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Topical Review

Regulation of Insulin Secretion in Islets of Langerhans by Ca^{2+} Channels

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Abstract. Insulin secretion from β-cells of the pancreatic islets of Langerhans is triggered by Ca^{2+} influx through voltage-dependent Ca^{2+} channels. Electrophysiological and molecular studies indicate that β-cells express several subtypes of these channels. This review discusses their roles in regulating insulin secretion, focusing on recent studies using β-cells, exogenous expression systems, and Ca^{2+} channel knockout mice. These investigations reveal that L-type Ca^{2+} channels in the β-cell physically interact with the secretory apparatus by binding to synaptic proteins on the plasma membrane and insulin granule. As a result, Ca^{2+} influx through L-type channels efficiently and rapidly stimulates release of a pool of insulin granules in close contact with the channels. Thus, L-type Ca^{2+} channel activity is preferentially coupled to exocytosis in the β-cell, and plays a critical role in regulating the dynamics of insulin secretion. Non-L-type channels carry a significant portion of the total voltage-dependent Ca^{2+} current in β-cells and cell lines from some species, but nevertheless account for only a small fraction of insulin secretion. These channels may regulate exocytosis indirectly by affecting membrane potential or second messenger signaling pathways. Finally, voltage-independent Ca^{2+} entry pathways and their potential roles in β-cell function are discussed. The emerging picture is that Ca^{2+} channels regulate insulin secretion at multiple sites in the stimulus-secretion coupling pathway, with the specific role of each channel determined by its biophysical and structural properties.

Key words: β-cells — Exocytosis — Patch clamp — Glucose — SNARE — Diabetes mellitus

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Introduction

Pancreatic β-cells, the principal cellular constituent of the islets of Langerhans, control whole-body metabolism by secreting insulin in response to elevations in the plasma glucose concentration. Discerning the cellular mechanisms involved in the regulation of insulin secretion is of considerable interest, since defective insulin secretion leads to disorders of blood glucose homeostasis such as type II diabetes mellitus. Furthermore, the β-cell serves as an interesting paradigm for the study of peptide hormone secretion in electrically excitable neuroendocrine cells. Typical of such cells, exocytosis in the β-cell is a Ca^{2+}-dependent process, and elevated intracellular Ca^{2+} ($[Ca^{2+}]_i$) is widely understood to be the principal trigger for insulin secretion. Since $[Ca^{2+}]_i$ in the β-cell is largely determined by the activity of voltage-dependent Ca^{2+} channels (VDCCs), the importance of these channels in stimulus-secretion coupling has long been appreciated. Nevertheless, only recently have combinations of electrophysiological, molecular and sub-cellular imaging approaches begun to shed light on the roles of each VDCC subtype in insulin secretion and the structural basis for these roles. Furthermore, mounting evidence indicates that voltage-independent Ca^{2+} entry pathways may also be involved in regulating β-cell function. This review summarizes recent findings in this field and their implications for understanding stimulus-secretion coupling in the β-cell.

Stimulus-Secretion Coupling in the β-Cell

Since Dean and Matthews first described pancreatic β-cell electrical activity in 1968 [17], voluminous experimental data have established that membrane depolarization and the resulting influx of Ca^{2+} through VDCCs are essential steps in the triggering

pathway of insulin secretion (reviewed in [2, 22, 42]). Glucose, the principal physiologic insulin secretagogue, is a potent regulator of β-cell membrane potential. As shown in Fig. 1*A*, stimulatory concentrations of the sugar (>~7 mM) depolarize the β-cell and initiate a rhythmic electrical activity called bursting. Bursting consists of active phases of Ca^{2+}-dependent action potentials interposed with hyperpolarized silent phases, with the relative duration of the active phase increasing with the prevailing glucose level [44]. Importantly, bursting electrical activity entrains simultaneous oscillations of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), owing to enhanced activity of VDCCs during the active phase [59]. The $[Ca^{2+}]_i$ oscillations in turn drive simultaneous oscillations of insulin secretion [4, 18].

Figure 1*B* identifies several ion channels that are crucial for glucose-induced electrical activity. Adenosine triphosphate-sensitive K^+ channels (K_{ATP}) mediate glucose-dependent depolarization by closing in response to increases in the cytosolic ratio of adenosine triphosphate to adenosine diphosphate (ATP:ADP) that accompany glucose metabolism [15, 32]. At stimulatory glucose concentrations the depolarization is sufficient to activate VDCCs, which mediate the upstrokes of the action potentials and produce the $[Ca^{2+}]_i$ rises that stimulate exocytosis. L-Type channels are particularly important in this process, since antagonists of these channels potently suppress glucose-induced bursting, $[Ca^{2+}]_i$ oscillations and insulin secretion [2, 42]. Repolarization of the action potentials is attributed to the activity of voltage-dependent K^+ channels [38]. The schematic in Fig. 1*B* does not account for oscillatory electrical activity in the β-cell, which likely requires complex interactions among many ion channels and cellular signals [9, 31]. Nevertheless, the simplified model clearly illustrates the importance of VDCCs for control of electrical activity, $[Ca^{2+}]_i$ and insulin secretion in the β-cell. The properties of these channels have therefore been the subject of intense study.

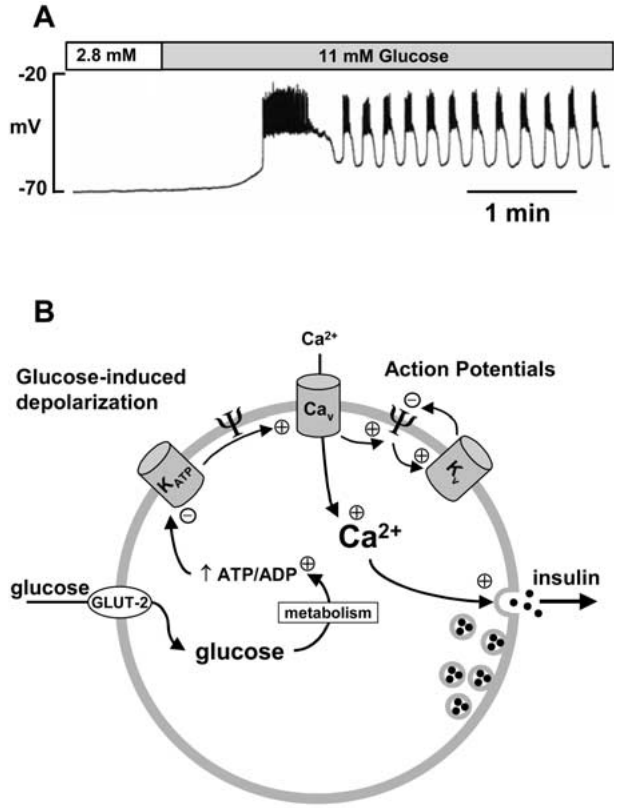

Fig. 1. Electrical activity in pancreatic β-cells. (*A*) Intracellular recording of membrane potential made from a β-cell within a microdissected mouse islet of Langerhans. The islet was perfused with a bicarbonate-buffered Krebs solution containing the indicated glucose concentrations. (*B*) Schematic of glucose-induced β-cell electrical activity. Abbreviations: GLUT-2, glucose transporter isoform-2; ATP/ADP, intracellular ratio of adenosine triphosphate to adenosine diphosphate; K_{ATP}, adenosine triphosphate-sensitive K^+ channel; Ca_v, voltage-dependent Ca^{2+} channel; K_v, voltage dependent K^+ channel; Ψ, membrane potential.

β-Cells Express Multiple Voltage-dependent Ca^{2+} Channels

The application of the patch-clamp technique [21] to excitable cells led to the identification of several classes of VDCCs, distinguishable by their biophysical properties and drug sensitivity. Molecularly, VDCCs are composed of four or five protein subunits (α_1, α_2, β, δ, and γ). The electrophysiological and pharmacological properties are conferred principally by the α_1 subunit, for which ten mammalian genes have been identified [12]. This section focuses on recent electrophysiological and molecular evidence that multiple VDCCs are expressed in pancreatic β-cells.

Whole-cell measurements of voltage-dependent Ca^{2+} currents in β-cells invariably reveal the presence of a high-voltage activated (HVA) component, activating at membrane potentials >−50 mV [2]. The peak amplitude (generally <15 pA/pF in isolated rodent and human β-cells bathed in 2.6 mM Ca^{2+}) is small compared to many other endocrine cells under similar ionic conditions [6]. A variable portion of the HVA current inactivates in a Ca^{2+}-dependent manner during sustained depolarization [51], and a slower, voltage-dependent component of inactivation (time constant ~1–7 s) has also been described in rodent β-cells [61]. The time course of the slow component of inactivation makes it a candidate process for controlling bursting electrical activity, although this hypothesis has not been verified [16].

L-Type Channels

Dihydropyridine (DHP)-sensitive, L-type Ca^{2+} channels are responsible for at least a portion of the

HVA Ca^{2+} current in β-cells from virtually all species [2, 60]. Since DHPs potently (but often not completely) suppress insulin secretion, L-type channels are considered crucial for β-cell function. In mouse β-cells the L-type current accounts for 50–90% of the total voltage-dependent Ca^{2+} current [19, 51, 64]. In contrast, L-type currents may comprise less than 25% of the Ca^{2+} current in INS-1 cells, an insulinoma line derived from rat. [36, 68]. In all, the data indicate that L-type channels are not the sole VDCC expressed in β-cells. Nevertheless, since DHPs usually block insulin secretion more strongly than they inhibit voltage-gated Ca^{2+} currents, L-type channels appear to be preferentially coupled to insulin secretion [62]. The molecular basis for this tight coupling is discussed in a subsequent section.

Of the four genes that encode α_1 subunits of L-type Ca^{2+} channels, either $Ca_v1.2$ (α_{1C}), $Ca_v1.3$ (α_{1D}), or both have been identified in mouse [7, 47, 64, 73], rat [23, 26, 65] and human islets [65], as well as various β-cell lines [23, 24, 50]. The relative expression levels of the two genes and their importance for insulin secretion remain controversial, and may differ among species or even strains. Analyses of mRNA and protein levels indicate that expression of $Ca_v1.3$ dominates over $Ca_v1.2$ in rat islets and INS-1 cells [23, 24, 26]. Conversely, immunoprecipitation of DHP-labeled subunits suggested that $Ca_v1.2$ represents $>50\%$ of the L-type channels in a different rat β-cell line (RIN) [57]. Barg and colleagues detected expression of both $Ca_v1.2$ and $Ca_v1.3$ in mouse islets by RT-PCR [7]. However, only $Ca_v1.2$ was detected in the majority of the islet by immunostaining, with $Ca_v1.3$ being detected in cells that appeared morphologically different from β-cells. In contrast, a separate immunohistochemical analysis of mouse islets revealed the presence of both $Ca_v1.2$ and $Ca_v1.3$, with $Ca_v1.3$ showing more intense staining throughout the islet [47]. As discussed further below, studies using mice lacking either $Ca_v1.2$ or $Ca_v1.3$ have since provided additional insights into the specific functional roles of these channels in mouse β-cells.

Non-L-Type Channels

Although the various components of the β-cell HVA Ca^{2+} current are difficult to distinguish based on kinetics and voltage-dependence, in many cases non-L-type Ca^{2+} currents have been identified pharmacologically. A portion of the voltage-gated Ca^{2+} current in the β-cell lines HIT and RIN is variably found to be sensitive to the N-type Ca^{2+} channel antagonist ω-conotoxin GVIA [40, 62, 63, 67]. Others report no effect of this agent in mouse and human β-cells [19, 53]. Interestingly, in one study, ω-conotoxin blocked 35% of the Ca^{2+} current in HIT cells but had no effect on glucose-induced insulin secretion, demonstrating that a VDCC subtype can be present without playing a role in exocytosis [62].

An agatoxin-sensitive, P/Q type Ca^{2+} current has been described in RIN, INS-1 and rat β-cells [23, 35, 39, 68]. Consistent with this finding, Ligon et al. identified two splice variants of $Ca_v2.1$ (α_{1A}) in rat pancreatic islets, and showed that agatoxin inhibited the majority of the DHP-insensitive component of glucose-induced insulin secretion in this preparation [35]. Hovarth and co-workers also identified P/Q type Ca^{2+} currents in INS-1 cells, but found that the glucose-induced increase in $[Ca^{2+}]_i$ in these cells was mediated entirely by L-type channels [23].

A recently described R-type Ca^{2+}-channel antagonist (SNX-482, a 41-amino acid peptide from *Hysterocrates gigas*) blocked a portion of the non-L, non-N type current in INS-1 cells, and also slightly inhibited glucose-mediated insulin secretion [68]. SNX-482 also reduced the voltage-gated Ca^{2+} current in mouse β-cells by 25% [64]. The R-type current in β-cells may be carried at least in part by $Ca_v2.3$ (α_{1E}), which has been identified in rat islets by RT-PCR [69], and in human islets by immunohistochemistry [20].

In rat and human β-cells, a low-voltage-activated (T-type) Ca^{2+} current can be distinguished from the HVA Ca^{2+} current by its lower activation threshold, slower rate of deactivation and susceptibility to inactivation at holding potentials >-100 mV [2, 45]. In addition, rat islets and INS-1 cells express two variants of the T-channel family member $Ca_v3.1$ (α_{1G}) [74]. Determining the functional roles of T-type currents is difficult since selective antagonists are lacking. However, the absence of a T-type current in normal mouse β-cells indicates that it is not necessary for bursting electrical activity, which is reliably observed in this preparation [2].

Table 1 summarizes data on VDCC expression in various β-cell preparations. Much of the remainder of this review is devoted to the mechanisms by which these channels regulate insulin secretion. The discussion begins with L-type channels, which play the most important and best understood role in β-cell function.

L-Type Ca^{2+} Channels Physically Interact with the Secretory Apparatus and Mediate First-Phase Isulin Secretion

In vivo and in vitro, pancreatic islets respond to step increases in extracellular glucose with a biphasic pattern of insulin release. The first phase lasts about ten minutes, during which the secretory rate rises to a peak and then declines. In mouse, the second phase consists of steady secretion at slower rate, while in rat and human the rate progressively increases during this phase [13]. The mechanisms controlling first-phase insulin release are of considerable interest,

Table 1. Calcium channel expression in islets of Langerhans and β-cell lines.

Calcium channel	Current	Antagonists	Islet species	Cell lines
$Ca_v1.2$ (α_{1C})	L-Type	Dihydropyridines	Mouse, rat, human	HIT, RIN
$Ca_v1.3$ (α_{1D})	L-Type	Dihydropyridines	Mouse, rat, human	HIT, RIN, INS-1, βTC3
$Ca_v2.1$ (α_{1A})	P/Q-Type	ω-Agatoxin IVA	Rat	INS-1
$Ca_v2.3$ (α_{1E})	R-Type	SNX-482	Rat, human	INS-1
$Ca_v3.1$ (α_{1G})	T-Type	?	Rat	INS-1

since its absence is among the first manifestations of type II diabetes.

At the single-cell level, the kinetics of exocytosis are most conveniently studied by measuring membrane capacitance changes resulting from granule fusion [1]. In such studies, voltage-clamp depolarization of mouse β-cells elicits a biphasic pattern of exocytosis, with 50–60 granules fusing within about 100 ms (~600 granules/s), followed by exocytosis at a steady rate of less than 15 granules/s [7]. Considering that processes such as formation of the fusion pore and dissolution of granule contents could cause significant delays between granule fusion and insulin release, the rapid exocytosis of 50–60 granules may well correspond to the first phase of insulin secretion [6]. The evidence described below suggests that these 50–60 granules constitute an immediately releasable pool (IRP) in close contact with L-type channels.

The fast exocytosis observed transiently upon depolarization of the β-cell requires a high level of Ca^{2+} (>20 μM), similar to that required for synaptic transmission [7]. In nerve terminals, fast exocytosis involves the formation of an 'excitosome' complex between N-type (or P/Q type) Ca^{2+} channels and synaptic proteins, including the plasma membrane SNARE proteins syntaxin and SNAP-25, and the likely Ca^{2+} sensor synaptotagmin I [3]. The interactions occur via the intracellular loop connecting domains II and III of the α_1 subunit (the "synprint" region) [66] and the resultant anchoring of vesicles to the Ca^{2+} entry sites ensures that the granules are exposed to large, spatially restricted increases in $[Ca^{2+}]_i$. The relatively low density of L-type Ca^{2+} channels in the β-cell suggests that a similar tethering of insulin granules to these channels may be required for fast exocytosis [7].

Early indications that insulin granules colocalize with L-type Ca^{2+} channels in mouse β-cells came from the observation that channel activity was highest in the region of the cell membrane near where insulin granules were clustered [11]. Later, Wiser et al. [71] provided more direct evidence that L-type channels physically interact with the secretory apparatus by showing that the II-III loop of $Ca_v1.2$ binds to syntaxin 1A, SNAP-25 and synaptotagmin I. Importantly, injection of an exogenous peptide corresponding to the II-III loop of $Ca_v1.2$ into mouse β-cells had dramatic effects on the kinetic pattern of exocytosis. Capacitance changes during the initial pulses of depolarizing train (which normally release the entire IRP) were dramatically reduced, whereas exocytosis during later pulses proceeded at rates similar to controls [7, 71]. These changes were not associated with reduction of Ca^{2+} current density or the number of granules that could be released when global $[Ca^{2+}]_i$ was increased by photolysis of caged Ca^{2+}. Presumably, the exogenous synprint peptide reduced the size of the IRP by competing with endogenous L-type channels for binding sites on synaptic proteins, thereby displacing the granules from the channels. The data indicate that formation of the excitosome complex serves to expose a pool of insulin granules to high Ca^{2+} concentrations in the vicinity of L-type Ca^{2+} channels. The rapid exocytosis of this IRP at the onset of depolarization likely accounts for first-phase insulin secretion (Fig. 2*A*).

The secretory rate during the second phase of insulin secretion most likely represents the rate of replenishment of the IRP from a reserve pool of granules [5]. However, in high-resolution capacitance measurements, the second phase of exocytosis is fairly insensitive to disruption of the excitosome complex with exogenous synprint [7]. This suggests that the second phase is partly mediated by global $[Ca^{2+}]_i$ rises, which could stimulate a low rate of exocytosis of release-competent granules located at considerable distance from the Ca^{2+} entry sites (Fig. 2*B*). In fact, since the slower phase of exocytosis is observed in the presence of L-type Ca^{2+}-channel antagonists and even in β-cells lacking L-type channels [64], non-L-type Ca^{2+} channels are likely to contribute to this process. It should be noted that the depolarizing pulses used in high-resolution exocytosis studies are much stronger than physiological, nutrient-induced depolarization. Therefore, the relative contribution of global $[Ca^{2+}]_i$ rises versus replenishment of the IRP to the second phase of glucose-induced insulin secretion is uncertain [64].

It warrants mention that the excitosome complex, in addition to tethering Ca^{2+} channels to the secretory apparatus, may be important for regulating Ca^{2+} influx in β-cells. Syntaxin 1A reduces the

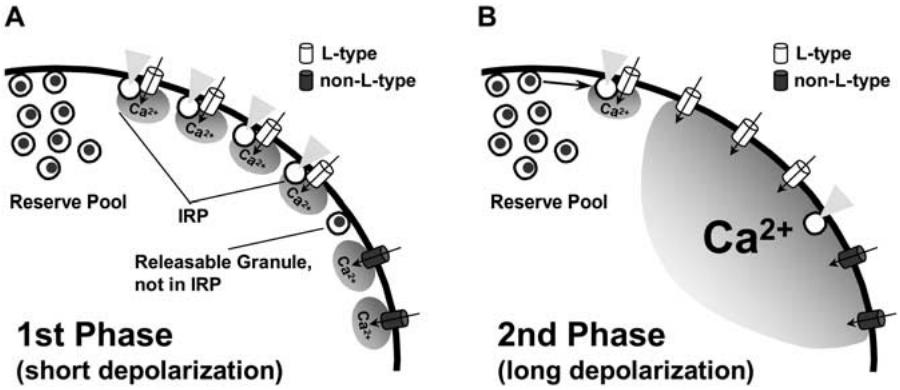

Fig. 2. Mechanisms underlying the two phases of insulin secretion. (*A*) First-phase insulin secretion reflects the release of a pool of granules in close contact with L-type Ca^{2+} channels (immediately releasable pool, IRP). These granules are exposed to spatially localized $[Ca^{2+}]_i$ increases near the mouth of open Ca^{2+} channels at the onset of depolarization, whereas releasable granules not in the IRP are not. (*B*) Two mechanisms that could contribute to second-phase insulin secretion are illustrated. These include replenishment of the immediately releasable pool from the reserve pool (*upper left*), and exocytosis of release-competent granules located far from Ca^{2+} channels, owing to widespread increases in $[Ca^{2+}]_i$ during prolonged depolarization (*lower right*). Both L- and non-L-type channels could contribute to the increase in global $[Ca^{2+}]_i$ for the latter mechanism.

magnitude and activation rate of L-type Ca^{2+} currents when co-expressed with $Ca_v1.2$ in *Xenopus* oocytes [70, 71]. Similarly, overexpression of syntaxin 1A or syntaxin 3 resulted in attenuated L-type currents in HIT cells [30]. SNAP-25 slightly inhibits L-type Ca^{2+} currents, and counteracts the effect of syntaxin on the inactivation rate of the channel [27, 70]. Finally, synaptotagmin restores the L-type current amplitude and activation rate in the presence of syntaxin 1A [71]. This functional crosstalk between L-type Ca^{2+} channels and synaptic proteins may increase the efficiency of Ca^{2+}-dependent exocytosis, by directing Ca^{2+} influx to sites occupied by insulin granules. Accordingly, plasma membrane-bound syntaxin (and perhaps SNAP-25) could inhibit L-type channels that are not associated with a secretory granule. The subsequent interaction of this complex with synaptotagmin on a docked insulin granule could then facilitate channel activation to induce fusion of the granule [5]. The prevention of superfluous Ca^{2+} entry through these interactions likely benefits the β-cell, in which secretory activity is tightly coupled to the metabolic state of the cell, by limiting the need to expend excessive metabolic energy in Ca^{2+} removal.

Finally, some evidence suggests that $Ca_v1.3$ couples to the secretory apparatus in some β-cell preparations. For example, Yang et al showed that $Ca_v1.3$ binds to syntaxin 1 in obese/obese mice, an animal model of type II diabetes [73]. In INS-1 cells, such interactions may preferentially couple $Ca_v1.3$ to exocytosis, as glucose-induced insulin secretion was inhibited by overexpression of the II-III loop of $Ca_v1.3$, but not that of $Ca_v1.2$ [36]. Whether this effect was associated with selective loss of first-phase insulin secretion was not reported.

What Are the Roles of Non-L-Type Channels in Insulin Secretion?

The frequent observation that a small fraction of glucose-induced insulin secretion is insensitive to saturating concentrations of DHPs suggests that non-L-type channels also play a functional role in the β-cell. The previous section introduced the possibility that Ca^{2+} influx through these channels could partially mediate second-phase insulin secretion by contributing to global $[Ca^{2+}]_i$ rises (Fig. 2*B*). In mouse β-cells, R-type channels may serve this role, since the antagonist SNX-482 attenuated the second phase of depolarization-evoked capacitance changes without significantly affecting the initial phase [64].

Another suggestion is that non-L-type channels affect insulin secretion indirectly, by enhancing the excitability of the β-cell membrane. For example, one model proposes that glucose-induced insulin secretion involves sequential opening of voltage-dependent Ca^{2+} channels with different activation thresholds [49]. In this scheme, the depolarization caused by K_{ATP} channel closure first activates T-type channels, which further depolarize the membrane to activate R-type channels. The combined depolarizing action of these channels ultimately activates L-type channels, which carry most of the Ca^{2+} required for insulin secretion. While this model could account for inhibition of insulin secretion by R-channel antagonists in INS-1 cells, to date it has not been shown that pharmacological or molecular ablation of R-type channel activity actually affects β-cell electrical activity.

Finally, non-L-type channels could mediate Ca^{2+}-dependent functions in the β-cell other than direct stimulation of exocytosis. These could include

mobilization of secretory granules from the reserve pool [64], activation of Ca^{2+}-dependent amplifying pathways [29] and regulation of gene expression [8].

Studies with Ca^{2+}-Channel Knockout Mice

Mice lacking specific VDCCs have been generated through targeted gene knockout. One caveat in interpreting currently available data from these animals is that most studies have used general, rather than tissue-specific knockout strategies. The resulting phenotypes may not be attributable to loss of Ca^{2+} channels in the β-cell per se. Nevertheless, the models provide interesting insights into the roles of VDCCs in insulin secretion and in vivo glucose homeostasis.

Schulla and co-workers used a model in which $Ca_v1.2$ was selectively ablated in β-cells using Cre/loxP recombination [64]. In control experiments, DHPs blocked 65% of the voltage-gated Ca^{2+} current in β-cells of $\beta Ca_v1.2^{+/+}$ mice, demonstrating the presence of both L-type and non-L-type channels. Strikingly, no DHP-sensitive Ca^{2+} current was observed in β-cells of $\beta Ca_v1.2^{-/-}$ mice. The absence of L-type Ca^{2+} channels in $\beta Ca_v1.2^{-/-}$ mouse β-cells was accompanied by glucose intolerance and loss of first-phase insulin secretion following intraperitoneal glucose injection. In vitro, the first phase of glucose-induced insulin secretion from perfused islets and the rapid component of exocytotic capacitance changes in isolated β-cells were both selectively attenuated by the mutation. These results demonstrate that $Ca_v1.2$ is the only L-type Ca^{2+} channel expressed in mouse β-cells (background strain C57BL/6), and that Ca^{2+} influx through these channels is indeed essential for first-phase insulin secretion. Interestingly, glucose-induced $[Ca^{2+}]_i$ oscillations and action potential firing persisted in β-cells of $Ca_v1.2^{-/-}$ mice. This would seem to indicate that L-type channels, while crucial for insulin secretion, play only a minor role in controlling β-cell electrical activity and $[Ca^{2+}]_i$ dynamics. However, this unexpected result contrasts with the typically potent inhibition of electrical activity and $[Ca^{2+}]_i$ rises in normal mouse β-cells by DHPs, and may be a consequence of compensatory overexpression of non-L-type channels in β-cells lacking $Ca_v1.2$. The fact that such compensation restores $[Ca^{2+}]_i$ signaling but not insulin secretion in $Ca_v1.2^{-/-}$ β-cells further emphasizes the preferential coupling of L-type channels to exocytosis.

Conflicting results were reported for mice lacking $Ca_v1.3$. In one study, β-cell Ca^{2+} currents were unaffected by loss of $Ca_v1.3$ [7]. In accord with this result, resting glucose and insulin levels were not altered in the mutant animals [52]. However, in another study, $Ca_v1.3$ null mice had low serum insulin levels and were glucose intolerant, owing to a reduction in β-cell mass [47]. In vitro glucose-induced insulin secretion was not affected by the mutation, and the only noticeable change in the properties of voltage-gated Ca^{2+} currents was a shift of the activation curve to more positive potentials. Based on these studies, it does not appear that $Ca_v1.3$ expression is necessary for normal stimulus-secretion coupling in mouse β-cells. The channels may be involved in regulating β-cell proliferation, but the mechanism underlying this function is not known.

Finally, two studies have reported the effects of loss of $Ca_v2.3$ on in vivo glucose homeostasis. In the first, mice lacking $Ca_v2.3$ were slightly glucose intolerant during IP injection, but this phenotype was attributed to a decrease in insulin sensitivity rather than defective insulin secretion [41]. In another study, glucose intolerance in $Ca_v2.3^{-/-}$ mice was associated with reduced glucose-stimulated insulin secretion in vivo and in vitro, suggesting a role for $Ca_v2.3$ in the β-cell [49]. However, since the effect of the $Ca_v2.3$ null mutation on Ca^{2+} currents, electrical activity and $[Ca^{2+}]_i$ signaling in β-cells was not reported, the mechanism underlying the insulin secretory defect following global knockout of $Ca_v2.3$ remains to be determined.

Voltage-independent Ca^{2+} Channels in the β-Cell

A number of non-selective, Ca^{2+}-permeable ion channels are present in β-cells and may serve as voltage-independent Ca^{2+} entry pathways. These include channels activated by glucose or agents that enhance glucose-induced insulin secretion, fueling speculation that they could play important physiological roles. For example, Rojas et al. identified a small, non-selective cation channel activated by glucose in human pancreatic β-cells [56]. The channel was permeable to Ca^{2+}, having a conductance of 4.9 pS with 25 mM Ca^{2+} in the pipette. These channels could account for the glucose-induced uptake of $^{45}Ca^{2+}$ that was observed in rat islets exposed to VDCC antagonists [28]. However, the contribution of this channel in glucose-induced $[Ca^{2+}]_i$ rises and insulin secretion has not yet been determined.

More recently, the potential roles of store-operated currents (SOCs) in the β-cell have received considerable attention. SOCs activate following release of Ca^{2+} from intracellular stores, and have been studied predominantly in non-excitable cells [48]. In the β-cell, agents that deplete IP_3-sensitive intracellular Ca^{2+} stores, such as the muscarinic agonist acetylcholine and the SERCA antagonist thapsigargin, enhance glucose-induced electrical activity [10, 72]. These agents also stimulate a sustained 'capacitative' Ca^{2+} influx, even when the cells are hyperpolarized [37, 46], suggesting that β-cells express a store-operated ionic current that is Ca^{2+}-permeable and voltage-insensitive. Worley and co-workers first

measured a candidate current in mouse β-cells, a non-selective cation current activated by removal of extracellular Ca^{2+} [72]. The current, called Ca^{2+} release-activated non-selective current (I_{CRAN}), is activated by maitotoxin (MIX) and blocked by SKF 96365 [55]. Mears and Zimliki later showed that acetylcholine and thapsigargin activate a similar current in HIT and mouse β-cells [43]. Although the non-selective, store-operated current in β-cells is Ca^{2+}-permeable, capacitative Ca^{2+} influx causes only a modest increase in $[Ca^{2+}]_i$, and does not stimulate secretion when β-cells are hyperpolarized. This suggests that the principal role of SOCs in insulin secretion is to provide additional depolarization in the presence of glucose to enhance voltage-dependent Ca^{2+} influx.

A Ca^{2+}-dependent, non-selective cation current, activated by glucagon-like peptide 1 (GLP-1), has been observed in HIT, mouse and human β-cells ($I_{CA\text{-}NS}$) [33, 34]. Like I_{CRAN}, $I_{CA\text{-}NS}$ is activated by MTX and blocked by SKF96365, but whether or not the currents are molecularly identical remains to be determined. A small channel that is activated by GLP-1 and MTX in cell-attached patches (30 pS with Na^+ as the permeating ion) is the likely carrier of $I_{CA\text{-}NS}$ [34]. The channel is active in the presence of low glucose, suggesting a role as a background conductance that depolarizes the membrane when K_{ATP} channels close [34].

Mammalian homologs of the *Drosophila* transient receptor potential channel (TRP) family have emerged as likely molecular candidates for voltage-independent, Ca^{2+}-permeable channels, including SOC channels [14]. Mouse β-cells express the TRP-related genes TRPC1 and/or TRPC4, both of which form non-selective, possibly store-operated ion channels in heterologous expression systems [55, 58]. However, the role of TRPC1 or TRPC4 as SOC channels in the β-cell is uncertain since overexpression of these genes in a mouse β-cell line (βTC3) did not produce detectable store-operated currents [54]. Future work in this emerging field, including the analysis of islet function in mice lacking specific TRP genes, is likely to shed new light on the molecular nature of voltage-independent Ca^{2+} channels in the β-cell and their roles in insulin secretion.

Conclusions and Perspectives

Nutrient-induced insulin secretion from pancreatic β-cells is critically dependent on membrane depolarization and Ca^{2+} influx. These cells are endowed with numerous Ca^{2+}-entry pathways, many of which have been identified molecularly. Among these, L-type voltage-gated Ca^{2+} channels unquestionably play the most prominent role in insulin secretion. However, the classical view that these channels control insulin secretion simply by regulating the bulk cytosolic Ca^{2+} concentration is an oversimplification. More precisely, these channels directly control the dynamics of stimulated insulin secretion through physical interactions with the cellular secretory machinery. The pool of granules associated with L-type channels is exposed to high Ca^{2+} levels upon depolarization, and their rapid exocytosis produces the first phase of insulin secretion. Calcium influx through other voltage-dependent and independent Ca^{2+} channels in the β-cell is responsible for, at most, a small fraction of glucose-stimulated insulin secretion. These channels may regulate exocytosis by contributing to global $[Ca^{2+}]_i$ rises during prolonged depolarization, by influencing β-cell electrical activity, or by activating Ca^{2+}-dependent amplifying pathways.

Ongoing studies of Ca^{2+}-channel structure-function relationships are likely to yield new insights into their roles in regulating insulin secretion. For example, it will be interesting to determine if Ca^{2+} channels interact with other proteins on the secretory granule or intracellular organelles. One intriguing suggestion is that L-type Ca^{2+} channels in the β-cell could couple directly to intracellular ryanodine receptors, as is well-documented for $Ca_v1.1$ in skeletal muscle cells [36]. Although the role of ryanodine receptors in the β-cell is controversial, such a coupling could allow L-type channels to directly activate an amplifying signal for Ca^{2+}-dependent exocytosis. Other nascent areas of investigation include the mechanisms that regulate Ca^{2+} channel trafficking and the functional roles of Ca^{2+} channel auxiliary subunits [24, 25]. Ultimately, it is likely that studies in this field will lead to a more complete understanding of the membrane events involved in metabolic regulation of insulin secretion, and how defects in these processes contribute to the development of type II diabetes.

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