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Review

Localized Calcium Influx in Pancreatic β-Cells

Its Significance for Ca^{2+}-Dependent Insulin Secretion from the Islets of Langerhans

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Ca^{2+} influx through voltage-dependent Ca^{2+} channels plays a crucial role in stimulus-secretion coupling in pancreatic islet β-cells. Molecular and physiologic studies have identified multiple Ca^{2+} channel subtypes in rodent islets and insulin-secreting cell lines. The differential targeting of Ca^{2+} channel subtypes to the vicinity of the insulin secretory apparatus is likely to account for their selective coupling to glucose-dependent insulin secretion. In this article, I review these studies. In addition, I discuss temporal and spatial aspects of Ca^{2+} signaling in β-cells, the former involving the oscillatory activation of Ca^{2+} channels during glucose-induced electrical bursting, and the latter involving $[Ca^{2+}]_i$ elevation in restricted microscopic "domains," as well as direct interactions between Ca^{2+} channels and secretory SNARE proteins. Finally, I review the evidence supporting a possible role for Ca^{2+} release from the endoplasmic reticulum in glucose-dependent insulin secretion, and evidence to support the existence of novel Ca^{2+} entry pathways. I also show that the β-cell has an elaborate and complex set of $[Ca^{2+}]_i$ signaling mechanisms that are capable of generating diverse and extremely precise $[Ca^{2+}]_i$ patterns. These signals, in turn, are exquisitely coupled in space and time to the β-cell secretory machinery to produce the precise minute-to-minute control of insulin secretion necessary for body energy homeostasis.

Key Words: Insulin secretion; calcium channels; islets of Langerhans; β-cells.

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Introduction

Although it has been known for some time that a rise in intracellular $[Ca^{2+}]_i$ due to extracellular Ca^{2+} influx is important for physiologic insulin secretion in pancreatic islet β-cells (for review, *see* refs. *1–4*), recent work has provided much more detailed insight into these processes. Specifically, much has been learned about the molecular identity, structure, and location of the Ca^{2+} channels that mediate β-cell Ca^{2+} influx, and how influx is coupled to insulin granule exocytosis. In addition, several alternative Ca^{2+} signaling mechanisms have been identified that may provide parallel pathways for increasing $[Ca^{2+}]_i$. These topics are the subject of the present review.

For the sake of brevity, I regrettably cannot discuss all the work done in this large area of research. And, although there is emerging evidence to suggest that not all insulin secretion is fully dependent on the closing of adenosine triphosphate (ATP)-sensitive K channels (K_{ATP}) and, concomitantly, Ca^{2+} influx *(5–7)*, the emphasis in this review is on Ca^{2+}-dependent insulin secretion, because the bulk of past, as well as current, data supports the hypothesis that Ca^{2+}-dependent insulin secretion is the primary mode of glucose-dependent secretion under physiologic conditions. My goal is to provide the reader with a current view of calcium-dependent insulin secretion, with an emphasis on the voltage-dependent channels that are spatially coupled to insulin secretion. In addition, I discuss evidence that intracellular Ca^{2+} release and novel Ca^{2+} influx pathways are involved in the control of insulin secretion.

Physiologic Insulin Secretion Is Calcium Dependent

Numerous experiments conducted over many years have established that insulin secretion is calcium depen-

dent. Most of the available data are based on experiments conducted using freshly isolated rodent islets or insulin-secreting cell lines. The arguments in support of the Ca^{2+} Hypothesis are as follows. First, the removal of extracellular calcium has generally been found to block glucose-dependent insulin secretion (reviewed in refs. *4, 8,* and *9; see also* refs. *10–12*). Second, pharmacologic inhibitors of voltage-gated Ca^{2+} channels block insulin secretion, often to near basal levels *(13–20)*. Third, as a direct test of the Ca^{2+} Hypothesis, experiments carried out using electrically permeable islets demonstrated that islets secrete insulin in response to elevated $[Ca^{2+}]_i$ *(21–23)*. Fourth, simultaneous measurements of insulin secretion and islet or β-cell $[Ca^{2+}]_i$ show a high temporal corellation between these variables, with changes in calcium leading secretion *(10,20,24–26)*. Finally, activation of whole-cell Ca^{2+} current by a brief depolarizing voltage pulse triggers a rapid phase of insulin granule exocytosis. Exocytosis can be monitored electrically in single cells by measuring cell capacitance. An increase in cell membrane capacitance following transient Ca^{2+} entry reflects the increased fusion and, ultimately, the release of secretory granules from the cell *(27–29)*.

Calcium-dependent insulin secretion is likely to be significant for understanding type II diabetes mellitus, because this disease is associated with impaired insulin secretion, as well as insulin resistance *(30)*, and a number of rodent models of diabetes have been shown to exhibit significant impairments in islet Ca^{2+} influx or Ca^{2+} handling mechanisms, which negatively impact their ability to secrete *(31–35)*. It has also been demonstrated that experimentally induced hyperglycemia can downregulate Ca^{2+} channel expression in islets *(36)*.

Concensus Model of Stimulus Secretion Coupling in β-Cells and Islet Bursting

Because the major physiologic stimulator of Ca^{2+}-dependent insulin secretion is glucose, we must consider how glucose metabolism can raise β-cell $[Ca^{2+}]_i$. The current consensus model is that glucose is taken up by the β-cell by the GLUT-2 transporter and is then metabolized to generate an increase in the ATP /adenosine 5'-diphosphate (ADP) ratio *(1,2)*. This, in turn, leads to the inhibition of K_{ATP} channels in the β-cell plasma membrane. Because K_{ATP} conductance is dominant in these cells, channel closure following glucose metabolism results in β-cell depolarization *(37–41)*. This depolarization leads to the opening of voltage-gated Ca^{2+} channels in the membrane, producing an increase in $[Ca^{2+}]_i$, which stimulates insulin granule exocytosis *(2,42)*.

Although this model is generally well accepted, it is an oversimplification. The "depolarization" of the cell is not a simple phenomenon involving one or two types of channels, but a complex process termed *bursting*, which involves many interacting β-cell ion channels. If the membrane potential of a whole islet bathed in low [glucose] is measured with a sharp microelectrode, the islet membrane potential resides at about –75 mV. As [glucose] is raised to near 7 m*M*, K_{ATP} channels close, producing a subthreshold depolarization. As [glucose] is further increased, the islet membrane potential begins to exhibit rhythmical electrical waves or plateau depolarizations lasting 10–20 s, which, in turn, trigger a burst of superimposed rapid Ca^{2+}-dependent action potentials *(43–47)*. If [glucose] is increased further, the membrane potentials of the interburst, plateau, or spike peaks do not change, but the relative amount of time spent in the active spiking phase increases, such that by 300 mg/dL, the islets continuously spike from the tops of the extended plateaus. The glucose dependence of islet electrical activity, expressed as the percentage of time the islets are in the spiking or plateau active phase, closely matches that of insulin secretion *(45)*.

The rapid depolarizing spikes and the slow plateaus that characterize islet electrical activity are mediated by the activation of voltage-dependent Ca^{2+} channels *(48–50)*. In addition, simultaneous measurements of $[Ca^{2+}]_i$ and electrical activity in mouse islets clearly demonstrate that oscillations in $[Ca^{2+}]_i$ occur in phase with the burst plateaus *(26,51)*. Corresponding oscillations in insulin secretion from islets have also been reported *(25,26,52,53)*. This supports the hypothesis that oscillations in $[Ca^{2+}]_i$ and concomitant oscillations in insulin secretion are at least partly regulated by glucose metabolism through modulation of bursting electrical activity *(54)*. The adaptive advantage of this mechanism likely stems from the very wide dynamic range provided by islet oscillatory behavior (when one considers electrical bursting as well as $[Ca^{2+}]_i$ oscillations), in which frequencies as low as 0.01 Hz and as high as 10 Hz have been observed *(46,47,54)*.

Voltage-Dependent Ca^{2+} Channels: Mediators of Extracellular Ca^{2+} Influx

We now know a considerable amount about the voltage-dependent Ca^{2+} channels that open following membrane depolarization in excitable cells. This knowledge originally stemmed from many electrophysiologic studies of whole-cell β-cell Ca^{2+} currents or single Ca^{2+}channels. More recently, new insights into the structure and regulation of voltage-dependent Ca^{2+} channels have resulted from the application of molecular biologic approaches.

Many cardiac, neuronal, and endocrine preparations have now been shown to possess multiple classes or subtypes of voltage-gated Ca^{2+} channels *(55–57)*. The original evidence for multiple types of Ca^{2+} channels came from studies showing that the whole-cell Ca^{2+}currents of cardiac or neuronal cells could be fractionated into different components having distinct biophysical characteristics and pharmacology *(57–61)*. Single-channel studies provided even clearer separation, because the single channel or unit

conductances of the different types of Ca^{2+} channels could be distinguished by their characteristic conductances, gating pattern, or drug sensitivity *(55,61)*.

Whole-cell patch clamp studies initially identified two current components having different activation voltage thresholds and dihydropyridine sensitivity: high- and low-voltage-activated Ca^{2+} currents *(55)*. High-voltage-activated "L-type" Ca^{2+} channels activate at potentials less than –40 mV, inactivate relatively slowly, and are selectively blocked by the dihydropyridine Ca^{2+} channel blockers nifendipine and nimodipine *(55,62)*. The single-channel conductance of these channels ranges from 20 to 25 pS (in 100–110 m*M* $BaCl_2$), and these channels are expressed in cardiac cells, skeletal muscle, endocrine cells, and neurons *(55,61)*.

By contrast, low-voltage-activated "T-type" Ca^{2+} channels (which are also expressed in certain cardiac, endocrine, and nerve cells) activate near –70 mV, inactivate rapidly through a voltage-dependent mechanism, and are insensitive to dihydropyridines *(57,63,64)*. Progress in the T-channel field has been hampered by the lack of selective T-channel blockers, although some blockers such as octanol and amiloride have been reported *(65–67)*.

Subsequent research revealed additional "high-voltage" Ca^{2+} channels, primarily in brain tissue. N-type Ca^{2+} channels are distinguishable from L-channels by their resistance to dihydropyridines and their selective blockade by ω-conotoxin GVIA. L-type channels are generally resistant to this toxin *(68,69)*. High-voltage P-type Ca^{2+} channels are insensitive to both dihydropyridines and ω-conotoxin GVIA, but are selectively blocked by agatoxin IVA *(70,71)*. A more recently described high-voltage-activated Ca^{2+} channel, the Q/R channel, is similar to the P-channel, but is agatoxin resistant *(69)*.

Molecular biology has contributed greatly to our understanding of the structural biology of the voltage-dependent Ca^{2+} channels. All of the major voltage-gated Ca^{2+} channels have now been cloned, and are classified as members of the voltage-gated ion channel superfamily based on their shared sequence homology and their homology with other voltage-gated channels *(10,57,58,72)*. The L-type channel, like the voltage-dependent Na^+ channel family prototype, is a multimeric protein consisting of five subunits: α_1, α_2, β, δ, and γ *(10,73)*. Expression of the L-channel α_1 subunit alone in heterologous expression systems results in functional Ca^{2+} current *(59)*. However, expression of the full complement of subunits increases the level of Ca^{2+} channel expression and more fully reconstitutes the native phenotype *(59)*.

The topology of the L-channel α_1 subunit consists of four repeated homologous domains, I–IV, each of which is composed of six transmembrane-helices, S1–S6 *(10,58,72,73)*. The entire subunit folds to form a functional aqueous pore, and the S4 elements with their characteristic charge spacing are believed to act as the voltage sensor of the channel *(10)*. This voltage sensor is the part of the ion channel that senses changes in the membrane electric field (e.g., depolarization), and then transduces these changes so as to change the gating state of the channel *(73)*. An intracellular loop between domains II and III is believed to mediate a specific interaction between the molecule, and SNARE proteins of the exocytotic pathway *(59,74)*.

At least 10 Ca^{2+} channel α_1-subunit genes have now been cloned, and many have been identified as being the molecular counterparts of specific Ca^{2+} channel phenotypes *(59,69,74,75)*. The expression of three of these α_1 subunits (along with their splice variants) results in functional dihydropyridine-sensitive, L-type channels: the α_{1C} isoform, which is predominant in cardiac tissue *(76)*; the α_{1D} isoform, the neuroendocrine type, which is present in nerve and endocrine cells *(77)*; and α_{1S}, the skeletal muscle isoform *[58,78]*; for very concise lists and classification, *see* refs. *75* and *79)*.

By contrast, ω-conotoxin GVIA-sensitive N-type channels are believed to result from the expression of the α_{1B} isoform, whereas P- or Q-channels appear to result from α_{1A} isoform expresion, depending on the specific tissue involved *(69)*. R-channels, which are insensitive to dihydropyridines, ω-conotoxin GVIA, and agatoxin, are mediated by the α_{1E} isoform *(69)*. Recently, Perez-Reyes et al. *(63)* identifed a gene in a rat brain library they called α_{1G}, whose expression in a heterologous oocyte system resulted in functional T-type Ca^{2+} currents.

The molecular as well as the phenotypic diversity of Ca^{2+} channels is likely very large not only because there are many α_1 genes, with corresponding splice variants, but because there are also at least four β-subunit genes, β_{1-4}, as well as the other subunits present in native Ca^{2+} channels *(57,59)*.

Voltage-Dependent Ca^{2+} Currents of Insulin-Secreting Cells

Progress in understanding the Ca^{2+} currents of heart and brain tissues has spurred progress in our understanding of the Ca^{2+} influx mechanisms of endocrine cells, including insulin-secreting islet or cell preparations. Broadly speaking, the major high-voltage component of whole cell Ca^{2+} current in insulin-secreting cells typically activates near –50 mV, reaches its peak amplitude (≤200 pA, depending on the ionic conditions) between –10 and +10 mV, and reverses beyond +50 mV. The transient (<0.1s) activation of Ca^{2+} channel current in β-cells produces sufficient Ca^{2+} influx to cause a corresponding transient elevation in $[Ca^{2+}]_i$ *(80,81)*, and insulin exocytosis *(29,42,82)*.

L-type Ca^{2+} channels have been found in virtually all islet β-cells and insulin-secreting cells lines, and appear to mediate the bulk of the Ca^{2+} influx required for sustained insulin secretion. The electrophysiologic evidence to support this conclusion is based on a multitude of whole-cell

patch clamp studies showing a high-voltage-activated and dihydropyridine-sensitive Ca^{2+} current in primary β-cells or insulinoma cells (mouse β-cells: *[83–86]*; human β-cells: *[87–89]*; dog β-cells: *[90]*; RINm5F cells: *[91–94]*; HIT cells: *[16,17,95,96]*; INS-1 cells: *[97]*; rat β-cells: *[98,99]*). A smaller number of single-channel studies, carried out using 100–110 m*M* Ba^{2+} as a charge carrier, have directly identified 21 pS L-type Ca^{2+} channels in β-cells and insulin-secreting cell lines (rat: *[87,100]*; mouse: *[101]*; RIN cells: *[102,103]*).

Molecular studies have identified multiple types of L-channel isoforms in β-cells and insulin-secreting cell lines. Perez-Reyes et al. *(77)* found that HIT cells contained three separate genes encoding the skeletal muscle L-channel isoform, α_{1s} (CaCh1 in their original nomenclature; *see 79)*; the cardiac isoform, α_{1C}, and a neuroendocrine L-channel isoform, α_{1D}. In addition, all three genes were found to undergo alternative splicing. Yaney et al. *(104)* confirmed that α_{1D} was expressed in HIT cells, an INRI-G9 hamster islet cell line, rat pancreas, βTC-3 cells, and two RIN cell lines. Seino et al. *(105)* cloned α_{1D} and α_{1C} from human islets, and confirmed that α_{1D} was present in RIN, βTC-3, and rat β-cells but, surprisingly, not HIT cells. Iwashima et al. *(36)* reported a 2.5-fold greater level of α_{1D} vs α_{1C} message in rat islets, consistent with the findings of Horvath et al. *(97)* that α_{1D} expression mediated the dominant Ca^{2+} channel of INS-1 cells. INS-1 cells were found to express α_{1D} and α_{1C} isoforms as well as α_{1S}, although α_{1S} expression was inconsistent *(97)*. Ihara et al. *(106)* functionally expressed the β-cell form of α_{1D} in Chinese hamster ovary cells, resulting in L-type Ca^{2+} current. Subsequent studies by Iwashima et al. *(36)* demonstrated that α_{1D} expression could be downregulated by glucose infusion. At present, the relative contribution of the three L-channel α_1 isoforms to insulin secretion (or islet electrical activity) is not known, owing to the lack of isoform-selective blockers or reagents. The generation of knockout mice lacking specific L-channel isoforms in their β-cells will likely address this area of question in the not-too-distant future.

Although most workers in the islet field agree that L-channels are important for insulin secretion, the identity and the putative role of non-L-type, high-voltage Ca^{2+} channels in β-cells and cell lines are more controversial *(97)*. Insulin-secreting cell lines and some primary cell preparations have been reported to express high-voltage, non-L-type Ca^{2+} channels, in addition to L-channels. Thus, several investigators obtained evidence for the coexistence of dihydropyridine-blockable L-channels, and ω-conotoxin-blockable N-channels in RINm5F cells *(93,94,107,108)*. Insulin secretion from these cells was partially inhibited by ω-conotoxin GVIA, leading these investigators to conclude that there was functional coupling between Ca^{2+} influx through N-channels and insulin exocytosis *(108)*. Furthermore, a residual current that was present after pharmacologic inhibition of both L- and N-type channels mediated about 30% of total RIN cell Ca^{2+} current. This residual current was carried by an unusual channel that activated above –20 mV, slowly inactivated, and was blocked by Aga-IV toxin, suggesting mediation by P- or Q-type Ca^{2+} channels *(102,109)*. In contradiction to these findings, Schmidt et al. *(93)* reported that RIN cell Ca^{2+} current was insensitive to GVIA, and Roenfeldt et al. *(110)* similarly found no effect of GVIA on KCl-induced insulin secretion from these cells.

In addition to multiple L-channel isoforms, Horvath et al. *(97)* obtained evidence supporting the existence of P/Q-type (α_{1A}), but not N-type channels in INS-1 cells. INS-1 cell Ca^{2+} current was blocked by nifedipine or agatoxin, but was insensitive to ω-conotoxin GVIA *(97)*. Interestingly, 1 μ*M* nifedipine was found to fully block the rise in $[Ca^{2+}]_i$ seen with stimulation by 10 m*M* glucose, suggesting the participation of L-type channels *(97)*.

The situation in HIT cells has also been controversial. Keahy et al. *(16)* concluded that only a single class of L-type Ca^{2+} channels mediates HIT cell Ca^{2+} current, because both this current and KCl-stimulated insulin secretion were nearly fully blocked by nimodipine. In accordance with these findings, Marchetti et al. *(111)* reported that conotoxin GVIA had no effect on HIT cell Ca^{2+} current. By contrast, Satin and Cook *(96)* found that a large fraction of HIT cell Ca^{2+} current was nimodipine insensitive, suggesting a role for some other, non-L-type channel. Satin et al. *(17)* subsequently reported that even 100 μ*M* nimodipine could only inhibit 55% of the total Ca^{2+} current in these cells. The application of the N-channel blocker ω-conotoxin MVIIA (or GVIA) at 1 μ*M* blocked 35% of the total Ca^{2+} current; this block was additive to that produced by nimodipine, such that the coapplication of these drugs blocked 85% of the total HIT cell Ca^{2+} current *(17)*. One assumes that a non-L, non-N-type Ca^{2+} current must mediate the small (≈15%) residual Ca^{2+} current that remains in the presence of saturating doses of nimodipine and MVIIA *(17)*. Although the reasons for the discrepancies between the findings of Satin et al. *(17)* and Keahy et al. *(16)* are not clear, one obvious possibility is that not all HIT cells (or RIN cells) express the same channels. Differences in the experimental conditions may also have contributed to the divergent results *(see* Discussion in ref. *17*).

Adult mouse islet β-cells have been shown to possess classic L-channels *(17,84,112),* but there has been some controversy as to whether an additional Ca^{2+} channel might also be present. Hopkins et al. *(85)* distinguished two current components in these cells that differed in their inactivation rates, mechanisms of inactivation, and pharmacology. Subsequent work in the field has emphasized the primacy of L-channels for mouse islet electrical activity and secretion *(1,3,113)*, yet it is difficult to rule out completely the possibility that other Ca^{2+} channels might contribute to the whole-cell Ca^{2+} current. Although recent studies have emphasized that ω-conotoxin GVIA is without effect on mouse β-cell Ca^{2+} current *(3,114)*, it is inter-

esting that dihydropyridine antagonists are usually unable to fully block mouse β-cell Ca^{2+}, suggesting that a residual, dihydropyridine-insensitive current may be present, as in HIT and RIN cells.

In rat β-cells, arachidonic acid-stimulated rises in $[Ca^{2+}]_i$ were observed that were GVIA sensitive, consistent with the participation of N-channels in this process *(115)*. Using measurements of insulin secretion, Komatsu et al. *(18)* found that 100 n*M* GVIA blocked second-phase, glucose-dependent insulin secretion from rat islets, but, paradoxically, did not alter first-phase secretion or the secretion elicited by KCl. Ohta et al. *(19)* reported results similar to those of Komatsu et al. *(18)*, but attributed their effects to drug actions on islet energetics.

Ligon et al. *(116)* found that following the blockade of α_{1D}-type L-channels in rat islets, a small fraction of insulin secretion remained that was insensitive to ω-conotoxin GVIA, but was blocked by ω-Aga-IVa, suggesting the participation of P-channels in stimulus-secretion coupling. These data are in accordance with reverse transcriptase polymerase chain reaction data revealing different α_{1A} isoforms in rat islets, and additional findings obtained using antibodies and cRNA probes for α_{1A} *(116)*.

Human islet β-cells possess L-channels, as already described, but also have a component of current that is resistant to blockage by nifedipine *(94)*. These cells do not appear to have N-channels, and the nature of this non-L- type current is presently unknown.

Low-Threshold Ca^{2+} Channels in β-Cells

The role of low-threshold T-type channels in islet physiology and pathophysiology has been less well studied than other Ca^{2+} channels. T-type channels have been reported in rat *(98,100,117)*, human *(88,89),* and nonobese diabetic (or NOD) mouse β-cells, as well as in INS-1 *(64)* and RINm5F cells. The channel appears to be absent from islets of Swiss-Webster or NMRI mice, and HIT and βTC-3 cells *(64,95,112)*.

It has been argued that the presence of a low-threshold Ca^{2+} current would be expected to facilitate islet excitability or bursting *(118)*, as is the case in thalamic neurons *(57,63,119)*. T-channels cannot be mandatory for bursting, because mouse β-cells lack T-channels but are the classic preparation for studying islet bursting. However, T-channels may play an important modulatory role in other species. For instance, T-current activation even under nonstimulatory conditions may raise basal $[Ca^{2+}]_i$ levels in β-cells from NOD mice, although other processes are also known to be involved in basal $[Ca^{2+}]_i$ regulation *(67)*. Interestingly, T-channel current can be upregulated by cytokine treatment of rodent cells, which has led to speculation that the activation of low-threshold T- current may be involved in β-cell apoptosis triggered by increased $[Ca^{2+}]_i$ in type I diabetes mellitus *(120)*. The recent cloning of the T-channel gene in INS-1 insulinoma cells *(64)* will likely help resolve some of these important issues, because it should now be possible to construct targeted T-channel knockout animals, and develop novel reagents or antisense oligonucleotides to definitively probe T-channel function in islets or cultured cell lines.

Spatial Colocalization of Ca^{2+} Channels with Exocytotic Machinery of β-Cells

Understanding the role of Ca^{2+} channels in islet stimulus secretion coupling is further complicated by the fact that the localization of these isoforms in the cell membrane may be crucial for efficient coupling to secretion. Several lines of evidence support the hypothesis that β-cell Ca^{2+} channels are spatially localized to be in close proximity to the insulin granule secretory apparatus. First, Ca^{2+} channel blockers differentially affect Ca^{2+} current and insulin secretion *(17)*. For instance, a saturating dose of the L-channel blocker nimodipine, which blocked 55% of HIT cell Ca^{2+} current, completely abolished fractional insulin secretion, which decreased from 4.5% of HIT cell insulin content per hour to near basal levels. Satin et al. *(17)* had originally hypothesized that nimodipine would only partially inhibit insulin secretion, because it only partly inhibits its Ca^{2+} current. In addition, ω-conotoxin MVIIA was without effect on glucose-dependent secretion at concentrations up to 10 μ*M*, despite the fact that even 1 μ*M* MVIIA blocked 35% of HIT cell Ca^{2+} current. In neurons, different Ca^{2+} channel subtypes are localized to different anatomical regions of the cell and have been found to differentially support neurosecretion *(59,61,121–125)*. Because central neurons mainly secrete neurotransmitters from their processes, rather than their somata, the differential localization of Ca^{2+} channel isoforms makes sense from a functional standpoint. Pancreatic β-cells are more simple in terms of their cytoarchitecture, but are also known to be polarized *(126,127)*.

Satin et al. *(17)* thus formulated the hypothesis that the Ca^{2+} that enters the β-cell through nimodipine-blockable L-type channels, but not Ca^{2+} permeating through conotoxin-blockable N-type channels, would be more tightly coupled to insulin exocytosis if the L-channels were relatively closer to the sites on the secretory pole of the cell where exocytosis occurs (*see also* ref. *127*). As mentioned earlier, even when Ca^{2+} influx through non-L-type Ca^{2+} channels has been shown to be coupled to insulin secretion, the relative amount of this secretion is small compared to L-channel-mediated secretion *(108,116)*. In this regard, the β-cell seems to be the inverse of the situation in central neurons, in which neurosecretion generally appears to be much more tightly coupled to Ca^{2+} influx through high-voltage, dihydropyridine-insensitive Ca^{2+} channels *(59,74,124,125)*.

Bokvist et al. *(127)* provided additional, elegant data to support the hypothesis that L-type Ca^{2+} channels are

colocalized near the mouse β-cell secretory apparatus. By exploiting the fact that insulin secretory granules accumulate the fluorescent dye quinacrine, they could visualize the granules by their fluorescence in living cells, and then patch clamp the cells in different regions *(127)*. Image analysis of quinacrine-loaded mouse β-cells revealed that the insulin-containing granules mainly resided near one pole of the cell. If cells were patch clamped in the vicinity of the secretory granules, membrane patches contained clusters or "hot spots" of single Ca^{2+} channel-opening events. By contrast, recording from regions of the cell that were more distant from the granules resulted in few if any Ca^{2+} channel-opening events *(127)*. This is consistent with the hypothesis that β-cell Ca^{2+} channels are selectively targeted to membrane domains close to the secretory apparatus of the cell, presumably in order to efficiently supply the apparatus with stimulatory levels of $[Ca^{2+}]_i$.

Another line of evidence in favor of this hypothesis comes from imaging $[Ca^{2+}]_i$ changes in single β-cells using Indo-1 following Ca^{2+} current activation *(127)*. $[Ca^{2+}]_i$ could be seen to rise in a small, restricted volume just under the plasma membrane 0.28 s after a pulse depolarization commenced. At later times, this local rise in $[Ca^{2+}]_i$ spread into the bulk of the cytoplasm. This confirms that Ca^{2+} influx is very likely highly localized, particularly immediately after the Ca^{2+} channels open. Although these data are consistent with the notion that there is punctate localization of Ca^{2+} channels in the plasma membrane, it cannot be ruled out that restrictive dye distribution, or the localization of the intracellular $[Ca^{2+}]_i$-handling mechanisms, could also account for the $[Ca^{2+}]_i$ pattern observed. However, on-cell patch clamp recordings of single L-channels in mouse β-cells typically contain multiple, rather than single, opening events per patch, and some patches are reported to lack any channel openings *(101)*. This suggests that there are hot spots of Ca^{2+} channels in the membrane.

Models of cellular $[Ca^{2+}]$ dynamics have suggested that when single Ca^{2+} channels open, there can exist highly restricted spatial domains near the channel mouth where $[Ca^{2+}]_i$ may rise to concentrations of 100 μ*M* or more *(128,129)*. This contrasts with recorded levels of whole-cell $[Ca^{2+}]_i$, which are typically 50–100 n*M*, and which typically double or triple following glucose stimulation. The "domain hypothesis" thus posits that microscopic regions exist that greatly elevate local $[Ca^{2+}]$ near the open mouth of individual Ca^{2+} channels, and that these domains have functional significance for the cell. The existence of $[Ca^{2+}]$ domains has been proposed to account for $[Ca^{2+}]$-dependent processes such as Ca^{2+} channel inactivation by $[Ca^{2+}]$ *(130),* as well as $[Ca^{2+}]$-dependent neurotransmitter release *(131)*. Several factors can affect whether domains will exert prominent effects or not, including the density of the single Ca^{2+} channels in the membrane, and the size of the single-channel current at the particular voltage being studied *(130)*.

How might the presence of very high, very localized changes in $[Ca^{2+}]_i$ be an advantage to the β-cell? The close proximity of the Ca^{2+} channels to the exocytotic release sites may be required for $[Ca^{2+}]_{domain}$ to remain elevated long enough to activate Ca^{2+}-dependent exocytotic proteins such as synaptotagmin (which may require tens of micromoles of $[Ca^{2+}]$) *(42)*, before rapidly dissipating *(132)*. In addition, the SNARE hypothesis stipulates that the targetting, docking, fusion, and release of secretory granules from cells during exocytosis requires the participation of interacting granule and plasma membrane SNARE proteins, as well as soluble proteins *(132,133)*. By supplying Ca^{2+} very locally to these exocytotic proteins, intimate spatial coupling might even be essential for Ca^{2+}-dependent exocytosis to occur at all. However, if only rapid (<1 s), microscopic changes in domain $[Ca^{2+}]$ are essential for Ca^{2+}-dependent insulin exocytosis, it is not clear how the islet would produce slower, more sustained phases of Ca^{2+} secretion because these domains dissipate quickly.

Recent studies suggest that Ca^{2+} channel activity may also modulate secretion via direct intermolecular interactions. A cytoplasmic loop between domains II and III of the α_1-subunit of voltage-dependent Ca^{2+} channels has been found to contain a segment known as a synprint sequence (for synaptic protein interaction domain; reviewed in refs. *59* and *134*) that is believed to physically interact with the SNARE proteins syntaxin, synaptotagmin, and SNAP-25, resulting in the modification of exocytosis (for β-cells *see* ref. *135*; for review *see* ref. *59*). Interestingly, this interaction may be bidirectional, because syntaxin and synaptotagmin, e.g., appear able to modify the gating of the plasmalemmal Ca^{2+} channels that are in contact with the secretory vesicles *(136,137)*. We are thus on the verge of a new era in our understanding of just how important the spatial localization of Ca^{2+} channels to secretory granules may be for the regulation of exocytosis in β-cells, as well as for other secretory cells.

Relative Importance of Ca^{2+} Influx vs Ca^{2+} Release from Intracellular Stores for Glucose-Dependent Insulin Secretion

The previous section demonstrated that islets possess a tightly controlled electrical bursting mechanism that can provide exquisite control of Ca^{2+} influx through Ca^{2+} channels over a wide dynamic range. Thus, β-cells have evolved an elaborate mechanism to ensure adequate Ca^{2+} influx, and, concomitantly, elevations in $[Ca^{2+}]_i$ for Ca^{2+}-dependent insulin secretion.

However, in addition to glucose-mediated increases in $[Ca^{2+}]_i$ due to Ca^{2+} influx, it has been proposed that glucose metabolism stimulates the release of Ca^{2+} from intracellular stores *(138,139)*. It is known that Ca^{2+} is stored within cells in the endoplasmic reticulum (ER) *(140)*. In β-cells, there is strong evidence for an inositol triphosphate (IP_3)-

sensitive intracellular Ca^{2+} pool that is discharged by increases in intracellular IP_3. IP_3 opens IP_3-receptor Ca^{2+} (IP_3-R) channels in the ER, allowing Ca^{2+} to enter the cytoplasm *(41,141–145)*. Additionally, there are data to support the existence of an IP_3-insensitive intracellular Ca^{2+} pool in β-cells that is sensitive to caffeine or ryanodine. This pool has been linked to Ca^{2+} or cyclic ADP ribose (c-ADPR) induced Ca^{2+} release in cells via the opening of ryanodine-receptor Ca^{2+} channels (RyR) in the ER *(145–148)*. IP_3-R channels are also regulated by Ca^{2+}, and both IP_3-R and RyR display calcium-induced Ca^{2+} release *(140)*.

Islet ER Ca^{2+} release has been widely accepted as a major, although not exclusive, pathway mediating the action of acetylcholine, which potentiates glucose-dependent insulin secretion by binding to muscarinic receptors on β-cells, and activating phospholipase C (PLC) *(41)*. However, the hypothesis that glucose releases stored Ca^{2+} has been far more controversial, despite the fact that it has been considered for at least a decade (*see* ref. *4* for a review of the older literature, ref. *149* for a newer review).

Several novel mechanisms have been proposed to couple glucose metabolism to intracellular Ca^{2+} release. Roe et al. *(138)* suggested that glucose released Ca^{2+} through a voltage-dependent mechanism in mouse β-cells, and that this pathway accounted for the majority of the rise in $[Ca^{2+}]$ observed with the sugar. They based this conclusion on their finding that glucose was able to increase $[Ca^{2+}]_i$ in islet cells even when the cells were bathed in solutions containing low extracellular $[Ca^{2+}]_i$, and that the effect of glucose could be mimicked by KCl depolarization *(138)*. The increases in $[Ca^{2+}]_i$ they observed were insensitive to ryanodine, but altered by caffeine, which potentiates release from RyR-mediated stores *(146)*. Curiously, the Na^+ channel blocker TTX abolished glucose- but not KCl-induced Ca^{2+} release.

Evidence obtained in βTC-3 cells supports a similar model in which glucose may release stored Ca^{2+} via the voltage-dependent activation of PLC, resulting in increased IP_3 *(139)*. Depolarizing conditions altered cellular inositol phosphate content and raised $[Ca^{2+}]$, although changes in the former were small, 5% of total content, and did not appear to clearly precede the changes in intracellular $[Ca^{2+}]$. Gromada et al. *(139)* speculated that membrane depolarization may directly activate PLC by facilitating its interaction with its plasma membrane-delimited phospholipid substrates.

Glucose metabolism has also been reported to increase the cytoplasmic concentration of c-ADPR, proposed as an endogenous ligand of the ER RyR *(150)*. Microsomes prepared from rat pancreatic β-cells released Ca^{2+} in response to c-ADPR, but not IP_3 *(150)*, suggesting that c-ADPR may be an important intracellular signal in the β-cell. Glucose increased c-ADPR levels in these microsomes as well *(150)*. At least two groups have reported low but measurable levels of RyR isoforms in β-cells, suggesting a functional role for RyR in β-cell Ca^{2+} signaling *(148,151)*. IP_3-Rs are coexpressed in the ER as well as the secretory granules of β-cells *(141)*.

However, the islet literature is conflicting regarding the effectiveness of caffeine and ryanodine on β-cell $[Ca^{2+}]_i$ signaling or islet electrophysiology, because it is replete with both positive *(138,146–148,151)* and negative findings *(144,152–154)*. A possible complexity in interpreting the literature is that protein kinase A-dependent phosphorylation of RyR which could be variable may enhance the ability of the stores to mediate Ca^{2+} release. This suggests that the state of the cells used in a particular study may in part determine how big a quantitative contribution RyR makes to the changes in $[Ca^{2+}]_i$ being studied *(147,148,151)*.

These results are intriguing, but there are also concerns about the hypothesis that glucose or membrane depolarization releases significant amounts of stored Ca^{2+}. First, most investigators have found that the addition of Ca^{2+} channel blockers, or the removal of external Ca^{2+}, abolishes increases in $[Ca^{2+}]_i$ or insulin secretion owing to depolarizing stimuli such as KCl or glucose (rat β-cells: *[9,155]*; HIT cells: *[10]*; βTC3: *[156]*; mouse β-cells: *[157]*). Second, proving that a rise in intracellular $[Ca^{2+}]$ results from Ca^{2+} release, rather than Ca^{2+} influx, requires that the rise be shown to persist even when the extracellular solutions lack Ca^{2+}. However, it can be difficult to completely eliminate Ca^{2+} influx experimentally, and even a reduction from millimolar to micromolar $[Ca^{2+}]$ levels could still permit a significant Ca^{2+} gradient to remain (*see* critique in ref. *47*). Note also that experimentally teasing apart Ca^{2+} influx from release is even more challenging if the release itself requires Ca^{2+} influx, as is the case for Ca^{2+}-induced Ca^{2+} release *(139,146,148)*. A further problem has been our inability to directly measure free Ca^{2+} in different cellular pools.

More recently, Maechler et al. *(145)* developed a method to directly measure ER $[Ca^{2+}]$ using a mutant form of the photoprotein aequorin that was targeted to the ER. When ACh was applied to βTC3 cells having mutant aequorin in their ER, $[Ca^{2+}]_{ER}$ decreased, as expected, following PLC activation, presumably owing to the opening of IP_3-gated ER Ca^{2+} channels *(145)*. The ryanodine receptor agonist 4-chloro *m*-cresol also lowered $[Ca^{2+}]_{ER}$, consistent with RyR-mediated Ca^{2+} release. However, in contradiction to the glucose-induced Ca^{2+} release hypothesis, the application of solutions containing stimulatory levels of glucose or KCl produced an increase in $[Ca^{2+}]_{ER}$ because the stores took up more Ca^{2+} from the cytosol, rather than releasing Ca^{2+} *(145)*. Although the technical approach used in this study is new, and is still being refined, the results suggest that glucose or KCl do not release stored Ca^{2+}.

Video imaging studies also do not support the release hypothesis, because stimulating glucose metabolism in rat β-cells results in elevations in submembrane $[Ca^{2+}]_i$, consistent with the main source of these rises being influx

through surface membrane Ca^{2+} channels, rather than Ca^{2+} release from the cell interior *(155)*. By contrast, the application of ACh, which is known to release Ca^{2+} from ER stores *(41),* produces a different pattern in which the observed rises in $[Ca^{2+}]_i$ occur in regions that are generally farther away from the submembrane space *(155)*.

An initial report cited earlier showed that cADPR stimulated Ca^{2+} release from ER microsomes in β-cells *(150)*, yet subsequent studies by numerous groups have failed to reproduce these findings (reviewed in detail in ref. *149*). It has also been very difficult for other groups to replicate the finding that glucose metabolism increases the amount of cADP-R in β-cells *(149,158)*, and the injection of cADP-R antagonist into β-cells did not alter glucose-induced elevations in $[Ca^{2+}]_i$ *(152)*. Using permeabilized pancreatic islet or INS-1 cells, Rutter et al. *(159)* reported a thapsigargin-sensitive Ca^{2+} pool that was released by IP_3 but was insensitive to cADP-R. Webb et al. *(154)* found that the injection of cADP-R or its antagonist failed to directly alter glucose-induced increases in $[Ca^{2+}]_i$ in *ob/ob* mouse cell aggregates. In addition, cADP-R failed to elicit secretion from permeabilized islets *(154)*.

As for data involving caffeine sensitivity, Islam and Berggren *(149)* and Islam et al. *(160)* have stressed that the effects of caffeine must be carefully interpreted, because this methylxanthine, being relatively nonselective, can, among other things, affect K_{ATP} channels. In their review, Islam and Berggren *(149)* concluded that although Ca^{2+} release may play some role in islet cell function, Ca^{2+} entry through voltage-gated channels appears to be quantitatively more important than Ca^{2+} release. In addition, it is their view that it has seldom been possible to demonstrate directly that glucose stimulation causes the net release of Ca^{2+} from its intracellular stores, and even when it has been observed, its physiologic consequences are probably small and transient compared to Ca^{2+} influx *(149)*. In the future, the development of more specific tests of this hypothesis, coupled with an increased understanding of the molecular biology of the IP_3-R and RyR channels and more selective release blockers, will likely clarify the relative roles of intracellular Ca^{2+} release mechanisms in islets.

Novel Ca^{2+} Entry Mechanisms Distinct from Voltage-Gated Ca^{2+} Channels

In addition to conventional voltage-gated Ca^{2+} channels, the β-cell plasma membrane contains other cation channels that may either directly (because they are Ca^{2+} permeable) or indirectly (because they depolarize the cell membrane and open voltage-gated Ca^{2+} channels) alter Ca^{2+} influx in β-cells. Of these novel cation channels, some have been reported to be permeable to only monovalent cations, some to divalents, and some to both types of ionic species. In addition, it has not yet been completely resolved which novel influx channels are linked to intracellular Ca^{2+} store depletion and which are independent of the filling status of these stores. Although the functional significance of non-selective cation channels is not known, channels having relatively depolarized reversal potentials are required to produce the subthreshold depolarization observed upon K_{ATP} closure. In addition, these channels might also assist in the generation of new plateau depolarizations at the end of the silent interburst phase of islet bursting, particularly if insufficient voltage-dependent Ca^{2+} current activates during the interburst phase *(54)*.

Sturgess and colleagues *(161-163)* provided some of the earliest electrophysiologic evidence for nonselective cation channels in β-cells. Using patches of membrane excised from insulin-secreting CRI-G1 cells (*161–163,* reviewed in ref. *2* and *3)*, they described a nonselective channel that had a unit conductance of 25 pS, was activated by millimolar levels of $[Ca^{2+}]_i$ ($EC_{50} \approx 1$ m*M*; *162)*, and was inhibited by a variety of nucleotides, including ATP and ADP. The physiologic relevence of the channel was not clear. Rojas et al. *(164)* described another nonselective channel in human β-cells, which was activated by glucose or the mitochondrial substrate ketoisocaproic acid. More recently, Leech and Habener *(165)* reported a 30-pS nonselective channel that likely mediates nonselective whole-cell currents in HIT and rat β-cells. This nonselective current was activated by a diverse collection of stimuli, including maitotoxin, the incretin hormone GLP-1, cyclic adenosine monophosphate, or sulfonylureas, and was somewhat dependent on basal $[Ca^{2+}]_i$ *(165)*. However, in contrast to the channel reported by Rojas et al. *(164)*, it was not glucose sensitive. The current was also unaffected by the IP_3-R blocker heparin, or the RyR blocker ryanodine, which is surprising because MTX and GLP-1 are believed to be linked to ER Ca^{2+} release *(166)*.

Roe et al. *(138,156)* and Worley et al. *(167)* have stimulated renewed interest in nonselective currents in the β-cell through their work showing that store depletion owing to glucose or thapsigargin in turn activates a nonselective cation current (CRAN current) that can depolarize the islet and indirectly potentiate electrical activity and Ca^{2+} influx. These CRAN channels would be expected to depolarize the membrane, because their reversal potential is near -30 mV *(156, 167)*. This, in turn, would open voltage-gated Ca^{2+} channels that would provide increased Ca^{2+} influx, probably in the form of Ca^{2+} spiking or bursting *(156,168)*. In the Capacitative Ca^{2+} Entry hypothesis of Putney and McKay *(169)* (reviewed in refs. *140,169*), the depletion of intracellular Ca^{2+} stores is linked to the activation of Ca^{2+} release activated Ca^{2+} channels (CRAC) in the surface membrane, which then open to refill the intracellular store with Ca^{2+}. In some systems, the surface membrane channels have been found to be either Ca^{2+}-permeable CRAC channels, or nonselective cation channels *(140)*. The nature

of the mechanism that couples the depletion status of the ER Ca^{2+} store to the activation of surface membrane Ca^{2+} channels has remained somewhat elusive, and is beyond the scope of this review. Interested readers are referred to recent reviews in this area *(140,169,170)*.

The identity, as well as the overall significance, of release-activated Ca^{2+} entry to glucose-dependent Ca^{2+} signaling in islet remains unresolved. Liu and Gylfe *(171)* obtained evidence for the CRAC pathway in *ob/ob* mouse β-cells, but concluded that it mediated a minor fraction of the Ca^{2+} entry in these cells. Liu et al. *(172)* had concluded earlier that the Ca^{2+} store plays no role in the production of slow $[Ca^{2+}]_i$ oscillations in *ob/ob* cells, because thapsigargin was without effect on these oscillations. Earlier data showed that ryanodine also did not affect slow oscillations in $[Ca^{2+}]_i$ in *ob/ob* islets *(153)*. Studies of β-cells carried out using diazoxide to hyperpolarize the cells and prevent Ca^{2+} entry through voltage-gated channels resulted in the identification of a store-operated CRAC, but this pathway could account only for a minor contribution to Ca^{2+} signaling under these conditions *(171)*.

In mouse β-cells, Miura et al. *(157)* found that store depletion evoked increased Ca^{2+} influx owing to a small contribution from the capacitance Ca^{2+} pathway, and through the potentiation of Ca^{2+} influx through voltage-gated Ca^{2+} channels, presumably mediated by cell membrane depolarization. This is conceptually the same argument as that made by Worley et al. *(167)* and Roe et al. *(156)*, but a complication is that in an earlier study, Miura et al. *(173)* found that Ca^{2+} pool depletion by muscarinic agonists, but not thapsigargin, raised $[Na^+]_i$ in β-cell clusters. This suggests that release-dependent depolarization was mediated by a mechanism that did not involve Na^+ *(156,173)*. Recently, Gilon et al. *(174)* conducted an elegant study of $[Ca^{2+}]_i$ dynamics in mouse β-cells, in which they found that slow ER Ca^{2+} release alternates between phases of Ca^{2+} influx. The slow dynamics of this release was suggested to be importantly involved in the control of islet $[Ca^{2+}]_i$ oscillations *(174)*. While oscillations in $[Ca^{2+}]_{ER}$ are predicted to occur in some islet bursting models, and could in turn produce the rhythmical activation of CRAN current *(155,168)*, Gilon et al. *(174)* found that Ca^{2+} release was not depolarization mediated, nor linked in any obvious way to the activity of IP_3 or RyR Ca^{2+} release channels. Instead, they suggested that Ca^{2+} release at the end of $[Ca^{2+}]_i$ oscillations results from a simple passive leak of Ca^{2+} *(174)*.

For one last mechanism to consider, Leech et al. *(175)* observed slow $[Ca^{2+}]_i$ oscillations in rat β-cells and HIT cells, which were attributed to the activation of a novel glucose-insensitive Ca^{2+} channel that was linked to Ca^{2+}-induced Ca^{2+} release *(175)*. The connection between this channel and CRAC remains unclear, although these investigators independently demonstrated that these cells are likely to have CRAC, because store depletion was linked to Mn^{2+}-induced FURA-2 quenching *(175)*. The glucose insensitivity of the current suggested that it was not mediated by the G channels described by Rojas et al. *(164)*. Leech et al. *(175)* suggested that a glucose-insensitive Ca^{2+} current might help maintain basal $[Ca^{2+}]$ under low glucose (i.e., fasting) conditions.

In mouse islets, Silva et al. *(176)* reported that raising extracellular $[Ca^{2+}]$ increased intracellular $[Ca^{2+}]$, despite the presence of 10 μ*M* nifedipine, suggesting mediation by a novel islet Ca^{2+} channel. This Ca^{2+}-raising effect was unaffected by changing glucose and was potentiated by ACh-mediated Ca^{2+} release. The novel pathway appeared to be sensitive to nickel, and to a blocker of nonselective cation channels *(176)*. Silva et al. *(176)* thus concluded that nonselective cation channels may provide β-cells with a background Ca^{2+} influx mechanism that can operate even at low levels of glucose.

There thus appears to be a host of novel channels or Ca^{2+} entry pathways in insulin-secreting preparations that, although interesting, will require much more study in order to determine their possible connection to ER Ca^{2+} store depletion and their overall significance for glucose-dependent islet electrical activity and insulin secretion.

Conclusion

We have seen that the influx of Ca^{2+} through voltage-gated Ca^{2+} channels, particularly those of the L-type, is important for glucose-dependent islet electrical activity, $[Ca^{2+}]_i$ signaling, and insulin secretion. The electrical activity of the islet takes the form of a stereotyped pattern of regular, glucose-dependent electrical oscillations. Islet electrical activity provides glucose metabolism with a mechanism to alter Ca^{2+} entry through voltage-dependent Ca^{2+} channels over a very wide dynamic range. It is attractive to think that precise timing of Ca^{2+} entry is necessary to produce appropriate and highly modulable $[Ca^{2+}]_i$ oscillations for efficient control of oscillatory insulin secretion. The overall relevance of cellular oscillations to those of plasma insulin observed in vivo is still not understood, because the oscillations in $[Ca^{2+}]_i$ and insulin observed in single β-cells vs whole islets do not fully agree in terms of their timing (*see* refs. *153, 177,* and *178* for reviews).

The literature has been more straightforward in its emphasis of the importance of Ca^{2+} channels for insulin secretion, but there is a growing interest in Ca^{2+} storage and release mechanisms in islet β-cells. It is likely that the controversies of the present will be resolved, as always, through advances in Ca^{2+} measurement technology (particularly the development of new probes that can report the $[Ca^{2+}]$ of different subcellular compartments), and in the molecular biology of nonselective cation channels, which is still in its nascent phase. It seems likely that the different results observed so far among different research groups are, in

fact, real, but reflect subtle differences in the experimental conditions or insulin-secreting preparations used in the studies.

At some point in the not-too-distant future, it is hoped that researchers studying the β-cell will arrive at a new consensus model, that is comprehensive in its ability to incorporate Ca^{2+} influx and release mechanisms, detailed spatial aspects of Ca^{2+} signaling obtained using confocal methods, and that can account for the two phases of insulin secretion that follow step changes in glucose, as well as insulin oscillations. In addition, this model may be able to identify which features of insulin secretion are Ca^{2+} dependent and channel dependent, and which occur in the distal domain without proximal channel involvement or possibly Ca^{2+} itself.

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