotrophic lateral sclerosis
ApoCIII	apolipoprotein CIII
BBW	Bio Bred/Worchester diabetic
Ca_V	voltage-gated calcium

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G. W. Zamponi, N. Weiss (eds.), *Voltage-Gated Calcium Channels*,
https://doi.org/10.1007/978-3-031-08881-0_15

GSIS	glucose-stimulated insulin secretion
PKA	protein kinase A
PKC	protein kinase C
SR-BI	scavenger receptor class B type I
T1D	type 1 diabetes
T2D	type 2 diabetes

Introduction

Nature has endowed the β cell with multiple types of voltage-gated calcium (Ca_V) channels including $Ca_V1.2$, $Ca_V1.3$, $Ca_V2.1$, $Ca_V2.2$, $Ca_V2.3$, $Ca_V3.1$, and $Ca_V3.2$ (Fig. 1). These Ca_V channels share some similar features but possess distinct characteristics in their intrinsic properties and functionalities (Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). As in other types of electrically excitable cells, β cell Ca_V channels transit from closed pores to Ca^{2+}-conduits upon membrane depolarization, resulting from ATP-sensitive potassium (K_{ATP}) channel closure by ATP produced in glucose metabolism. This glucose-stimulated conformational transition of Ca_V channels allows extracellular Ca^{2+} to rapidly enter the cytoplasm of β cells, initially generating electrical signals of the β cell and subsequently raising cytoplasmic-free Ca^{2+} concentration ($[Ca^{2+}]_i$) (Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). $[Ca^{2+}]_i$ that serves as a versatile and ubiquitous second messenger and controls almost all known molecular and cellular events in the β cell, like glucose metabolism, insulin secretion, β cell endocytosis, differentiation, maturation, survival, growth, protein phosphorylation, gene expression, protein and organelle trafficking (Fig. 1) (Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). Therefore, physiologically orchestrated β cell Ca_V channels mediate appropriate Ca^{2+} influx to ensure adequate β cell function, identity, and viability, thereby maintaining satisfactory insulin release and glucose homeostasis in the body (Fig. 1) (Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). Pathological disturbances in β cell Ca_V channels, resulting from their mutation, altered expression, and genetic mutations or undergoing phenotype switch, altered expression, and dysregulation under pathological conditions, β cell Ca_V channels become inadequate, hyperactivated, accumulated, or dislocated resulting in impaired GSIS, β cell dedifferentiation, poor expansion, and death due to insufficient, excessive, misspaced or ill-timed Ca^{2+} influx. Consequently, insulin deficiency, hyperglycemia, and diabetes occur. *CAC* citric acid cycle, *Ca_V* voltage-gated calcium, *EV* endocytotic vesicles, *GLUT* glucose transporter, *IG* insulin-containing granule, *$InsP_3R$* $InsP_3$ receptor, *K_{ATP}* ATP-sensitive, *RyR* ryanodine receptor, *ψ* depolarization

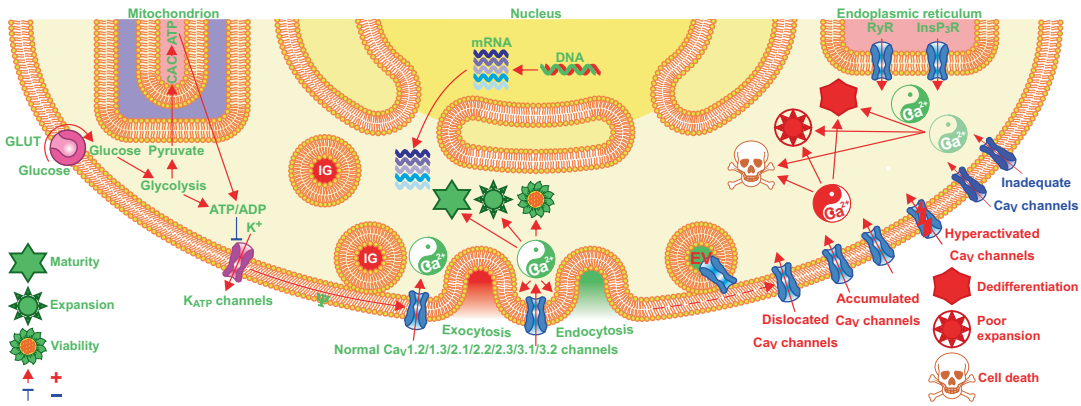

Fig. 1 A scheme illustrating pancreatic β cell Ca_V channels in health and disease. The β cell is equipped with $Ca_V1.2$, $Ca_V1.3$, $Ca_V2.1$, $Ca_V2.2$, $Ca_V2.3$, $Ca_V3.1$, and $Ca_V3.2$ channels. It converts glucose into ATP via cytosolic glycolysis and mitochondrial citric acid cycle bringing about K_{ATP} channel closure and plasma membrane depolarization to open Ca_V channels. In physiological contexts, β cell Ca_V channels take center stage in GSIS and a part in β cell maturity, viability, and expansion by mediating spatiotemporally distinct Ca^{2+} entries, which trigger Ca^{2+}-dependent protein-protein interactions and enzymatic responses and are involved in almost all known molecular and cellular events in the β cell. When suffering

dysregulation, impair insulin secretion and even drive β cell destruction due to insufficient or excessive Ca^{2+} influx (Fig. 1). Consequently, this brings about insulin deficiency, resultant hyperglycemia and diabetes (Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). Effective treatments for diabetes depend on molecular target- and mechanism-based therapies. β Cell Ca_V channels and their regulation mechanisms and signaling pathways most likely serve as druggable targets for medical intervention of Ca^{2+}-dependent β cell dedifferentiation and death associated with diabetes. Therefore, β cell Ca_V channels, their regulation mechanisms and signaling pathways have intrigued numerous researchers and clinicians including us. A series of seminal findings have been gained from basic studies by combining the patch-clamp technique, fluorescence microscopy, biochemical methods, pharmacological approaches, cellular and molecular techniques, and clinical investigations in patients with diabetes (Ovalle et al., 2018; Yang & Berggren, 2005a, b, 2006; Yang et al., 2014).

This chapter focuses on the physiological and pathological aspects of Ca_V channels in β cells.

β Cell Ca_V Channels Under Healthy Circumstances

The role of β cell Ca_V channels in the regulation of glucose-stimulated insulin secretion (GSIS) and the participation of Ca_V channels in the control of β cell maturity, viability, and expansion as well as signaling networks of β cell Ca_V channel subunits have thoroughly been characterized under healthy circumstances (Fig. 1) (Yang & Berggren, 2005a, b, 2006; Yang et al., 2014).

Ca_V Channel Regulation of Glucose-Stimulated Insulin Secretion

β Cell Ca_V channels serve as the pivot of a molecular network controlling GSIS where they exert insulinotropic action in a complex manner (Fig. 1) (Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). They not only directly steer insulin-secretory granule trafficking and trigger its exocytosis by mediating Ca^{2+} influx, but also indirectly regulate these two processes by facilitating the generation of glucose metabolism-derived signals. These allosteric actions are the most important for β cell Ca_V channel regulation of GSIS (Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). In addition, the β cell Ca_V channel-mediated Ca^{2+} influx in response to glucose stimulation also acts as an indispensable player in guaranteeing β cell maturity, growth and viability and ultimately adequate β cell mass and function by controlling β cell transcriptome, proteome, signalome, and metabolome (Fig. 1). This is well accounted for by the findings from a series of studies showing that Ca^{2+} passing through β cell Ca_V channels takes an eminent part in β cell gene expression, protein phosphorylation, proliferation, differentiation, and metabolism (for details, see section "Involvement of Ca_V channels in β cell maturity, viability, and expansion") (Benes et al., 1998; Berridge et al., 1998; Efrat et al., 1991; Jing et al., 2005; Namkung et al., 2001; Popiela & Moore, 1991; Sjoholm, 1995; Yu et al., 2020). Moreover, some of the β cell Ca_V channel subunits are also endowed with non-channel functionality that regulates GSIS (Berggren et al., 2004; Rajagopal et al., 2014). In fact, $Ca_V\beta_3$ subunits represent a good paradigm for the non-channel functionality of Ca_V channel subunits. $Ca_V\beta_3$ subunit-deficient β cells release significantly more insulin when subjected to glucose stimulation (Berggren et al., 2004; Rajagopal et al., 2014). This is attributed to the facts that the $Ca_V\beta_3$ subunit physically interacts with $InsP_3$ receptors and serves as a brake for intracellular Ca^{2+} mobilization from endoplasmic reticulum stores and that this subunit acts as a protein kinase C (PKC) inhibitor, thereby reducing GSIS (see section "Ca_V channel-centered interaction networks in the β cell") (Belkacemi et al., 2018; Berggren et al., 2004). Differential regulation of insulin-secretory granule trafficking and exocytosis in GSIS by distinct types of β cell Ca_V channels are discussed as follows.

In the process of GSIS, many steps such as glucose catabolism, insulin-secretory granule trafficking, docking and priming are involved in

$[Ca^{2+}]_i$ contributed from β cell Ca_V channel-mediated Ca^{2+} entry and importantly the final and key step, i.e., the fusion of insulin-secretory granule with the plasma membrane, is triggered by Ca^{2+} influx through Ca_V channels. This brings β cell Ca_V channels onto center stage in regulation of GSIS (Salinno et al., 2019; Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). Of particular importance is that distinct types of β cell Ca_V channels are likely to mediate spatiotemporally dissimilar Ca^{2+} influxes to differentially regulate insulin-secretory granule trafficking and exocytosis in GSIS (Barg et al., 2002; MacDonald et al., 2005; Rorsman & Braun, 2013; Rorsman & Renstrom, 2003; Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). In general, all known types of β cell Ca_V channels including $Ca_V1.2$, $Ca_V1.3$, $Ca_V2.1$, $Ca_V2.2$, $Ca_V2.3$, $Ca_V3.1$, and $Ca_V3.2$ channels are engaged in the regulation of GSIS by mediating Ca^{2+} influx (Lang, 1999; Yang & Berggren, 2005a).

The current consensus is that the Ca_V1 channel takes a predominant role over other types of Ca_V channels in Ca^{2+} triggered insulin exocytosis, the most distal step of GSIS (Yang & Berggren, 2005a). Studies with Ca_V channel blockers reveal that Ca^{2+} influx through the Ca_V1 channel brings about 60–80% of GSIS from mouse, rat, and human islets whose β cells possess various types of Ca_V channels (Davalli et al., 1996; Ohta et al., 1993; Schulla et al., 2003). Similarly, Ca_V1 channel-mediated Ca^{2+} entry also acts as a major player in triggering insulin exocytosis in insulin-secreting cell lines (Satin, 2000; Sher et al., 2003; Yang & Berggren, 2005a). Extensive investigations elucidated the role of Ca_V1 channels in dynamic insulin secretion following glucose stimulation (Davalli et al., 1996; Ohta et al., 1993; Schulla et al., 2003; Wollheim & Sharp, 1981). In an early study, the Ca_V1 channel blocker verapamil was shown to selectively inhibit the second phase of GSIS (Wollheim et al., 1978). However, accumulated experimental results verified that Ca_V1 channels regulate both phases of insulin secretion, predominantly triggering insulin release during the first phase in the mouse islet (Davalli et al., 1996; Ohta et al., 1993; Schulla et al., 2003). Dynamic perifusion in combination with pharmacological manipulation showed that three Ca_V1 channel antagonists nifedipine, diltiazem, and verapamil significantly reduce both the first and second phase of GSIS from perifused rat islets (Ohta et al., 1993). The Ca_V1 channel agonist Bay K8644 prominently elevated GSIS at both phases in human perifused islets (Davalli et al., 1996). Furthermore, islets from $Ca_V1.2$ subunit knockout ($Ca_V1.2^{-/-}$) mice drastically reduced their first phase insulin secretion when perifused with stimulatory glucose. Capacitance measurements verified that the $Ca_V1.2^{-/-}$ resulted in a selective impairment in the initial rapid component of insulin exocytosis (Schulla et al., 2003).

Two subtypes of Ca_V1 channels, $Ca_V1.2$ and $Ca_V1.3$ channels, operate in the β cell (Namkung et al., 2001; Schulla et al., 2003; Wiser et al., 1999; Yang et al., 1999). They indeed distinctly contribute to GSIS, but the distinctive contribution has not been thoroughly characterized in different species and still stands as a controversial issue. Rat β cells express a higher level of $Ca_V1.3$ subunit mRNA that is 2.5 times greater than that of $Ca_V1.2$ subunit mRNA (Iwashima et al., 1993). In $Ca_V1.2^{-/-}$ mice, β cell Ca_V currents were reduced by about 45% and their first phase insulin secretion decreased by about 80%, while the remaining Ca_V channel currents and insulin release were insensitive to Ca_V1 channel blockers (Schulla et al., 2003). Another study showed that mouse islet β cells displayed negative $Ca_V1.3$ subunit immunoreactivity (Barg et al., 2001). Comparison of the data obtained from $Ca_V1.2^{-/-}$ mice and those acquired from $Ca_V1.2^{+/+}$ mice have given the impression that $Ca_V1.2$ channels alone mediate Ca^{2+} influx to couple glucose stimulation to insulin secretion in mouse islet β cells. However, other observations provide evidence that both $Ca_V1.3$ subunit mRNA and protein are present in islet β cells from some strains of mice (Namkung et al., 2001; Yang et al., 1999, 2015). $Ca_V1.3^{-/-}$ β cells compensatorily increase the expression of $Ca_V1.2$ subunit proteins (Namkung et al., 2001). Patch-clamp analysis witnessed that although both the total voltage-gated Ba^{2+} current

density and L-type current density did not differ between $Ca_V1.3^{-/-}$ and $Ca_V1.3^{+/+}$ mouse islet β cells, the biophysical properties of L-type Ca^{2+} currents in $Ca_V1.3^{-/-}$ mouse islet β cells were markedly altered. Genetic ablation of the $Ca_V1.3$ subunit only resulted in a shift of about 10 mV towards more positive potentials at the lower voltage range (Namkung et al., 2001). Mutant islets released less insulin than wild-type islets when bathed in a buffer containing 3 mM glucose, but displayed insulin secretion similar to that of wild-type islets when exposed to 6 mM or higher concentrations of glucose. These observations suggest that compensatory upregulation of $Ca_V1.2$ channels occurs in $Ca_V1.3^{-/-}$ mouse islet β cells to maintain insulin secretion capacity (Namkung et al., 2001). The findings reflect that $Ca_V1.3$ subunits appear to mediate Ca^{2+} entry into wild-type mouse β cells for basal insulin secretion and stimulus-secretion coupling at the lower range of glucose concentrations (Namkung et al., 2001). The distinct contribution of $Ca_V1.2$ and $Ca_V1.3$ subtypes to insulin exocytosis remains to be further investigated.

Electrophysiological and pharmacological studies substantiate that $Ca_V2.1$ channels participate in GSIS from rat islet β cells (Ligon et al., 1998). Partial high voltage-activated Ca^{2+} currents disappeared in rat islet β cells exposed to the $Ca_V2.1$ channel blocker ω-Aga IVA. This pharmacological blockade inhibited the DHP-resistant component of GSIS by about 30% (Ligon et al., 1998). The involvement of $Ca_V2.1$ channels in GSIS is also true in rat insulin-secreting RINm5F cells (Sher et al., 2003). Moreover, $Ca_V2.1$ channels play a prominent role in the regulation of GSIS from human islet β cells. Human β cells exposed to ω-Aga IVA showed about a 65% reduction in GSIS (Sher et al., 2003). However, the participation of $Ca_V2.1$ channels in the regulation of GSIS from mouse islet β cells remains to be examined. The regulation of GSIS by $Ca_V2.2$ channels is still controversial. A study observed that the $Ca_V2.2$ channel blocker ω-CTX GVIA did not appreciably alter GSIS in human islets (Davalli et al., 1996). On the contrary, another observation showed that this blocker significantly inhibited GSIS in these islets (Sher et al., 2003). In addition, ω-CTX GVIA produced inhibitory effects on second phase GSIS from rat islets but did not affect first phase GSIS and high K^+-evoked insulin exocytosis. This inconsistency led to speculation that the ω-CTX GVIA-induced impairment of second phase GSIS occurred due to toxic effects rather than blockade of $Ca_V2.2$ channels (Komatsu et al., 1989). However, this speculation was made without considering the possibility that the β cell $Ca_V2.2$ channel-mediated Ca^{2+} influx does not directly trigger insulin exocytosis per se, but rather is engaged in signaling critical for second phase insulin secretion. Indeed, further work on the effect of ω-CTX GVIA on second phase insulin secretion verifies that ω-CTX GVIA significantly decreased the ATP/ADP ratio (Ohta et al., 1993). This suggests that Ca^{2+} entry through $Ca_V2.2$ channels participates in Ca^{2+}-dependent glucose metabolism, thereby promoting later phase production of ATP, which is critical for second phase insulin secretion (Ainscow & Rutter, 2002; Rorsman & Renstrom, 2003). It appears that an increase in glucose concentration can only result in a limited initial increase in cytosolic ATP, which then brings about the sequential events, K_{ATP} channel closure, Ca_V1 channel opening and first phase insulin secretion. In addition, non-Ca_V1 channel-mediated Ca^{2+} influx induced by glucose stimulation is likely to promote mitochondrial metabolism resulting in later production of ATP, which activates the signaling pathway specific for second phase insulin secretion in a K_{ATP} channel-independent manner (Ainscow & Rutter, 2002; Rorsman & Renstrom, 2003). Of note, Ca^{2+}-dependent regulation of mitochondrial metabolism is complex. As an example, the oxidative phosphorylation inhibitors carbonyl cyanide mchlorophenylhydrazone and sodium azide elevate $[Ca^{2+}]_i$, but reduce mitochondrial membrane potential and ATP production in pancreatic β cells (Rustenbeck et al., 1997). It is worthwhile to point out that $Ca_V2.2$ channel-mediated Ca^{2+} entry is likely to directly trigger insulin exocytosis in insulin-secreting RINm5F and INS-1 cells. Experimental evidence

for this comes from an observation that shows that ω-CTX GVIA inhibits both Ca_V currents and insulin secretion in these insulin-secreting cell lines, the latter being stimulated not only by glucose but also by high K^+ (Sher et al., 1992, 2003).

The use of the $Ca_V2.3^{-/-}$ mouse model and the $Ca_V2.3$ channel selective peptide blocker SNX-482 greatly advanced our understanding of the role of $Ca_V2.3$ channels in the regulation of GSIS (Jing et al., 2005; Newcomb et al., 1998; Pereverzev et al., 2002a; Yang & Berggren, 2005b). It is clear that Ca^{2+} entry through $Ca_V2.3$ channels regulates insulin secretion from both the pancreatic β cell line INS-1 and primary mouse β cells (Jing et al., 2005; Pereverzev et al., 2002a, b; Vajna et al., 2001; Yang & Berggren, 2005b). An initial study showed that $Ca_V2.3^{-/-}$ does not influence β cell mass and insulin content. Importantly, however, $Ca_V2.3$-deficient mice exhibited impaired insulin secretion, glucose intolerance and hyperglycemia. Unfortunately, this study did not examine dynamic insulin granule exocytosis and phasic insulin secretion from $Ca_V2.3^{-/-}$ β cells (Pereverzev et al., 2002a). Later, capacitance analysis showed that SNX-482 significantly reduces the late component of depolarization-induced exocytotic events without appreciable influence on the exocytotic response to the first depolarization in mouse pancreatic β cells (Schulla et al., 2003). Akin to the pharmacological manipulation with SNX-482, the genetic ablation of the $Ca_V2.3$ subunit gene gives rise to selective suppression of the late component without altering the early component of the depolarization-induced capacitance responses (Jing et al., 2005). This β cell capacitance analysis offers an indication that $Ca_V2.3$ channels are likely to selectively control second phase insulin secretion. The indication has been verified by performing a phasic insulin secretion assay. It revealed that either genetic deletion of the $Ca_V2.3$ subunit gene or pharmacological block of the $Ca_V2.3$ channel with SNX-482 resulted in significant impairment in second phase GSIS without affecting first phase insulin secretion. These findings demonstrate that $Ca_V2.3$ channels mediate a specific form of Ca^{2+} entry whereby they selectively regulate second phase GSIS (Jing et al., 2005; Schulla et al., 2003). Taken together, it is quite plausible to postulate that as distinct from the β cell Ca_V1 channels, which are physically associated with and functionally coupled to the exocytotic machinery, the $Ca_V2.3$ channel appears to be distant from exocytotic sites in the β cell. On the basis of this, the $Ca_V2.3$ channel-mediated Ca^{2+} influx is likely to specifically recruit insulin-containing granules from the reserve pool to the readily releasable pool/immediately releasable pool to govern second phase GSIS (Jing et al., 2005; Yang & Berggren, 2005b). Of particular interest is the finding showing that $Ca_V2.3^{-/-}$ islets displayed a 20% decrease in integral $[Ca^{2+}]_i$, a 30% decrease in $[Ca^{2+}]_i$ oscillation frequency, and a 50% decrease in insulin secretion. This points out that both the amount of $[Ca^{2+}]_i$ and the $[Ca^{2+}]_i$ oscillation frequency serve to regulate insulin secretion, especially in the second phase. They do so by activating Ca^{2+}-dependent adenylyl cyclase (AC) and phospholipase C resulting in the specific generation of cAMP and diacylglycerol (DAG), important insulinotropic signals, in second phase insulin secretion in pancreatic β cells (Jing et al., 2005; Yang & Berggren, 2005b).

Ca_V3 channels are likely to be engaged in stimulus-secretion coupling in the β cell. The Ca_V3 channel blocker $NiCl_2$ reduced insulin secretion from INS-1 cells in a dose-dependent manner (Bhattacharjee et al., 1997). The nonselective Ca_V1 and Ca_V3 channel antagonist flunarizine inhibited both glucose- and K^+-induced insulin secretion in perifused rat islets (Ohta et al., 1993). Ca_V3 channels appear to serve as key players in pacemaking in human islet β cells. This is most strikingly seen at membrane potentials around the threshold for action potential firing (−55 mV) and particularly important for insulin secretion induced by glucose at its threshold concentration. Blockade of Ca_V3 channels with NNC 55-0396 results in a slowdown of action potential firing and reduced insulin secretion in response to stimulation with 6 mM glucose (Braun et al., 2008). The exact role of the Ca_V3 channel in insulin secretion from human islet β cells remains for further research.

Involvement of Ca_V Channels in β Cell Maturity, Viability, and Expansion

Guaranteeing the adequate functional β cell mass under different metabolic conditions requires not only a reasonably high number of viable mature β cells, but also the sufficient capacity of β cell expansion including β cell proliferation, neogenesis, and hypertrophy (Bell & Polonsky, 2001; Mathis et al., 2001; Pipeleers et al., 2008; Rhodes, 2005; Wajchenberg, 2007). Naturally, β cells reach their full maturity through intrauterine differentiation and postnatal maturation (Liu & Hebrok, 2017; Salinno et al., 2019). Mature β cells are equipped with β cell-specific transcriptome, proteome, interactome, signalome and metabolome, and are characterized by exquisite glucose sensitivity and adequate insulin secretory capacity (Liu & Hebrok, 2017; Salinno et al., 2019). β Cells not only expand during intrauterine differentiation and postnatal maturation but also after postnatal development in some situations, e.g., pregnancy, where insulin demand increases (Liu & Hebrok, 2017; Salinno et al., 2019). Available evidence indicates that ion channels and in particular Ca_V channels are involved in β cell maturity, viability, and expansion by altering membrane potentials and mediating Ca^{2+} influx (Fig. 1). This is because β cell maturity, viability, and expansion rely on a number of Ca^{2+}-dependent cellular and molecular events like protein phosphorylation, transcription, metabolism, cell cycle, proliferation, differentiation, and maturation (Fig. 1) (Berridge et al., 1998; Yang & Berggren, 2005a, 2006; Yang et al., 2014).

Genetic ablation of $Ca_V\alpha1$ subunits significantly impairs the postnatal expansion of β cells (Jing et al., 2005; Namkung et al., 2001). The $Ca_V1.3^{-/-}$ mouse model shows that the $Ca_V1.3$ channel is indispensable for postnatal islet β cell expansion. Newborn $Ca_V1.3^{-/-}$ and $Ca_V1.3^{+/+}$ mice possess an equivalent number of islets when normalized to body weight although the former weighs less than the latter. In adulthood, however, differences between adult mutant and wild-type mice become obvious, the number and the size of islets in adult mutant mice drastically decline due to impaired β cell proliferation rather than β cell death (Namkung et al., 2001). Interestingly, $Ca_V2.3$ subunit deletion markedly impedes islet cell differentiation and maturation. This is suggested by an immunocytochemical observation showing that the majority of the glucagon-positive cells are also insulin-positive in $Ca_V2.3^{-/-}$ mouse islets. Such a type of polyhormonal cells has been considered immature fetal β cells (Hrvatin et al., 2014). This immunocytochemical phenotype indicates that the $Ca_V2.3$ channel-mediated Ca^{2+} influx is likely to drive the expression of some genes critical for β cell differentiation and maturation. Furthermore, pharmacological manipulation of Ca_V channel opening and closure markedly influences β cell proliferation and viability (Popiela & Moore, 1991; Sjoholm, 1995). It has been demonstrated that depolarization and hyperpolarization of β cells with the selective K_{ATP} channel blocker glibenclamide and opener diazoxide, which result in subsequent Ca_V channel opening and closure, induce facilitation of DNA synthesis and an impediment in β cell proliferation, respectively (Sjoholm, 1995). Importantly, the Ca_V1 channel blockers D-600 and diltiazem can produce a similar effect, i.e., a striking inhibition of β cell proliferation, as glibenclamide (Popiela & Moore, 1991; Sjoholm, 1995).

Ca_V channel-dependent regulation of β cell maturity, viability, and expansion is based on Ca^{2+}-dependent maintenance of normal transcriptome and proteome in the β cell. The expression of a range of genes in the β cell is associated with Ca^{2+} influx through Ca_V channels (Efrat et al., 1991; German et al., 1990; Lee & Laychock, 2000; Macfarlane et al., 2000). For example, the insulin gene, the most specific β cell gene, is regulated by glucose. This glucose-stimulated regulation depends on Ca^{2+} influx through β cell Ca_V channels. It is effectively abolished by the Ca_V channel blockers, such as D-600 and verapamil (Efrat et al., 1991; German et al., 1990). This is also true for the islet amyloid polypeptide amylin. This peptide is co-localized and co-secreted with insulin and regulates glucose metabolism as well as insulin resistance. The β cell elevates amylin gene transcription when exposed to

stimulatory glucose. Such glucose-induced transcription can no longer occur in the presence of the Ca_V1 channel blocker verapamil (Macfarlane et al., 2000). Inositol 1,4,5-trisphosphate ($InsP_3$) receptor gene expression is also under the influence of Ca_V channel-mediated Ca^{2+} influx. The Ca_V1 channel blocker nimodipine effectively ablates the expression of $InsP_3$ receptor type II and III genes induced by protein kinase A (PKA) activation (Lee & Laychock, 2000).

All factors that are able to alter β cell Ca_V1 channel activity or density may affect β cell maturity, viability, and expansion. Thus far, a limited number of factors have been examined. In fact, β cells fail with regard to maturity, viability, and expansion in a significant proportion of patients with diabetes. Understanding the special involvement of Ca_V channels in these processes may lead to the development of new preventive measures and curative treatments for diabetes.

Ca_V Channel-Centered Interaction Networks in the β Cell

Cellular proteins interact with each other in concert to constitute a plethora of cellular signaling networks (Bennett et al., 1992; Bezprozvanny et al., 1995; Ji et al., 2002; Kang et al., 2002; Leveque et al., 1992; Mochida et al., 1996; Rettig et al., 1996; Sheng et al., 1994, 1996, 1997; Wiser et al., 1999; Yang et al., 1999). As a typical example, Ca_V channel subunits interact with many other proteins to create complex signaling networks in addition to forming Ca^{2+} conducting pores in the plasma membrane (Belkacemi et al., 2018; Yang & Berggren, 2005a, 2006; Yang et al., 2014). In a Ca_V channel subunit complex, Ca_V channels no longer sense and respond only to voltage depolarization, but are also modulated by their interacting partners (for additional information, please see chapter "Modulation of VGCCs by G-protein Coupled Receptors and Their Second Messengers" by Mark and colleagues). They signal not only by altering membrane potentials and conducting Ca^{2+}, but also by physically acting on their interacting partners. Exceptionally, Ca_V channel subunits can even stay away from the Ca_V channel subunit complex to function as non-channel proteins and crosstalk with other signaling molecules (Belkacemi et al., 2018; Berggren et al., 2004; Hibino et al., 2003; Yang & Berggren, 2005a, 2006; Yang et al., 1999, 2014). This is well exemplified by the fact that a short splice variant of the $Ca_V\beta_4$ subunit enters the nucleus where it directly acts on the nuclear protein chromobox protein 2 and controls gene silencing (Hibino et al., 2003). Here we discuss some Ca_V channel-centered interaction networks in the β cell including Ca_V1 channel-exocytotic proteins, $Ca_V\beta_3$ subunit-$InsP_3$ receptor, and $Ca_V\beta$ subunit-PKC signaling pathways.

Ca_V1 Channel-Exocytotic Protein Interaction Networks

The first evidence for interactions between Ca_V channels and exocytotic proteins results from observations of the pull-down of ω-CTX GVIA binding proteins by antibodies against syntaxin or synaptotagmin (Bennett et al., 1992; Leveque et al., 1992). Soon after that, Ca_V channels were found to physically associate with exocytotic proteins and thereby forming an exocytotic signalosome. This exocytotic signalosome in combination with their upstream and downstream molecules makes up a complex signaling network whereby Ca_V channels and exocytotic proteins interact with each other to efficiently and precisely orchestrate complex cellular and molecular processes (Bezprozvanny et al., 1995; Mochida et al., 1996; Rettig et al., 1996; Sheng et al., 1994, 1996, 1997). In distinct cell types, specific types of Ca_V subunits selectively interact with exocytotic proteins likely due to their subcellular localization. In neurons, $Ca_V2.1$ or $Ca_V2.2$ subunits are not only colocalized, but also physically associated with exocytotic proteins in active zones, but Ca_V1 subunits are not (Sheng et al., 1998). By contrast, a Ca_V1 subunit-exocytotic protein signaling network indeed operates in pancreatic β cells (Ji et al., 2002; Kang et al., 2002; Wiser et al., 1999; Yang et al.,

1999). Both $Ca_V1.2$ and $Ca_V1.3$ subunits have similar interaction with the exocytotic machinery as the neuronal $Ca_V2.1$ and $Ca_V2.2$ subunits (Ji et al., 2002; Kang et al., 2002; Wiser et al., 1999; Yang et al., 1999).

The interaction of $Ca_V1.3$ subunits with syntaxin 1A in the pancreatic β cell has been experimentally verified (Yang et al., 1999). This interaction indeed has clear functional significance. Interrupting the formation of a syntaxin 1A/$Ca_V1.3$ subunit complex not only induces the rundown of β cell Ca_V1 channel activity but also impairs insulin exocytosis independently of this rundown. These findings corroborate that the interaction between the β cell Ca_V1 channel and syntaxin 1A is required for a proper β cell function (Yang et al., 1999). The β cell $Ca_V1.2$ subunit also interacts with exocytotic proteins (Wiser et al., 1999). Its II-III loop is physically associated with syntaxin 1A, SNAP-25 and synatotagmin. Among these interaction partners, syntaxin 1A dampens the amplitude of $Ca_V1.2$ subunit-mediated currents. This inhibitory effect is partially counteracted by synaptotagmin (Wiser et al., 1999). Disruption of this $Ca_V1.2$ subunit-exocytotic protein complex almost completely abolishes depolarization-evoked exocytosis (Kang et al., 2002; Wiser et al., 1999). β Cell Ca_V1 channels functionally interact with distinct domains within SNAP-25 (Ji et al., 2002). Interestingly, the long N-terminus (SNAP-$25_{(1–197)}$) and the short C-terminus of this exocytotic protein (SNAP-$25_{(198–206)}$) produce opposite effects on Ca_V1 channel activity, namely stimulation and inhibition, respectively. The stimulatory effect of SNAP-$25_{(1–197)}$ is weaker than the inhibitory effect of SNAP-$25_{(198–206)}$ (Ji et al., 2002).

In conclusion, the β cell Ca_V1 channel physically associates with the exocytotic machinery to constitute Ca_V1 channel-exocytotic protein interaction networks. Such networks serve to anchor β cell Ca_V1 channels and exocytotic machinery in close proximity to each other within the site of insulin exocytosis and to fine-tune β cell Ca_V1 channel function. This allows them to gain the most effective use of Ca^{2+} influx for insulin exocytosis and avoid adverse effects of unnecessary excessive Ca^{2+} influx.

$Ca_V\beta_3$ Subunit-$InsP_3$ Receptor Signaling Pathway

Both the plasma membrane Ca_V channels and intracellular $InsP_3$ receptors serve to create the exquisitely fine-tuned dynamics of $[Ca^{2+}]_i$, namely, the precise spatiotemporal arrangement of $[Ca^{2+}]_i$, by mediating Ca^{2+} influx from the extracellular space and Ca^{2+} mobilization from the intracellular store (Catterall, 2000; Taylor et al., 2004). The dynamics of $[Ca^{2+}]_i$ act as a ubiquitous second messenger encoding a rich assortment of information to control Ca^{2+}-dependent protein-protein interactions and enzymatic responses in the cell (Berridge et al., 2000, 2003).

Unexpectedly, the $Ca_V\beta_3$ subunit does not behave as a required building block of β cell Ca_V channels. Instead, it promiscuously crosstalks with the intracellular Ca^{2+} release machinery to establish a $Ca_V\beta_3$ subunit-intracellular Ca^{2+} store network (Berggren et al., 2004). This is strongly supported by the following findings: genetic ablation of $Ca_V\beta_3$ subunits ($\beta_3^{-/-}$) did not alter the activity and gating properties of Ca_V channels at both the single channel and whole-cell level in the β cell, but prominently enhanced $InsP_3$-induced Ca^{2+} release and markedly increased the frequency of glucose-induced $[Ca^{2+}]_i$ oscillations in islets. Intact $Ca_V\beta_3^{-/-}$ islets significantly increased their GSIS, whereas permeabilized $Ca_V\beta_3^{-/-}$ islets showed unaltered Ca^{2+}-evoked insulin secretion in comparison to wild-type islets. Restoration of the $Ca_V\beta_3$ subunit effectively counteracted the enhancement of GSIS induced by the deletion of this subunit. Importantly, mutant mice displayed a better glucose tolerance and more efficient glucose homeostasis. Furthermore, ectopic expression of the $Ca_V\beta_3$ subunit in COS-7 cells, where there are no endogenous Ca_V channels, significantly dampened Ca^{2+} release from $InsP_3$-sensitive stores. The endogenous $Ca_V\beta_3$ subunit in pancreatic β cells and ectopically expressed $Ca_V\beta_3$ subunit in COS-7 were mainly localized in intracellular compartments resembling ER where $InsP_3$ receptors reside. In addition to the above findings, the large cytoplasmic region of the $InsP_3$ receptor

subunit carries numerous recognition sites for a range of small molecules and proteins like $InsP_3$, Ca^{2+}, nucleotides, protein kinases and phosphatases, calmodulin, apoptotic proteins, transient receptor potential channels, and G-protein βγ subunits. (Taylor et al., 2004). Such a molecular organization opens the possibility for $InsP_3$ receptors to interact with $Ca_V\beta_3$ subunits. This prompted us to propose that physical and functional interactions of the $Ca_V\beta_3$ subunit with $InsP_3$ receptors operate in the pancreatic β cell to act as a brake on $InsP_3$ receptor-mediated Ca^{2+} mobilization from intracellular stores (Berggren et al., 2004).

Recently, the aforementioned proposal, by taking advantage of $Ca_V\beta_3^{-/-}$ mouse model and heterologous expression systems, was closely examined and substantiated (Belkacemi et al., 2018). Co-immunoprecipitation and immunoblot assay revealed that antibodies against $Ca_V\beta_3$ subunits or $InsP_3$ receptor subtypes 1–3 effectively co-immunoprecipitated $InsP_3$ receptor subtypes 1–3 or $Ca_V\beta_3$ subunits, but were unable to do so when the SH3-HOOK domain of $Ca_V\beta_3$ subunits was deleted. $[Ca^{2+}]_i$ measurements showed that the presence of $Ca_V\beta_3$ subunits resulted in inhibition of Ca^{2+} mobilization from $InsP_3$-sensitive stores, whereas the absence of $Ca_V\beta_3$ subunits gave rise to sensitization of $InsP_3$-sensitive Ca^{2+} stores to low $InsP_3$ levels. The inhibitory effect required the $Ca_V\beta_3$ SH_3-HOOK domain binding to $InsP_3$ receptors. Furthermore, radioligand binding assays revealed that $InsP_3$ receptors reduced their sensitivity to $InsP_3$ in the presence of $Ca_V\beta_3$ subunits. Overall, these findings demonstrate that the $Ca_V\beta_3$ subunit indeed physically and functionally interacts with $InsP_3$ receptors and acts as a brake on $InsP_3$ receptor-mediated Ca^{2+} mobilization from intracellular stores (Belkacemi et al., 2018). Releasing the brake by ablating the $Ca_V\beta_3$ subunit is beneficial for glucose homeostasis and skin wound healing, which is relevant for patients with diabetes (Belkacemi et al., 2018; Berggren et al., 2004). However, the physiological significance of the interaction between $Ca_V\beta_3$ subunits and $InsP_3$ receptors remains enigmatic and interesting.

$Ca_V\beta$ Subunit-PKC Signaling Pathway

Interestingly, a promiscuous interaction of $Ca_V\beta_2$ or $Ca_V\beta_3$ subunits with PKCα has been found to limit insulin secretion (Rajagopal et al., 2014). Following injection of $Ca_V\beta_2$ or $Ca_V\beta_3$ siRNAs into the tail vein, mice showed elevated serum insulin levels and improved glucose homeostasis. The same did not hold true for mice subjected to tail vein injection of $Ca_V\beta_1$ or β_4 siRNAs. A clear $Ca_V\beta_2$- or $Ca_V\beta_3$-immunoreactive band appeared in cytoplasmic fractions of islets isolated from control mice, but not from mice treated with $Ca_V\beta_2$ or $Ca_V\beta_3$ siRNAs. The treatment indeed effectively silenced $Ca_V\beta_2$ or $Ca_V\beta_3$ subunit expression. Enhanced GSIS occurred in islets and islet cells from mice treated with $Ca_V\beta_2$ or $Ca_V\beta_3$ siRNAs, but not in those from controls (Rajagopal et al., 2014). Furthermore, there was a clear translocation of PKCα, PKCβII, and PKCθ, to the plasma membrane of islet β cells obtained from $Ca_V\beta_2$- or β_3 siRNA-treated mice. This reflects released $Cav\beta_2$ and β_3 subunit inhibition on PKCα, PKCβII, and PKCθ. Moreover, mice treated with PKCα, but not with PKCβII or PKCθ siRNAs displayed a reduction in serum insulin levels and an elevation in blood glucose concentrations. This raises the possibility that PKCβII and PKCθ are not as important as PKCα in the regulation of GSIS. Taken together, these findings demonstrate that $Ca_V\beta_2$ or $Ca_V\beta_3$ subunits interact with PKCα inhibiting GSIS (Rajagopal et al., 2014).

β Cell Ca_V Channels in Pathology

Massive efforts have been made to understand β cell Ca_V channels in pathology (Yang & Berggren, 2005a, 2006; Yang et al., 2014). The resulting findings provide strong evidence that β cell Ca_V channels suffer deterioration in several aspects under unhealthy circumstances and especially in diabetes (Fig. 1). Here we discuss pathological alterations of β cell Ca_V channel phenotypes, associations of Ca_V channel gene mutation and polymorphism with abnormal insulin secretion

and diabetes, engagement of Ca_V channels in β cell dedifferentiation and death as well as Ca_V channel-mediated coupling of diabetogenic serum components to Ca^{2+}-dependent β cell demise.

Pathological Alterations of β Cell Ca_V Channel Phenotypes

A series of papers verify that the phenotypic switch of β cell Ca_V channels happens in a diabetic milieu although β cell Ca_V channelome is genetically inherited. In fact, T-type Ca^{2+} currents are not detectable in the normal mouse pancreatic β cell (Ashcroft & Rorsman, 1989; Satin, 2000; Schulla et al., 2003; Sher et al., 2003; Yang & Berggren, 2005a). However, such Ca^{2+} currents appear in NOD mouse pancreatic β cells (Wang et al., 1996). Importantly, the newly appeared T-type Ca^{2+} currents elevate basal $[Ca^{2+}]_i$ and thereby driving the Ca^{2+}-dependent apoptosis of NOD mouse β cells (Wang et al., 1996, 1999). This is undoubtedly involved in the development of type 1 diabetes (T1D) in the NOD mice, an animal model of human T1D characterized by leukocytic infiltration in islets and β cell death (Solomon & Sarvetnick, 2004; Yoshida & Kikutani, 2000). Experimental evidence shows that inflammatory cytokines, produced by the immune cells in the peri-islet and intra-islet infiltrate, induce abnormal expression of Ca_V3 channels, resultant excessive $[Ca^{2+}]_i$ and consequent Ca^{2+}-dependent apoptosis in β cells, but not in a glucagon-secreting α cell line (Wang et al., 1999). The above findings clearly pinpoint cytokines as the main culprit causing the phenotypic switch of β cell Ca_V channels and Ca^{2+}-dependent β cell death, thereby playing an important role in the development of T1D. However, some deep-seated problems remain to be addressed. For example, what are the mechanistic details whereby cytokines selectively initiate the appearance of β cell Ca_V3 channels in the T1D situation? Do cytokines turn on de novo expression of β cell Ca_V3 channel genes or just activate functionally concealed Ca_V3 channels in β cells?

Association of Ca_V Channel Gene Mutations and Polymorphisms with Abnormal Insulin Secretion and Diabetes

Type 2 diabetes (T2D) is a complex polygenic trait that is phenotypically characterized by a progressive loss and/or impairment in functional β cell mass (American Diabetes Association, 2014; Mathis et al., 2001; Saltiel, 2001; van Belle et al., 2011). It is rational to deem genes critical for GSIS and/or β cell viability, as exemplified by Ca_V channel genes, as potential diabetes risk genes (American Diabetes Association, 2014; Ashcroft & Rorsman, 2004; Holmkvist et al., 2007; Mathis et al., 2001; Reinbothe et al., 2013; Saltiel, 2001; van Belle et al., 2011). Efforts have been put into research understanding the association of Ca_V channel gene mutation and polymorphism with abnormal insulin secretion and diabetes. Some interesting findings on the association of Ca_V channel gene mutations and polymorphisms with abnormal insulin secretion and diabetes are discussed below.

It has been clearly verified that $Ca_V1.2$ missense mutations are the cause of the monogenetic disorder Timothy syndrome. This inherited disease manifests with a wide clinical spectrum that involves the cardiac, endocrine, immune and central nervous systems (Splawski et al., 2004). This complex clinical spectrum stems from a single nucleotide G > A transition at position 1216 or substitution of glycine with arginine at residue 406 (G406R) in the $Ca_V1.2$ subunit gene. Such mutant $Ca_V1.2$ subunits almost completely lose voltage-dependent inactivation. The loss of voltage-dependent inactivation in $Ca_V1.2$ channels should enhance insulin secretion in patients with Timothy syndrome. In fact, 36% of the patients suffered from episodic hypoglycemia, reflecting that excessive insulin secretion occurs due to the exaggerated Ca^{2+} influx through the mutant $Ca_V1.2$ channels in the pancreatic β cell. Moreover, such episodic hypoglycemia caused by inadequate insulin secretion has led to the decease of some affected patients (Splawski et al., 2004).

Investigation of human $Ca_V1.3$ subunit gene variation in 918 Japanese type 2 diabetics and 336 control subjects identified an ATG repeat expansion of this gene in type 2 diabetics (Yamada et al., 2001). However, this mutation appears at low frequency and is not closely associated with the development of common T2D. Nevertheless, it may give rise to a subgroup of this polygenic disease (Yamada et al., 2001; Yang & Berggren, 2005a, 2006). Furthermore, the association of the single nucleotide polymorphisms (SNPs) rs312480, rs312486 and rs9841978 in the $Ca_V1.3$ subunit gene in humans with impaired insulin secretion and T2D has been characterized in 8987 non-diabetic Finnish and Swedish people and 2830 Finnish and Swedish patients with T2D. The obtained data show that the SNP rs312480 is associated with decreased mRNA expression of the $Ca_V1.3$ subunit gene and impaired GSIS and that the SNPs rs312486 and rs9841978 are linked to T2D (Reinbothe et al., 2013). Moreover, the SNP rs673391 in the human $Ca_V2.3$ subunit has also been identified by the Botnia Study Group in collaboration with others (Holmkvist et al., 2007). It is closely associated with T2D and impaired insulin secretion and in particular reduced second-phase insulin secretion (Holmkvist et al., 2007).

The association of $Ca_V2.1$ subunit gene mutation-caused spinocerebellar ataxia type 6 with T2D has been found in a Japanese family (Takiyama et al., 1998; Yang & Berggren, 2005a, 2006). In this five-generation family, thirteen members are diagnosed with $Ca_V2.1$ subunit mutation and suffer from spinocerebellar ataxia type 6. Three out of the five patients examined also have overt T2D. This $Ca_V2.1$ subunit mutant carries abnormal CAG repeat expansion encoding a polyglutamine tract (Takiyama et al., 1998; Yang & Berggren, 2005a, 2006). Characterization of biophysical properties and surface expression of this mutant shows contradictory results from two groups (Piedras-Renteria et al., 2001; Toru et al., 2000; Yang & Berggren, 2005a, 2006). One group reveals that the polyglutamine-containing $Ca_V2.1$ subunit expressed in HEK 293 cells displayed a negative shift of voltage-dependent inactivation resulting in reduced Ca^{2+} influx (Toru et al., 2000; Yang & Berggren, 2005a, 2006). In contrast, the other witnesses that the mutant $Ca_V2.1$ subunit does not alter its biophysical properties, but instead is expressed more abundantly in the plasma membrane in HEK 293 cells, giving rise to higher Ca_V channel current density as compared to the wild-type $Ca_V2.1$ subunit (Piedras-Renteria et al., 2001; Yang & Berggren, 2005a, 2006). It remains to be seen how the mutant $Ca_V2.1$ channel behaves in the β cell to induce T2D concurrent with spinocerebellar ataxia type 6 (Takiyama et al., 1998; Yang & Berggren, 2005a, 2006).

Interestingly, a genomewide linkage analysis has found a novel neonatal diabetes locus mapped to chromosome 10p12.1-p13 in a large consanguineous family with autosomal recessively inherited neonatal diabetes (Sellick et al., 2003; Yang & Berggren, 2005a, 2006). This region contains the $Ca_V\beta_2$ subunit gene, which is predominantly expressed in the β cell (Berggren et al., 2004). All affected individuals in this family phenotypically manifested low or even undetectable levels of circulating insulin indicating inadequate β cell mass and impaired insulin synthesis and secretion (Sellick et al., 2003; Yang & Berggren, 2005a, 2006). These findings indicate that the $Ca_V\beta_2$ subunit gene should be considered as one of the potential susceptibility genes for neonatal diabetes (Sellick et al., 2003; Yang & Berggren, 2005a, 2006).

Engagement of Ca_V Channels in β Cell Dedifferentiation and Death

Correctly-localized and properly-functioning Ca_V channels with an appropriate density serve as central players in the creation and orchestration of dynamics, homeostasis, and remodeling of $[Ca^{2+}]_i$ in the β cell (Yang & Berggren, 2005a, 2006; Yang et al., 2014). This is critical for β cell maturity, viability, and expansion, as discussed earlier. Conversely, in pathological contexts, β cell Ca_V channels undergo hypoactivation or hyperactivation, become abnormally scarce or abundant, or are out of position (Fig. 1). These pathological disturbances make β cell Ca_V

channels mediate either deleteriously high or harmfully low Ca^{2+} influx, resulting in exaggerated and minimized $[Ca^{2+}]_i$, respectively, or have them conduct Ca^{2+} into wrong subcellular locations. This leads to activating detrimental signaling pathways driving β cell dedifferentiation or immaturity and β cell death (Fig. 1) (Yang & Berggren, 2005a, 2006; Yang et al., 2014).

Ca_V Channels in β Cell Dedifferentiation

Strong evidence has accumulated to support that abnormal Ca_V channels can contribute to β cell dedifferentiation (Fig. 1). In islets of GK rats, a non-obese, T2D model, pathological hyperactivation of β cell Ca_V3 and Ca_V1 channels appear coincidentally with lower levels of insulin and exocytotic proteins, including vesicle-associated membrane protein-2 (VAMP-2), synaptotagmin III, cysteine string protein (CSP), the mammalian homolog of the unc-18 gene (Munc-18), α-soluble N-ethylmaleimide-sensitive attachment protein (α-SNAP), N-ethylmaleimide-sensitive factor (NSF) and synaptosomal-associated protein of 25 kDa (SNAP-25) (Kato et al., 1996; Rose et al., 2007; Salinno et al., 2019; Yang & Berggren, 2005a, b, 2006; Yang et al., 2014; Zhang et al., 2002). Furthermore, GK rats suffer impairment of GSIS, which has thus far been considered the most important and most specific parameter representing the metabolic and functional maturity of the β cell (Kato et al., 1996; Rose et al., 2007; Salinno et al., 2019; Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). It is most likely that pathological Ca^{2+} influx through hyperactivated β cell Ca_V3 and Ca_V1 channels drives β cell dedifferentiation by decreasing expression of exocytotic proteins, resulting in impaired GSIS of GK islets. There is also a possibility that such a GK rat β cell dedifferentiation occurs due to hyperactivated Ca_V channels being redistributed apart from exocytotic sites to instead serve as a Ca^{2+}-dependent β cell dedifferentiation signal (Yang & Berggren, 2005a, 2006; Yang et al., 2014).

A recent study demonstrates that enhanced expression of β cell $Ca_V3.1$ channels drive the dedifferentiation of these cells, as evidenced by impaired GSIS and aberrant glucose homeostasis (Yu et al., 2020). Detailed analysis shows that de novo expression of a recombinant adenovirus encoding enhanced green fluorescent protein-$Ca_V3.1$ subunit (Ad-EGFP-$Ca_V3.1$) in the β cell increases typical T-type Ca^{2+} currents and basal $[Ca^{2+}]_i$. Islets transduced with Ad-EGFP-$Ca_V3.1$ secrete less insulin under both basal and first phases in response to glucose stimulation and display the inability to normalize hyperglycemia in diabetic rats. Furthermore, Ad-EGFP-$Ca_V3.1$-transduced β cells exhibit decreased phosphorylated FoxO1 in their cytoplasm, increased FoxO1 nuclear retention and reduced syntaxin 1A, SNAP-25 and synaptotagmin III. Importantly, these disturbances are reduced by inhibiting $Ca_V3.1$ channels or the Ca^{2+}-dependent phosphatase calcineurin. These findings corroborate that enhanced expression of β cell $Ca_V3.1$ channels induces β cell dedifferentiation via a signaling pathway consisting of initial excessive Ca^{2+} influx, subsequent activation of calcineurin, consequent dephosphorylation, and nuclear retention of FoxO1 and eventual FoxO1-mediated downregulation of β cell exocytotic proteins. This Ca^{2+}-dependent β cell dedifferentiation is strong enough to interfere with glucose homeostasis and hence being a pathogenic mechanism in diabetes (Yu et al., 2020).

Ca_V Channels and β Cell Death

A set of molecules responsible for cell survival can no longer function and another set of molecules being in charge of cell demise becomes activated when $[Ca^{2+}]_i$ goes below or over a physiological range or becomes spatiotemporally impaired owing to the pathological conductivity, abnormal density or dislocation of β cell Ca_V channels. Such pathologically-disturbed $[Ca^{2+}]_i$ drives β cells to death (Fig. 1) (Chang et al., 2004; Juntti-Berggren et al., 1993, 2004; Wang et al., 1999).

Pathologically exaggerated $[Ca^{2+}]_i$ mediated by hyperactivated β cell Ca_V channels can drive apoptotic and necrotic β cell death by activating

various Ca^{2+}-sensitive enzymes, e.g., calcineurin, endonucleases, transglutaminase, and calpains (Chang et al., 2004; Efanova et al., 1998; Huo et al., 2003; Juntti-Berggren et al., 1993, 2004; Wang et al., 1999; Zaitsev et al., 2001). The serine- and threonine-specific protein phosphatase (PP) calcineurin is conserved in all eukaryotes and undergo activation in a Ca^{2+}- and calmodulin-dependent manner. It acts as a key player in cell death signaling. Activated calcineurin catalyzes dephosphorylation of the Bcl-2 family member Bad to play its pro-apoptotic role (Orrenius et al., 2003). It has been shown that calcineurin orchestrates β cell Ca_V1 channel-mediated Ca^{2+} influx and the cytokine signal transduction cascade in the process of β cell death. Treatment with interleukin 1β induces pancreatic β cell apoptosis. This apoptotic event is largely counteracted by either the Ca_V1 channel inhibitor D-600 or the calcineurin inhibitor deltamethrin (Zaitsev et al., 2001). Furthermore, hyperactivated Ca_V channels can activate calpain by overloading insulin-secreting MIN6N8 cells with Ca^{2+} and subsequently calpain initiates activation of calcineurin. Upon activation, calcineurin directly dephosphorylates Bad bringing about apoptosis of the insulin-secreting cells (Chang et al., 2004).

Ca^{2+}-dependent endonucleases induce programmed cell death i.e., apoptosis by catalyzing the cleavage of chromosomal DNA into oligonucleosomal size fragments, a biochemical hallmark of apoptosis (Orrenius et al., 2003). This happens in pancreatic β cells where these DNA cutting enzymes become activated to evoke Ca^{2+}-dependent β cell apoptosis as pathologically excessive Ca^{2+} ions enter β cells subsequent to Ca_V channel activation (Efanova et al., 1998; Yang & Berggren, 2006). This is well exemplified by the fact that high glucose exposure induces typical Ca^{2+}-dependent apoptosis which is mimicked by the K_{ATP} channel blocker tolbutamide, abolished by either the K_{ATP} channel opener diazoxide or the Ca_V1 channel blocker D-600, and prevented by the endonuclease inhibitor aurintricarboxylic acid (Efanova et al., 1998; Yang & Berggren, 2006).

Transglutaminases are widely distributed in various organs, tissues, and body fluids to catalyze Ca^{2+}-dependent post-translational modification of proteins. Transglutaminase 2, also known as tissue transglutaminase, is ubiquitously expressed in mammalian cells including β cells where it extensively cross-links various cytoskeletal proteins and nucleosomal histones as the distal steps of apoptosis (Ballestar et al., 1996; Esposito & Caputo, 2005; Yang & Berggren, 2006). Transglutaminase 2 is activated by Ca^{2+} and inhibited by GTP. The activated enzyme extensively cross-links various cytoskeletal proteins, such as microtubule protein tau, β-tubulin, actin, myosin, spectrin, thymosin β, troponin T, and vimentin. This Ca^{2+}-dependent cross-linking of cytoskeletal proteins brings the apoptotic process into its final steps (Esposito & Caputo, 2005). The activated transglutaminase 2 also regulates post-translational modification of nuclear proteins, like core histones. The resultant histone cross-linking is speculated to mediate the chromatin condensation in apoptosis (Ballestar et al., 1996). Importantly, activated transglutaminase 2 also induces apoptotic cell death in a Ca^{2+}-dependent fashion (Orrenius et al., 2003). Experimental data show that insulin-secreting HIT-T15 cells subjected to depletion of GTP with mycophenolic acid display increased transglutaminase 2 activity and in turn undergo apoptosis. Of particular interest is that β cell apoptosis induced by activated transglutaminase 2 is effectively counteracted by reducing extracellular Ca^{2+} concentrations (Huo et al., 2003). Most likely transglutaminase 2 acts as a potential decoder of excessive Ca^{2+} entry through hyperactivated Ca_V channels in β cell apoptosis.

Calpains are Ca^{2+}-activated neutral cysteine proteases. Among them, the two best characterized isoforms are μ- and m-calpain, which are so named because they are activated in vitro by concentrations of Ca^{2+} in μM and mM ranges, respectively. The activated μ- and m-calpains proteolyze a number of cytoskeletal, membrane-associated, and regulatory proteins involved in a range of cellular processes including necrosis and apoptosis (Goll et al., 2003; Liu et al., 2004). An interesting study shows that apoptotic signaling cascade proceeds in a sequence of cytokine, PKC, Ca_V channels, calpain, calcineurin, Bad,

cytochrome c, and caspases in insulin-secreting MIN6N8 cells (Chang et al., 2004). Co-treatment with interferon-γ and TNF-α significantly enhances high Ca_V channel activity, prominently increases $[Ca^{2+}]_i$ and consequently induces apoptotic death of these insulin-secreting cells. The effects are effectively abolished by the PKC inhibitor chelerythrine. It is likely that PKC phosphorylation results in the enhanced activity of high Ca_V channels through which excessive Ca^{2+} influx occurs triggering Ca^{2+}-dependent apoptosis. In fact, the excessive Ca^{2+} influx first activates the Ca^{2+}-activated protease calpain. Then, the activated calpain induces activation of calcineurin, which catalyzes the dephosphorylation of Bad at S112. Subsequently, Bad dephosphorylation leads to the release of cytochrome c from the mitochondria to the cytoplasm. Eventually, caspases 9, 3, and 7 are cleaved and activated resulting in apoptosis (Chang et al., 2004).

In general, there are two ways for Ca_V channels to be engaged in β cell apoptosis (Fig. 1). On one hand, Ca_V channels undergo upregulation of their conductivity and/or density in the β cell. The upregulated Ca_V channels mediate excessive Ca^{2+} influx into the β cell resulting in abnormally exaggerated $[Ca^{2+}]_i$ which in turn initiates apoptotic cascades in the β cell. This is exemplified by the case of calpain-mediated β cell apoptosis triggered by hyperactivated Ca_V channel-mediated Ca^{2+} entry (Fig. 1) (Chang et al., 2004). On the other hand, physiological Ca^{2+} influx through β cell Ca_V channels can serve as a permissive condition for initiation of apoptosis under some conditions. This is well illustrated by the fact that interleukin 1β cannot induce β cell apoptosis without Ca^{2+} influx through Ca_V1 channels that are not directly affected by this cytokine (Zaitsev et al., 2001).

The loss of β cells occurs in both T1D and T2D. The former is characterized by the absolute loss of pancreatic β cells, whereas the latter is defined by not only the progressive loss of β cell function but also increased β cell apoptosis (Mathis et al., 2001). As illustrated above, hyperactivation of β cell Ca_V channels plays an important role in β cell apoptosis (Fig. 1) (Chang et al., 2004; Juntti-Berggren et al., 1993, 2004; Wang et al., 1999). These findings pinpoint β cell Ca_V channels as potential therapeutic targets for the prevention of β cell loss during the development of diabetes. In fact, it has been demonstrated that systemic application of the Ca_V1 channel blocker verapmil ameliorates and even prevents low-dose streptozotocin-induced progressive diabetes in mice through reduction of β cell apoptosis and promotion of β cell survival and function (Xu et al., 2012).

Ca_V Channel-Mediated Coupling of Diabetogenic Serum Components to Ca^{2+}-Dependent β Cell Demise

T1D sera contain diabetogenic components that hyperactivate β cell Ca_V1 channels to provoke Ca^{2+}-dependent β cell death (Fig. 1) (Juntti-Berggren et al., 1993, 2004; Shi et al., 2014). In fact, diabetogenic components are also present in sera from patients with neurodegenerative disorders like amyotrophic lateral sclerosis (ALS) (Shi et al., 2019).

Originally, T1D serum has been verified to hyperactivate β cell Ca_V1 channels driving pathological Ca^{2+} influx into β cells and consequent Ca^{2+}-dependent β cell apoptosis (Juntti-Berggren et al., 1993). In cell-attached single-channel recordings, unitary Ca_V1 currents display more frequent openings in β cells exposed to T1D serum. Consistent with single Ca_V1 channel analysis, whole-cell patch-clamp recordings reveal that Ca_V1 currents are massively increased in T1D serum-treated β cells. As a direct consequence of the hyperactivation of β cell Ca_V1 channels, excessive Ca^{2+} ions enter β cells resulting in pathologically exaggerated $[Ca^{2+}]_i$ in these cells. The Ca^{2+} overload eventually causes β cell apoptosis as verified by the appearance of typical apoptotic DNA "ladder". Interestingly, the T1D serum-induced β cell apoptosis disappears upon pharmacological ablation of Ca_V1 channels. This demonstrates that hyperactivated Ca_V1 channels serve to convey apoptotic cues from T1D serum to the intrinsic apoptotic machinery in β cells (Juntti-Berggren et al., 1993).

More recently, the subtypes of Ca_V1 channels hyperactivated by T1D serum and the biophysical mechanisms responsible for T1D serum-induced hyperactivation of β cell Ca_V1 channels have been clarified by using $Ca_V1.2$ and $Ca_V1.3$ knockout mice in combination with patch-clamp recordings and single-cell RT-PCR analysis (Yang et al., 2015). Interestingly, a subgroup of islet β cells from $Ca_V1.2$ knockout mice express functional $Ca_V1.3$ channels. Importantly, T1D sera increase whole-cell Ca_V currents in islet β cells from $Ca_V1.2$- or $Ca_V1.3$-deficient mice. They boost the open probability and number of functional unitary Ca_V1 channels in $Ca_V1.2$- and $Ca_V1.3$-deficient β cells. It is clear that T1D sera hyperactivate both $Ca_V1.2$ and $Ca_V1.3$ channels by increasing their conductivity and number (Yang et al., 2015). These findings pinpoint $Ca_V1.2$ and $Ca_V1.3$ channels as potential targets for anti-diabetes therapy.

To nail down the actual factor(s), i.e. molecular component(s), responsible for T1D serum-induced hyperactivation of β cell Ca_V1 channels, high-performance liquid chromatography of T1D sera has been carried out. The obtained data show that T1D sera contain significantly higher levels of apolipoprotein CIII (ApoCIII). Interestingly, patch-clamp recordings show that this apolipoprotein is capable of mimicking T1D serum to hyperactivate β cell Ca_V channels. Like T1D sera, ApoCIII markedly increases whole-cell Ca_V channel currents and $[Ca^{2+}]_i$ in the β cell and prominently provokes β cell apoptosis. Neutralization of ApoCIII with anti-ApoCIII antibodies effectively abolishes both T1D serum- and ApoCIII-induced elevation in $[Ca^{2+}]_i$ and apoptosis (Juntti-Berggren et al., 2004). The findings provide promising evidence that ApoCIII is likely to act as the actual factor in T1D sera to hyperactivate β cell Ca_V channels.

Unfortunately, the above findings cannot reveal which Ca_V channel type(s) are targeted by ApoCIII and whether Ca_V channel conductivity, density, or both are influenced by ApoCIII treatment? These two issues have been addressed by combining patch-clamp techniques and pharmacological manipulation (Shi et al., 2014). Cell-attached single-channel recordings reveal that ApoCIII increases both the open probability and density of unitary Ca_V1 currents. Whole-cell patch-clamp analysis visualizes that ApoCIII enhances whole-cell Ca^{2+} currents and the enhancement can no longer occur in the presence of the Ca_V1 channel blocker nimodipine. The findings corroborate that ApoCIII selectively targets β cell Ca_V1 channels and increases their conductivity and density (Shi et al., 2014).

The key issue of what the signaling mechanisms are whereby ApoCIII hyperactivates β cell Ca_V channels has drawn great attention. The findings obtained in other cell types suggest that plasma membrane-standing scavenger receptor class B type I (SR-BI) can directly sense extracellular ApoCIII and subsequently signals downstream to the SR-BI interaction partner β1 integrin that activates PKA, PKC, and Src kinase to phosphorylate their substrates like Ca_V1 channels. Indeed, SR-BI physically associates with ApoCIII in a CHO heterologous expression system and also interacts with β1 integrin in microglial cells (Bamberger et al., 2003; Xu et al., 1997). ApoCIII activates PKC through indirect interaction with β1 integrin in monocytic cells (Kawakami et al., 2006). Furthermore, β1 integrin activation leads to increased Ca_V1 channel activity in neurons, ventricular myocytes, and vascular smooth muscle cells through stimulation of PKA, PKC, and Src kinase (Gui et al., 2006; Rueckschloss & Isenberg, 2004; Waitkus-Edwards et al., 2002; Wu et al., 2001). In fact, all these components are expressed in β cells (Bosco et al., 2000; Kantengwa et al., 1997; Mukai et al., 2011; Nikolova et al., 2006; Yang & Berggren, 2006). Hyperactivation of Ca_V1 channels by ApoCIII is hardly influenced by individual inhibition of PKA, PKC, or Src, but significantly abolished by a cocktail of PKA, PKC, and Src or by coinhibition of PKA and Src. Moreover, knockdown of β1 integrin or SR-BI effectively prevents ApoCIII from hyperactivating β cell Ca_V channels. These findings demonstrate that ApoCIII hyperactivates β cell Ca_V1 channels through SR-BI/β1 integrin-dependent coactivation of PKA and Src (Shi et al., 2014).

Moreover, additional factors in T1D sera may signal upstream of β cell Ca_V channels to drive β

cell apoptosis as suggested by a study performed in neuroblastoma cells (Pittenger et al., 1997). This study shows that Fas-specific antibodies in T1D sera induce neuroblastoma cell apoptosis similar to T1D-induced β cell apoptosis. This indicates that Fas-specific antibodies in T1D sera may act as another candidate factor involved in the hyperactivation of β cell Ca_V channels and Ca^{2+}-dependent β cell death (Pittenger et al., 1997). As a matter of fact, N1E-115 murine neuroblastoma cells show a gradual increase in $[Ca^{2+}]_i$ followed by Fas-mediated apoptosis characterized by the appearance of condensed chromatin, shrunken cytoplasm, and DNA fragmentation when exposed to T1D sera (Pittenger et al., 1997). Given that β cell apoptosis happens following activation of the Fas signaling pathway or exposure to T1D sera, it is intriguing to clarify if Fas-specific antibodies in T1D sera drive β cell apoptosis through hyperactivation of β cell Ca_V1 channels (Maedler et al., 2001).

Possible involvement of inhibitory G proteins in T1D serum-induced hyperactivation of β cell Ca_V channels is suggested by studies with serum from the TID animal model, Bio Bred/Worchester diabetic (BBW) rat. BBW rat serum-induced hyperactivation of neuronal Ca_V channels is very similar to human T1D-induced hyperactivation of β cell Ca_V channels. Nondiabetic rat dorsal root ganglion neurons display a drastic increase in Ca_V channel activity following treatment with the BBW rat serum. This occurs most likely due to impaired regulation of the inhibitory G protein-Ca_V channel complex (Ristic et al., 1998). Interestingly, both high voltage-activated and low voltage-activated Ca^{2+} channels in dorsal root ganglion neurons from the BBW rats undergo an enhancement due to a decrease in opiate-mediated inhibition of PTX-sensitive, G protein-coupled Ca_V channels (Hall et al., 1995, 1996, 2001). The β cell also accommodates inhibitory G proteins that down-regulate β cell Ca_V channel activity (Ammala et al., 1992; Robertson et al., 1991). There is a high likelihood that human T1D serum-induced hyperactivation of β cell Ca_V channels involves inhibitory G proteins.

T1D serum has been found to promote the expression of Ca_V3 channels in a particular type of neurons with triangular soma in cerebellar granule cell cultures (Chandra et al., 2001). This is interesting because T1D serum does not affect the Ca_V3 channel in RINm5F cells, an insulin-secreting cell line (Juntti-Berggren et al., 1993; Shi et al., 2014). Maybe this is due to differences in signaling pathways upstream of Ca_V3 channels between nonproliferating primary cells like cerebellar granule neurons and islet β cells and the highly proliferative RINm5F cell line. As a matter of fact, current available data on islet β cells cannot fully rule out the possibility that TID sera also attack β cell Ca_V3 channels to provoke Ca^{2+}-dependent β cell death.

Indeed, a T-lymphocyte-mediated autoimmune attack is crucial for β cell death in T1D (Mathis et al., 2001). In addition, the above-discussed findings add another layer of complexity to the mechanism of β cell death in T1D and suggest that factors such as ApoCIII and Fas-specific antibodies in T1D sera can attack β cell-cell Ca_V channels and initiate cell death (Juntti-Berggren et al., 2004; Pittenger et al., 1997; Shi et al., 2014). The complete repertoire of diabetogenic factors in T1D sera and their involvement in β cell Ca_V channels are of therapeutic interest and need further investigation.

In addition to T1D sera, sera from patients with amyotrophic lateral sclerosis (ALS) and T2D (ALS-T2D) also express diabetogenic factors (Shi et al., 2019). About 60% ALS-T2D patients have positive sera that exaggerate $[Ca^{2+}]_i$ responses induced by K^+ depolarization in mouse islet cells. The component responsible for the effect has been identified as pathogenic immunoglobulin Gs (IgGs). These pathogenic IgGs not only immunocapture $Ca_V\alpha_2\delta1$ subunits in the β cell plasma membrane, but also hyperactivate β cell Ca_V1 channels. The hyperactivated Ca_V1 channels conduct excessive Ca^{2+} entry leading to pathologically exaggerated increases in $[Ca^{2+}]_i$ that activate multiple Ca^{2+}-dependent events detrimental to β cell function and viability. These detrimental events include impaired mitochondrial function, disturbed $[Ca^{2+}]_i$ dynamics,

reduced GSIS, and nonviable β cells. The findings verify that cytotoxic ALS-T2D-IgG autoantibodies appear in sera from ALS-T2D patients, behaving as a causal link between two closely-associated disorders ALS and T2D by immunoattacking $Ca_V\alpha_2\delta1$ subunits. Interestingly, this study suggests that in a fraction of patients T2D is likely to result from immune destruction of β cells by cytotoxic ALS-T2D-IgG autoantibodies reflecting altered humoral immunity. This may suggest a new pharmacological treatment strategy for patients suffering from a combination of ALS and T2D.

Conclusions

Over decades, persistent attempts and continuous efforts to understand pancreatic β cell Ca_V channels in health and disease have led to significant progress (Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). Electrically excitable β cells accommodate at least seven $Ca_V\alpha1$ subunits including $Ca_V1.2$, $Ca_V1.3$, $Ca_V2.1$, $Ca_V2.2$, $Ca_V2.3$, $Ca_V3.1$, and $Ca_V3.2$. These pore-forming subunits and certain Ca_V auxiliary subunits are noncovalently assembled into different types of Ca_V channels that conduct L-, P/Q-, N-, R-, and T-type Ca_V currents, respectively (Braun et al., 2008; Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). As reflected by the name of these channels, they undergo conformational transitions from an impermeable state to a highly permeable pore upon membrane depolarization initiated by the glucose-induced closure of K_{ATP} channels, mediating rapid Ca^{2+} entry into β cells (Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). Such a Ca^{2+} entry not only generates electrical signals in the β cell plasma membrane but also the versatile intracellular messenger $[Ca^{2+}]_i$ (Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). This makes Ca_V channels different from other ion channels in promoting Ca^{2+}-dependent molecular and cellular events besides producing electrical signals (Catterall, 2000).

In physiological contexts, β cell Ca_V channels take center stage in GSIS and are engaged in the control of β cell maturity, viability, and expansion, thereby acting as a multifaceted player in β cell signaling (Fig. 1) (Yang & Berggren, 2005a, b, 2006; Yang et al., 2014). Impressively, β cell Ca_V channels interplay with other non-channel proteins to create complex signaling networks in addition to promoting Ca^{2+} influx over the plasma membrane (Belkacemi et al., 2018; Berggren et al., 2004; Ji et al., 2002; Rajagopal et al., 2014; Wiser et al., 1999; Yang & Berggren, 2005a, 2006; Yang et al., 1999, 2014). This renders β cell Ca_V channels versatile, efficient, and even specific in β cell signaling. β Cell Ca_V1 subunits and exocytotic proteins specifically interact at the site of exocytosis to drive insulin release, thus preventing detrimental actions of unnecessary excessive Ca^{2+} influx (Ji et al., 2002; Wiser et al., 1999; Yang & Berggren, 2005a, 2006; Yang et al., 1999). Of interest is that promiscuous $Ca_V\beta$ subunits are networking with $InsP_3$ receptors and PKC to limit GSIS (Belkacemi et al., 2018; Berggren et al., 2004; Rajagopal et al., 2014; Yang & Berggren, 2005a, 2006; Yang et al., 2014). It seems difficult to reconcile the evolutionary conservation of $Ca_V\beta$ subunits and their negative action on GSIS, but it becomes understandable why these signaling networks are conserved in β cells if adding pathological roles of insulin hypersecretion into the context. They likely function as protective mechanisms against insulin hypersecretion and its obesogenic and even carcinogenic effects (Erion & Corkey, 2017; Gallagher & LeRoith, 2020). Surprisingly, $Ca_V\beta_3$ subunits do not appear as a required building block for β cell Ca_V channels, but instead an interaction partner of $InsP_3$ receptors to serve as a brake on Ca^{2+} mobilization from $InsP_3$-sensitive stores (Belkacemi et al., 2018; Berggren et al., 2004; Yang & Berggren, 2005a, 2006; Yang et al., 1999, 2014).

β Cell Ca_V channels suffering from inherited defects or acquired impairments conduct pathological Ca^{2+} influx, being insufficient, excessive, or spatiotemporally disorganized, causing β cell dysfunction, dedifferentiation, destruction, aberrant expansion, and eventual diabetes (Fig. 1) (Yang & Berggren, 2005a, 2006; Yang

et al., 2014). They undergo either hyperactivation or hypoactivation under diabetic conditions (Yang & Berggren, 2005a, 2006; Yang et al., 2014). Excessively-expressed $Ca_V\beta_3$ subunits drive diabetic phenotypes in obese mice, whereas $Ca_V\beta_3$ gene silencing reverses these phenotypes (Lee et al., 2018). Ca_V3 channels show no functional operation in healthy mouse β cells, but become active in NOD mouse β cells (Yang & Berggren, 2005a, 2006; Yang et al., 2014). Ca_V channel gene mutation and polymorphism are closely associated with abnormal insulin secretion and diabetes (Holmkvist et al., 2007; Splawski et al., 2004). For example, the $Ca_V1.2^{G406R}$ mutant in patients with Timothy syndrome displays little voltage-dependent inactivation and mediates exaggerated Ca^{2+} influx that enhances insulin secretion resulting in lethal hypoglycemia (Splawski et al., 2004). The close association of the SNPs rs312480, rs312486, and rs9841978 in the $Ca_V1.3$ subunit gene with impaired insulin secretion and T2D is verified in Finnish and Swedish patients (Holmkvist et al., 2007). Interestingly, $Ca_V3.1$ channels overexpressed in β cells activate detrimental signaling pathways driving β cell dedifferentiation or maturity loss through FoxO1-mediated downregulation of β cell exocytotic proteins (Yu et al., 2020). In worst-case scenarios, β cell Ca_V channels mediate exaggerated Ca^{2+} entry resulting in excessive $[Ca^{2+}]_i$ and consequent Ca^{2+}-dependent β cell death by activating various Ca^{2+}-sensitive enzymes, e.g., calcineurin, endonucleases, transglutaminase and calpains (Chang et al., 2004; Efanova et al., 1998; Huo et al., 2003; Juntti-Berggren et al., 1993, 2004; Wang et al., 1999; Zaitsev et al., 2001). In general, β cell Ca_V channels serve to couple diabetogenic factors, such as ApoCIII, $Ca_V\alpha_1\delta2$-specific, and Fas-specific antibodies in sera from patients with T1D or ALS-T1D to Ca^{2+}-dependent β cell demise (Juntti-Berggren et al., 1993, 2004; Shi et al., 2014, 2019; Yang et al., 2014, 2015; Yang & Berggren, 2005a, 2006). More specifically, Ca_V1 channels increase their density and activity in the β cell plasma membrane in response to ApoCIII through SR-BI/β1 integrin-dependent coactivation of PKA and Src kinase (Shi et al., 2014). Cytotoxic IgG autoantibodies in sera from ALS-T2D patients immunocapture $Ca_V\alpha_2\delta1$ subunits and hyperactivate β cell Ca_V channels leading to impairments in $[Ca^{2+}]_i$ dynamics, mitochondrial function, insulin secretion, and survival of β cells, thereby serving as a causal link between ALS and T2D (Shi et al., 2019).

The current knowledge on β cell Ca_V channels have been gained mostly from studies with rodent β cells, but seldom from those with human ones (Yang & Berggren, 2005a, 2006; Yang et al., 2014). Caution should be exercised in generalizing such knowledge to human β cells. Human β cell Ca_V channelome, physiology, pathology, regulation mechanisms, and therapeutic potentials should be subjected to further high-priority research. The findings from studies with rodent β cells support that β cell Ca_V1 and Ca_V3 channels and their associated interplayers ApoCIII, $InsP_3$ receptors, PKC and calcineurin most likely serve as druggable targets for clinical intervention of Ca^{2+}-dependent β cell dedifferentiation and death associated with diabetes (Belkacemi et al., 2018; Berggren et al., 2004; Juntti-Berggren et al., 2004; Rajagopal et al., 2014; Shi et al., 2014, 2019; Yang & Berggren, 2005a, 2006; Yang et al., 2014; Yu et al., 2020). Of particular interest are clinical trials of Ca_V3 channel blockers for diabetes treatment. Thus far, the visualization of the ultra-high resolution structure of Ca_V channels has indeed been turned into reality with the development of advanced technologies, like x-ray crystallography, cryo-electron microscopy, stimulated emission depletion microscopy, and single-molecule microscopy. However, vivid super-ultra high-resolution imaging of instantaneous switch in Ca_V channel conformation and dynamic redistribution of Ca_V channels in the plasma membrane is still infeasible. This has created a wide range of physiological enigmas and medical dilemmas and prevented in-depth understanding of β cell Ca_V channels in health and disease. To resolve these issues, one has to develop and apply super-ultra high spatiotemporal resolution technologies.

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