Chapter 11
Calcium Signaling in the Islets

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Abstract Easy access to rodent islets and insulinoma cells and the ease of measuring Ca^{2+} by fluorescent indicators have resulted in an overflow of data that have clarified minute details of Ca^{2+} signaling in the rodent islets. Our understanding of the mechanisms and the roles of Ca^{2+} signaling in the human islets, under physiological conditions, has been hugely influenced by uncritical extrapolation of the rodent data obtained under suboptimal experimental conditions. More recently, electrophysiological and Ca^{2+} studies have elucidated the ion channel repertoire relevant for Ca^{2+} signaling in the human islets and have examined their relative importance. Many new channels belonging to the transient receptor potential (TRP) family are present in the β-cells. Ryanodine receptors, nicotinic acid adenine dinucleotide phosphate channel, and Ca^{2+}-induced Ca^{2+} release add new dimension to the complexity of Ca^{2+} signaling in the human β-cells. A lot more needs to be learnt about the roles of these new channels and CICR, not because that will be easy but because that will be difficult. Much de-learning will also be needed. Human β-cells do not have a resting state in the normal human body even under physiological fasting conditions. Their membrane potential under physiologically relevant resting conditions is ~−50 mV. Biphasic insulin secretion is an experimental epiphenomenon unrelated to the physiological pulsatile insulin secretion into the portal vein in the human body. Human islets show a wide variety of electrical activities and patterns of $[Ca^{2+}]_i$ changes, whose roles in mediating pulsatile secretion of insulin into the portal vein remain questionable. Future studies will hopefully be directed toward a better understanding of Ca^{2+} signaling in the human islets in the context of the pathogenesis and treatment of human diabetes.

Keywords CICR · Transient receptor potential channels · Calcium oscillation · Depolarization · TRP channels · TRPV1 · Ryanodine receptor · TRPV4 · Basal

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11.1 Introduction

Changes in the concentration of the free Ca^{2+} in the cytoplasm ($[Ca^{2+}]_i$) or in subcellular compartments can act as signals for many cellular processes. Increase in $[Ca^{2+}]_i$ may be local (e.g., Ca^{2+} "sparks"), which may give rise to global $[Ca^{2+}]_i$ changes [1]. $[Ca^{2+}]_i$ changes take the forms of oscillations and propagating waves. Generation and shaping of the Ca^{2+} signals require participation of different membranes, channels, pumps, stores, other organelle, as well as many Ca^{2+}-binding-proteins. $[Ca^{2+}]_i$ changes are often loosely termed "Ca^{2+} signals", although it is likely that all $[Ca^{2+}]_i$ changes do not have a signaling role. Ca^{2+} signals control events such as exocytosis that take place in seconds and events like gene transcription that take place over minutes to hours. In this review, I shall not attempt to compile a catalog of all of the molecules and phenomena that are known in connection with Ca^{2+} signaling in the islets; instead, I shall depict some emerging and intriguing areas and give my views. The review is structured and dimensioned to deliver selected messages rather than to dilute them by writing a complete treatise on Ca^{2+} signaling.

When it comes to Ca^{2+} signaling in the islets, the literature is dominated by data obtained from in vitro experiments that have used islets or insulinoma cells from rodents. We, therefore, understand how rodent islets behave in Petri dishes or in in vitro perfusion systems, better than we understand how human islets behave in their native environment in the pancreas in the normal human body. If we want to learn how mountain gorillas behave, we can do that by poking a monkey in a cage or by watching real mountain gorillas in their social and natural environments in Rwanda. In practice, however, we tend to draw far-reaching conclusions not only about the function of normal human islets but also about the dysfunctions of human islets in diabetes, from in vitro studies done on rodent islets. It is therefore important that we carefully examine what key experiments were done, what conditions were used in those experiments, and what results were obtained. This may enable us to reinterpret the existing data and draw our own conclusions about some fundamental issues some of which are illustrated in the following paragraphs.

11.2 Human β-Cells as a Group Are Never Resting

The notion that β-cells have a "resting" state is a myth arising from in vitro experimental protocols. In vitro experimentalists find it convenient to work with a stable low rate of insulin secretion and a stable low basal $[Ca^{2+}]_i$ at the beginning of an experiment. They want to ensure that the $[Ca^{2+}]_i$ or insulin curves show a stable baseline, which reviewers like to see. To achieve this, investigators expose islets to

low concentrations of glucose (often 2–3 mM, sometimes 0–1 mM), and no other nutrients are included in the solution. Human islets are incubated in zero glucose for as long as an hour to force them to rest [2]. Under such conditions, β-cells are largely depleted of energy, and consequently, a high proportion of K_{ATP} channels are open. β-Cells in the human body, however, even after overnight fast, are bathed in ~4–6 mM glucose, other nutrients like the amino acids, and the hormone glucagon that is present in high concentration in the fasting plasma. The availability of these nutrients ensures that human β-cells, even under fasting conditions, are not energy depleted. The K_{ATP} channels of many β-cells in the human islets are thus mostly in a closed state even under fasting conditions. In in vitro experiments, human β-cells secrete insulin even when they are exposed to only 3–5 mM glucose as the only nutrient [2]. When human β-cells are exposed to only 5–6 mM glucose, as the sole nutrient, they keep firing action potentials from a baseline membrane potential of ~−50 to ~−45 mV, at rates ranging from one every 4 s to one every 2 s [3]. Complex patterns of membrane potential oscillations are seen in human islets even when they are exposed to only 2.8 mM glucose (and no other nutrients) and even when the experiments are performed at 34°C [4]. (To the cell biologists 34°C or even 21°C is O.K.; to the clinicians, a patient with 34°C body temperature poses a real emergency). Thus, in the normal human body, β-cells are not resting even after overnight fast. Under fasting conditions, the concentration of insulin in the portal vein of human is 440 ± 25 pmol/L [5]. Under such conditions, islets secrete not only insulin but also glucagon, which protects against hypoglycemia. This is evident from the observations that total pancreatectomy in human leads not only to diabetes but also to a rather more difficult complication, namely hypoglycemia due to the lack of glucagon [6].

It is accepted that the $[Ca^{2+}]_i$ of "resting" β-cells is ~25–100 nM and that the membrane potential of "resting" β-cells is ~−70 mV. These values are obtained from experiments where β-cells are forced to artificial "resting conditions" that are different from the physiological resting conditions. If β-cells are, instead, kept in a solution that mimics the human plasma after an overnight fast (i.e., physiologically relevant resting condition), then their resting membrane potentials will be different (perhaps ~−50 to ~−45 mV). Consequently, their resting $[Ca^{2+}]_i$ will also be different (perhaps ~300 nM and perhaps in the form of oscillations). In other words, β-cells in the normal human body spend most of their life-time with a much higher $[Ca^{2+}]_i$ and secretory activity than can be guessed from conventional experiments.

11.3 Biphasic Insulin Secretion Is an Experimental Epiphenomenon

In experiments where β-cells are first forced to rest (often by incubating in ~2–3 mM glucose, as the only nutrient), and then *suddenly* exposed to a high concentration of glucose (often >10 mM, sometimes 30 mM!), continuously for a prolonged period, then one sees what has been called "biphasic insulin secretion" over the past

decades. Biphasic refers to two phases of insulin secretion: the first phase consists of the initial large insulin secretion that peaks at 5–6 min after increasing the concentration of glucose and the second phase consists of the subsequent lower rate of insulin secretion that remains stable or slowly rises as long as the glucose concentration remains high (over a period of 1–2 h or more) (see Fig. 6.3). (Electrophysiologists have a different definition of "biphasic," their first phase peaking in <500 ms! [7].) Human β-cells in normal human body encounter conditions of stimulations that are substantially different from the experimental conditions that are used to elicit the biphasic nature of insulin secretion. As mentioned before, normal human β-cells are not in a resting state even under fasting conditions. They are seldom subjected to a sudden increase of glucose to a very high concentration (or sudden increase of $[Ca^{2+}]_i$ to 30 μM by UV flash [7]). They are usually triggered by lower concentrations of glucose (usually by ~7–<10 mM glucose after a mixed meal), and normally glucose concentrations in the plasma do oscillate. The result is that normal insulin secretion in the human portal vein is oscillatory and not biphasic as elicited by artificial experimental conditions. Experiments that are designed to demonstrate biphasic nature of insulin secretion are not usually designed to detect oscillations of insulin secretion (e.g., samples for insulin assay are not collected at 1 min or more frequent intervals). Thus normal secretion in normal human being during fasting states and after mixed meals may employ a set of molecular mechanisms that may be substantially different from those involved in mediating biphasic insulin secretion elicited by experimental protocols described above.

11.4 Glucose Increases Insulin Secretion by Increasing $[Ca^{2+}]_i$ and by Providing ATP in the Face of Energy-Consuming Processes Triggered by Ca^{2+} Influx Through the Voltage-Gated Ca^{2+} Channels (VGCC)

When 30 mM KCl is applied to islets in the presence of low concentration of glucose (or zero glucose [8], and no other nutrients are included in the solution), there is an increase of both $[Ca^{2+}]_i$ and insulin secretion with a biphasic time course [2, 8]. A large and persistent increase of $[Ca^{2+}]_i$ in a cell that is kept at 1 mM glucose (and no other nutrients) reduces cytoplasmic [ATP] [9]. This is due to the fact that plasma membrane Ca^{2+}-ATPase and other Ca^{2+}-sensitive biochemical cascades that link Ca^{2+} influx to insulin secretion consume ATP of the cell which is kept in only 1 mM glucose (and which has a high-K_m glucokinase to phosphorylate the sugar) [10]. In fact, in the later part of the second phase, $[Ca^{2+}]_i$ increases slowly since the cell can no longer pump out Ca^{2+} adequately because of energy deficiency [8]. Consequently, Ca^{2+}-mediated insulin secretion (which is an energy-consuming process) is progressively reduced in the second phase of prolonged $[Ca^{2+}]_i$ increase by KCl [8]. If one now applies 15 mM glucose (and thereby improves energy status of the cells) to these "$[Ca^{2+}]_i$-clamped" islets, a larger amount of secretion is obtained [2, 8]. So, to recapitulate, in the first scenario, insulin secretion increases because

of an increase of $[Ca^{2+}]_i$, but the magnitude of the increase is low and it declines further over time because of inadequate energy availability to support secretion. In the second scenario, glucose does what it is supposed to do, i.e., it performs its universal fuel function by supplying energy to the cells and thereby it increases insulin secretion further. Of course, glucose metabolism produces many other molecules too, e.g., cAMP (via ATP), which can increase insulin secretion [11].

That glucose can stimulate insulin secretion from human β-cells in vivo, without inducing further closure of K_{ATP} channels, is evident from cases of severe poisoning with sulfonylureas. In these patients, the K_{ATP} channels are presumably completely closed and $[Ca^{2+}]_i$ of β-cells is certainly high. However, when glucose is infused into such patients (as an attempt to correct hypoglycemia), the β-cells secrete even more insulin, making the hypoglycemia recurrent and difficult to treat [12]. Similarly, people with $SUR1^{-/-}$, who do not respond to tolbutamide, do respond to glucose by insulin secretion [13].

Thus, while investigating signaling roles of glucose, the more universal role of glucose as a fuel needs to be considered explicitly. In experimental conditions where glucose is the only nutrient, its role as a fuel becomes even more critical. If concentration of glucose in the human plasma is reduced to less than 3 mM (and all other nutrients are kept normal), one will become unconscious within seconds, a vivid example of the role of glucose as a fuel in the central neurons. Similarly, if there is no glucose or only very low glucose in the perfusion medium (and no other nutrients are present), muscle cells will eventually fail to contract, heart will stop beating, and not surprisingly, islets will fail to secrete insulin properly.

11.5 Mechanism of Initial Depolarization of β-Cells by Glucose

Initial depolarization of plasma membrane to the thresholds for activation of voltage-gated Ca^{2+} channels is one of the most critical signaling events leading to Ca^{2+} signaling and insulin secretion. The most important function of β-cells is to prevent death due to hypoglycemia. If your fasting plasma glucose concentration is raised from 5 mM to 8 mM (i.e., you have diabetes), you will not die immediately. You may not even feel for years that your glucose is high. On the other hand if your fasting plasma glucose drops from 5 mM to 3 mM, you will have hypoglycemic symptoms and you may become unconscious and die. Other hormones in the body are not like insulin; if your pituitary or adrenal hormones are acutely low, it will not kill you immediately. β-Cells, thus, secrete a hormone that is potentially a killer. Nature has, therefore, equipped β-cells with powerful brakes to immediately stop insulin secretion, when glucose concentration is inappropriately low. Key elements of this brake system are the high-K_m glucokinase and the K_{ATP} channels. When plasma glucose concentration is reduced to near hypoglycemic levels, there is less glucose metabolism via glucokinase, leading to a reduced cytoplasmic MgATP/MgADP, opening of the K_{ATP} channels, and repolarization of plasma membrane potential (see chapter by Clark and Proks in this book). Thus, K_{ATP} channels

play a crucial role in stopping insulin secretion quickly, and its main function is to mediate quick repolarization of plasma membrane potential. Defects in these two brake systems, namely inactivating mutations of the K_{ATP} channels or activating mutations of glucokinase, lead to hypoglycemia [14].

At low glucose concentration (provided that no other nutrients are present), a high proportion of the K_{ATP} channels are in the open state. This situation occurs only in in vitro experiments that are often done at ~21°C [15] and by using cells or tissues that are to a varying degree "metabolically stunned." It has no resemblance to any in vivo situation in any living human being, where β-cells are at 37°C and are constantly bathed in a variety of nutrients including 20 different amino acids and fatty acids, even under normal fasting conditions when plasma glucose concentration is ~4–6 mM. In vivo, a healthy β-cell, thus, has enough ATP to keep almost 100% of the K_{ATP} channels closed. In the normal human body, where plasma glucose concentration changes only between ~4 mM in the fasting conditions and ~8 mM after meals, further closure of the K_{ATP} channels is thus not the likely mechanism for bringing about depolarization to the threshold for the activation of VGCCs. Glucose depolarizes β-cells in Sur1 or Kir6.2 knocked-out mice [16, 17]. Thus, under normal fasting conditions, the input resistance of β-cells is high and depolarization to the thresholds for the activation of VGCCs is brought about by various inward depolarizing currents mainly carried by Na^+. Here we are talking about tiny winy currents which are difficult to measure in native β-cells. Thus, mere anticipation, sight, or smell of food will depolarize β-cells and stimulate insulin secretion by vagus-mediated acetylcholine-induced depolarizing Na^+ current [18, 19]. Similarly, after a mixed meal, the incretin hormone GLP-1 depolarizes β-cells by triggering a cAMP-activated Na^+ current [20]. There is an urgent need to elucidate the molecular identity of the channels that mediate inward depolarizing currents in the β-cells. In this respect, there is currently, legitimate interest in the transient receptor potential (TRP) channels, which is the topic of the next paragraphs.

11.6 TRP Channels

More and more TRP channels are being identified in the β-cells. It is thought that these channels may account for the background depolarizing current (often called "leak" current) carried mostly by Na^+. Activation of some of these channels leads to an increase of $[Ca^{2+}]_i$ directly or by way of membrane depolarization. Examples of Ca^{2+}-permeable TRP channels in the islets include TRPC1, TRPC4, TRPV1, TRPV2, TRPV4, TRPV5, TRPM2, and TRPM3. TRPs are tetrameric ion channels and many form heterotetramers giving rise to a variety of ion channels with a variety of regulatory mechanisms. Expression of some TRP channels in the native cells is often low and their regulation is often studied in heterologous systems where the channels are overexpressed, an approach not entirely without problem. In the following paragraphs, I will write a few lines about each of the TRP channels that have been described in the β-cells.

Examination of formalin-fixed paraffin-embedded tissue shows strong TRPC1 immunoreactivity in the human islets (www.hpr.se). By RT-PCR, TRPC1 mRNA can be readily detected in mouse islets, MIN6 cells, INS-1 cells, and rat β-cells [21, 22]. TRPC1 is the only TRPC channel that is expressed at high level in MIN6 cells and mouse islets [22]. In contrast, another mouse insulinoma cell line βTC3 does not express TRPC1 mRNA. The only TRPC channel that can be detected by Northern blot in βTC3 cells is TRPC4 [23]. TRPC4 is also abundant in INS-1 cells and rat β-cells [21]. TRPC4 has two abundant splice variants: the full-length TRPC4α and a shorter TRPC4β that lacks 84 amino acids in the C-terminus. In INS-1 cells, TRPC4α is the dominant isoform, whereas in rat β-cells, TRPC4β dominates [21]. TRPC4α is inhibited by phosphatidylinositol 4,5-bisphosphate (PIP2) [24]. TRPC1 and TRPC4 are nonspecific cation channels with about equal permeability to Na^+ and Ca^{2+}. As alluded to earlier, it is possible that TRPC1 and other TRP channels mediate the inward depolarizing currents in β-cells. TRPC1 and TRPC4 are also molecular candidates for nonselective cation currents activated by Gq/PLC-coupled receptors or by store depletion [25]. From studies in other cells, it appears that TRPC1 together with STIM1 and Orai1 can mediate store-operated Ca^{2+} entry (SOCE), but the issue remains controversial [26]. TRPC5, which is closely related to TRPC4, is not expressed in mouse islets [23].

In the islets, TRPV1 is present mainly in a subset of sensory nerve fibers that are thought to be involved in mediating local islet inflammation in autoimmune diabetes [27, 28]. The TRPV1-expressing fibers secrete calcitonin gene-related peptide, which inhibits insulin secretion. In Zucker diabetic rats, it has been demonstrated that ablation of the TRPV1-expressing fibers by capsaicin treatment improves insulin secretion [27]. Insulinoma cell lines RIN and INS-1 express TRPV1. The picture is less clear for primary β-cells. TRPV1 has been demonstrated in primary β-cells of Sprague Dawley rats [29] but not in those of Zucker diabetic rats [27] or NOD mice [28]. It remains unknown whether the human β-cells express TRPV1.

Studies on TRPV2 channel in different cells show that it is activated by physical stimuli like noxious heat (>52°C), membrane stretch, and osmotic swelling. The roles of such stimuli in the regulation of TRPV2 channel of β-cells are unclear. TRPV2 channel of β-cells is in fact constitutively active [30]. It may thus be one of the channels responsible for the background depolarizing current in β-cells. Another remarkable feature of the TRPV2 channel of β-cells is its ability to translocate from cytoplasm to the plasma membrane, upon stimulation by insulin [30]. Such translocation of TRPV2 and the resulting increase in Ca^{2+} entry through the channel increases insulin secretion and β-cell growth. This observation implies that hyperinsulinemia, which is common in type 2 diabetes, may act as a positive feed-back to increase insulin secretion further. High concentration of glucose also induces translocation of TRPV2 to the plasma membrane. It appears that while glucose closes K_{ATP} channel by increasing cytoplasmic MgATP/MgADP ratio, it, at the same time, increases inward depolarizing current through TRPV2 channel by inducing translocation of the channel to the plasma membrane.

Immunohistochemistry of formalin-fixed paraffin-embedded tissues shows that the TRPV4 protein is highly expressed in the human islets, in contrast to the

pancreatic acinar cells, where it is almost absent (www.hpr.se). Even though TRPV4 is known to be a plasma membrane channel, the immunoreactivity is mostly in the cytoplasm, a situation apparently similar to that of TRPV2 in the β-cells. TRPV4 acts as a mechano-sensor and osmo-sensor, but it can be activated by various ligands including 4α-Phorbol 12,13-didecanoate, anandamide, arachidonic acid, and epoxyeicosatrienoic acids. Aggregated human islet amyloid polypeptide (hIAPP) induces changes in the plasma membrane leading to the activation of TRPV4, membrane depolarization, increase in $[Ca^{2+}]_i$, induction of ER stress, and apoptosis [31]. hIAPP-induced $[Ca^{2+}]_i$ changes and β-cell death are reduced by siRNA against TRPV4 [31].

By immunohistochemistry it has been shown that TRPV5 (formerly called ECaC1) protein is expressed in rat β-cells but not in the α-cells nor in the pancreatic acinar cells [32]. In the β-cells, the TRPV5 immunoreactivity is mostly in the secretory granules. TRPV5 is highly selective for Ca^{2+} and the tissues where it is typically expressed are the ones that are responsive to 1,25-dihydroxyvitamin D_3 (e.g., kidney and intestine), where it plays crucial roles in transepithelial (re)absorption of Ca^{2+} [33]. It is noteworthy that β-cells are also 1,25-dihydroxyvitamin D_3-responsive (see chapter by Leung and Cheng in this book). The antiaging protein klotho deglycosylates TRPV5 and thereby entraps the channel in the plasma membrane and ensures durable channel activity [34]. Islets of klotho mutant mice have decreased insulin content and secrete reduced amount of insulin [35]. However, there is so far only one study which has shown TRPV5 protein in the β-cells and the data, apparently, cannot be reproduced [32]. Native TRPV5 current has not been demonstrated in β-cells or indeed in any other cells because of low expression of the channel. TRPV5 protein has not been demonstrated in human β-cells.

The presence of functional TRPM2 channels in rodent insulinoma cells as well as in primary β-cells is well established [36]. In human islets, there are at least two main isoforms of the channel: the full-length form (TRPM2-L) and a short form (TRPM2-S), where the four C-terminal transmembrane domains, the putative pore region, and the entire C-terminus are deleted [37]. TRPM2-S does not form a functional channel. There are other splice variants of TRPM2 which form channels and are differentially regulated [38]. TRPM2 is activated by intracellular ADP ribose, β-NAD^+, nitric oxide, H_2O_2, free radicals, and Ca^{2+}. ADP ribose formed by the degradation of NAD^+ by poly(ADP ribose) polymerase is an important activator of the TRPM2 channel. The nonselective cation channel activated by the diabetogenic agent alloxan is probably TRPM2 [39]. The channel can be gated also by warm temperature (>35°C). Arachidonic acid, which is produced on stimulation of β-cells by glucose, is a positive modulator of TRPM2 channel [40–42]. Cyclic ADP ribose potentiates activation of the channel [43], but this is not a universal observation [44]. Perhaps the most important physiological regulator of TRPM2 is Ca^{2+}. All of the splice forms of TRPM2 that form a channel are activated by Ca^{2+}; Ca^{2+} released from the intracellular stores can activate the channel [45]. TRPM2 is located also on the lysosomal membranes and activation of intracellular TRPM2 releases Ca^{2+} from the lysosomes [36]. The role of TRPM2 channels in the regulation of insulin secretion and in mediating β-cells death in diabetes is an active area of research.

TRPM2 knock-out mice are apparently not diabetic [46]. This may mean that the channel is not important in mice β-cells or that other ionic mechanisms compensate for its absence in the knock-out mice. The channel may provide a mechanism for eliminating β-cells that have been severely damaged by oxidative stress [47].

The TRPM3 channel has many splice variants which differ in their functional properties including their permeabilities for divalent cations [48]. Micromolar concentrations of the steroid pregnenolone directly activate TRPM3 channel of β-cells leading to increase of $[Ca^{2+}]_i$ and augmentation of glucose-stimulated insulin secretion [49]. The channel is activated by nifedipine, commonly used as a blocker of L-type VGCCs.

TRPM4 is permeable to monovalent cations but not to Ca^{2+} [50]. It is activated by elevated $[Ca^{2+}]_i$ and its activity is regulated by voltage. Immunohistochemistry shows that TRPM4 protein is present in human β-cells [51]. In rodent insulinoma cells, increased $[Ca^{2+}]_i$ activates TRPM4 and generates a large depolarizing membrane current [52]. An increase in $[Ca^{2+}]_i$ in β-cells upon stimulation by glucose or activation of PLC-linked receptors activates TRPM4 channel [51]. An important regulator of TRPM4 is PIP2, which sensitizes the channel to the activation by $[Ca^{2+}]_i$, whereas depletion of PIP2 inhibits the channel [53]. Glucose, by increasing cytoplasmic MgATP/MgADP ratio, increases the concentration of PIP2 in the plasma membrane of β-cells [54]. This is a potential mechanism by which glucose may sensitize TRPM4 channel. On the other hand, glucose increases cytoplasmic [ATP], which has inhibitory effect on TRPM4 channel [55]. Amino acid sequence of TRPM4 shows two motifs that look like ABC transporter signature motif [56]. Consistent with this, TRPM4 is inhibited by glibenclamide [57]. Another voltage-modulated intracellular Ca^{2+}-activated monovalent-specific cation channel, which is closely related to the TRPM4 channel, is the TRPM5 channel [58]. Compared with TRPM4, TRPM5 is even more sensitive to activation by $[Ca^{2+}]_i$, but in contrast to TRPM4, it is not inhibited by ATP [55]. TRPM5 mRNA is present in MIN6 cells, INS-1 cells, and in whole human islets [58]. Reportedly, glucose-induced insulin secretion is reduced in TRPM5 knock-out mice. TRPM4 and TRPM5 may mediate Na^+ entry into the β-cells by sulfonamides, muscarinic agonists, and glucose and thereby depolarize membrane potential.

11.7 Store-Operated Ca^{2+} Entry (SOCE)

The filling state of the ER Ca^{2+} store may trigger Ca^{2+} entry across the plasma membrane in β-cells as in many other cells [59]. Thus, depletion of ER Ca^{2+} pools by SERCA inhibitors induces Ca^{2+} entry and depolarizes the plasma membrane potential of β-cells [60]. The ER Ca^{2+} store thus plays a role in the regulation of membrane potential [61, 62]. Two important molecular players involved in SOCE are stromal interaction molecule (STIM) and Orai1. STIM1 has an intraluminal EF-hand domain which enables it to act as a sensor of $[Ca^{2+}]$ in the ER lumen. STIM1, by its association with Orai1 or TRPC, regulates SOCE in some cells. Pancreatic

islets express STIM1. In MIN6 cells, it has been shown that EYFP-STIM1 is delivered to the peri-plasma membrane location when the ER Ca^{2+} pool is depleted [63]. 2-aminoethoxydiphenyl borate (2-APB) prevents SOCE and translocation of STIM1 to peri-plasma membrane locations. It is not known whether STIM1 interacts with Orai1 or TRPC channels in β-cells. The roles of TRPCs and the roles of STIM1 and Orai1 in mediating SOCE remain unsettled. Some results support the view that STIM1-Orai1-TRPC1 complex provides an important mechanism for SOCE [26]; others demonstrate that TRPC channels operate by mechanisms that do not involve STIM1 [64]. It should be noted that in β-cells, activation of muscirinic receptors leads to the activation of nonselective cation currents that have a store-operated and a store-independent component [19]. We demonstrated that activation of RyRs of β-cells leads to Ca^{2+} entry through TRP-like channels by mechanisms that apparently do not involve store depletion [65].

11.8 Voltage-Gated Ca^{2+} Channels of β-Cells

In β-cells, the most robust mechanism for the entry of extracellular Ca^{2+} across the plasma membrane is the Ca^{2+} entry through VGCCs. Opening of VGCCs leads to a large increase of $[Ca^{2+}]_i$ in microdomains near the plasma membrane and triggers exocytosis of insulin [66]. Both high-voltage-activated (HVA) and low-voltage-activated (LVA) Ca^{2+} currents are detected in human β-cells [67, 68]. The major component of the HVA current is L-type that is blocked by dihydropyridine antagonists and enhanced by BAYK8644. A second component of HVA current is resistant to inhibition by dihydropyridines and ω-conotoxin GVIA, an inhibitor of N-type Ca^{2+} channel but is blocked by P/Q channel blocker ω-agatoxin IVA. Consistent with this, 80–100% of glucose-induced insulin secretion from human islets is blocked by saturating concentration of dihydropyridine antagonists [68, 69]. Such dramatic inhibition is thought to be due to the fact that the L-type channels play essential role in the generation of electrical activity (however, these inhibitors also block NAADP receptor). In contrast, their roles in mediating exocytosis are less pronounced [69]. The L-type Ca^{2+} current in human β-cells is mediated mainly by $Ca_v1.3$ (α_{1D}) channel and to a lesser extent by $Ca_v1.2$ (α_{1C}). Compared to $Ca_v1.2$, the $Ca_v1.3$ channels activate at lower membrane potential (~−55 mV), which suggests that the latter may be the more important isoform in human β-cells. This is in contrast to mouse β-cells where $Ca_v1.2$ plays a central role in insulin secretion [70]. Compared to the $Ca_v1.2$ channels, the $Ca_v1.3$ channels are less sensitive to the dihydropyridine antagonists [71]. Identical de novo mutation (G406R) in this channel causes prolonged inward Ca^{2+} currents and causes episodic hypoglycemia [72].

The P/Q type Ca^{2+} channels ($Ca_v2.1$, α_{1A}) account for 45% of integrated whole-cell Ca^{2+} current in human β-cells. These channels are blocked by ω-agatoxin IVA. Compared to the L-type Ca^{2+} channels, the P/Q type Ca^{2+} channels are more tightly coupled to exocytosis.

The LVA current is of T-type which is activated at −50 mV and reaches a peak between −40 and −30. It inactivates within less than 1 s of sustained depolarization to −40 mV. The T-type current in human β-cells is mediated by $Ca_V3.2$ (α_{1G}). T-type channels are involved in insulin release induced by 6 mM but not by 20 mM glucose [69]. T-type current is blocked by NNC 55-0396.

If all of these ion channels are present in a given β-cell, one can envisage that closure of the K_{ATP} channels depolarizes membrane potential to above −55 mV, which then leads to the activation of T-type Ca^{2+} channels (which open at voltage above −60 mV) and then to the activation of the L-type Ca^{2+} channels (which open at voltage above −50 mV), which generates the action potential. Further depolarization occurs due to the activation of the voltage-gated Na^+ channels (which open at above −40 mV) leading finally to the activation of P/Q type Ca^{2+} channels (which opens at above −20 mV) [69].

R-type Ca^{2+} channels ($Ca_v2.3$, α_{1E}) are not present in human β-cells [69]. Mice lacking the R-type Ca^{2+} channels exhibit impaired insulin secretion. In this context, it is noteworthy that polymorphisms in the gene encoding the R-type Ca^{2+} channels $Ca_v2.3$ (CACNA1E) are associated with impaired insulin secretion and type-2 diabetes in human too [73, 74]. It is possible that, in human, R-type Ca^{2+} channels are involved in insulin secretion by operating other glucose-sensing cells like central neurons or GLP-1-producing L-cells in the gut [75].

11.9 Intracellular Ca^{2+} Channels of β-Cells

Among the channels that release Ca^{2+} from the ER or the secretory vesicles, the roles of the inositol 1,4,5-trisphosphate receptors (IP_3R) in the β-cells are well accepted. From immunohistochemistry pictures of paraffin-embedded formalin-fixed human tissues in the human protein atlas (www.hpr.se), it is evident that human islets express mainly the IP_3R2 and to a lesser extent the IP_3R3 but no IP_3R1. INS-1 and rat β-cells express predominantly IP_3R3 and IP_3R2 and to a lesser extent IP_3R1 [21]. It is evident from the same atlas that the tissue distribution of RyRs is wider than that of the IP_3Rs. In fact all of the three RyRs (i.e., RyR1, RyR2, and RyR3) are expressed to a variable degree, in almost all human tissues examined. All of the three RyRs are present also in the human islets. By RT-PCR, the mRNAs of the three types of RyRs can be detected in whole human islets [76]. β-cells certainly express the RyR2 and probably also the RyR1 isoform [76–78]. By RT-PCR, mRNA for RyR1 was not detectable in INS-1 cells and rat islets, whereas mRNA for RyR2 was readily detected [21]. By immunofluorescence using a monoclonal antibody that detects RyR1 and RyR2, Johnson et al. show that RyRs are present in ~80% of β-cells in dispersed human islets [79, 80]. Earlier studies on the RyRs in the β-cells and regulation of these channels have been reviewed [81].

In MIN6 cells, it has been shown that RyR1 is located mainly on the insulin-containing dense-core secretory vesicles, whereas RyR2 is located mainly on the ER [78]. Dantrolene, a blocker of RyR1, inhibits Ca^{2+} release from the vesicles and

inhibits insulin secretion [78]. By using a variety of approaches, including siRNA technology, Rosker et al. show that RINm5F cells express RyR2 also on the plasma membrane [82]. These putative plasma membrane RyR channels have conductance properties that are different from those reported for RyR2 in the literature, which makes one speculate that it could be a different nonspecific cation channel [83].

Low concentration of ryanodine (e.g., 1 nM) increases $[Ca^{2+}]_i$ and stimulates insulin secretion from human β-cells [80]. Another activator of RyR, 9-Methyl-7-bromoeudistomin D increases insulin secretion in a glucose-dependent manner [84]. Four molecules of FKBP12.6 are tightly associated with the four RyR2 protomers, whereby it stabilizes and modulates activity of the channel [85]. In FKBP12.6 knock-out mice, glucose-induced insulin secretion is impaired [86]. Among the glycolytic intermediates, fructose 1,6 diphosphate activates RyR2 [87]. Stimulation of β-cells by glucose increases the concentration of arachidonic acid which can activate RyRs [40]. Other molecules that can link glucose metabolism to the RyRs are cADPR, long chain Acyl CoA, and of course ATP [88].

A mathematical model to explain mechanism of glucose-induced changes in membrane potential of β-cells postulates that RyR stimulation changes the pattern from "bursting" to "complex bursting" [89]. The term "complex" or "compound" bursting refers to cyclic variations in the duration of the slow waves of depolarization and repolarization intervals observed in some islets, when they are stimulated by glucose [90, 91]. In mouse islets, compound bursting gives rise to mixed $[Ca^{2+}]_i$ oscillations (i.e., rapid $[Ca^{2+}]_i$ oscillations superimposed on slow ones) [91]. If Ca^{2+} release from the ER (through RyRs or IP_3Rs) is responsible for compound bursting and consequent mixed $[Ca^{2+}]_i$ oscillations, then both of them should be abolished if the ER Ca^{2+} pool is kept empty. In fact that is exactly what happens. Thus if the ER Ca^{2+} pool is emptied by thapsigargin in the normal mice, or by knocking out SERCA3, then there is no compound bursting and no mixed $[Ca^{2+}]_i$ oscillations [91]. Analysis of electrical activity shows a higher percentage of active phases in SERCA3$^{-/-}$ mice [91], which suggests that Ca^{2+} release (through RyRs or IP_3Rs) from SERCA3-equipped ER Ca^{2+} pool terminates the active phase (for instance, by activating Kca channels).

Glinides are a group of drugs used to stimulate insulin secretion in the treatment of type 2 diabetes. These drugs stimulate exocytosis even in SUR1 knock-out mice [92]. One of the mechanisms by which glinides induce insulin secretion is activation of the RyRs [93]. GLP-1 stimulates insulin secretion by cAMP-dependent mechanisms that include sensitization of RyR-mediated CICR [94].

11.10 Cyclic ADP Ribose (cADPR) and Nicotinic Acid Adenine Dinucleotide Phosphate (NAADP)

These two intracellular messengers are formed from β-NAD^+ and $NADP^+$ by several ADP ribosyl cyclases including CD38 [95]. These messengers release Ca^{2+} from intracellular stores. While cADPR releases Ca^{2+} from the ER, NAADP

releases Ca^{2+} from acidic Ca^{2+} stores like lysosomes and even from insulin secretory vesicles [78]. Several groups have reported important roles for cADPR and NAADP in the regulation of Ca^{2+} signaling and insulin secretion. In β-cells, cADPR not only releases Ca^{2+} from the ER but also triggers Ca^{2+} entry across the plasma membrane by activating the TRPM2 channel [43]. High concentrations of glucose increase cADPR level in the β-cells. PKA phosphorylation activates CD38 and thereby increases formation of cADPR [96]. Thus, incretins like GLP-1 lead to an increased formation of cADPR [97]. Abscisic acid is a proinflammatory cytokine released by β-cells upon stimulation by glucose. It acts in an autocrine/paracrine fashion on a putative receptor that is coupled to a pertussis-toxin sensitive G protein and increases cAMP level which via PKA phosphorylation of CD38 increases formation of cADPR. Nanomolar concentration of abscisic acid increases glucose-stimulated insulin secretion from human islets [96].

Glucose increases NAADP level in MIN6 cells and uncaging of microinjected caged NAADP increases $[Ca^{2+}]_i$ in these cells by releasing Ca^{2+} from a thapsigargin-insensitive pool [98]. NAADP-induced Ca^{2+} release is blocked by nifedipine and some other blockers of L-type VGCCs. One of the organelle that constitutes the NAADP-sensitive Ca^{2+} stores in these cells is the dense-core insulin secretory vesicles [78]. Microinjection of NAADP into human β-cells induces Ca^{2+} release from intracellular stores in an oscillatory manner [99]. Insulin increases $[Ca^{2+}]_i$ in about 30% of human β-cells by a NAADP-dependent mechanism [99]. It is not known whether insulin increases NAADP level in human β-cells. It does not increase NAADP in mouse β-cells [100]. NAADP releases Ca^{2+} by activating a relatively new group of voltage-gated ion channels called "two-pore channels" (TPCs also termed TPCNs) [101]. TPC2 is located on the lysosomal membranes and releases Ca^{2+} when activated by low nanomolar concentration of NAADP. Micromolar concentration of NAADP inhibits the channel. As expected, in TPC2 knock-out mice, NAADP fails to release Ca^{2+} from the intracellular stores of β-cells [101].

The most well-known enzyme that synthesizes cADPR and NAADP is CD38. However, studies using CD38-knock-out mice suggest that CD38 does not play an essential role in glucose stimulation of Ca^{2+} signals or insulin secretion. In CD38-knock-out mice, the islets are more susceptible to apoptosis suggesting that CD38/cADPR/NAADP system may be important for β-cell survival [102].

11.11 Ca^{2+}-Induced Ca^{2+} Release (CICR)

Just as there are voltage-gated Ca^{2+} channels (VGCC) in the plasma membrane, there are Ca^{2+}-gated Ca^{2+} channels (CGCC) on the intracellular Ca^{2+} stores. Both IP_3Rs and RyRs are CGCCs [103, 104] and both can mediate CICR, making the process a universal one [105]. It is easy to study VGCCs on the plasma membrane by patch clamp. Nevertheless, to activate a given VGCC, one has to carefully choose the holding potential, the voltage jump, and its duration depending on which VGCC

one is looking for. Availability of potent and specific inhibitors of VGCCs has made it further easier to study these channels. This is why the literature on Ca^{2+} signaling in the islets is hugely dominated by VGCCs. The situation is far more difficult when it comes to the study of CGCCs. In analogy with VGCCs, for triggering CGCCs by Ca^{2+}, one has to carefully choose the magnitude and the duration of the Ca^{2+} trigger [106]. In practice, this is not easy. Activation of CGCCs is further dependent on the filling state of the Ca^{2+} store, phosphorylation status, and co-agonists, e.g., IP_3 and cADPR. The pharmacology of CGCCs is also more complex than that of VGCCs. Thus, low nanomolar concentration of ryanodine activates RyRs and high concentration of ryanodine irreversibly locks the RyRs in a subconductance state. Inhibition of Ca^{2+} release by ryanodine is a use-dependent process and needs attention to appropriate protocols [107].

Measurement of spatially averaged $[Ca^{2+}]_i$ by using nonlinear Ca^{2+} indicators like fura-2 and indo-1 is not particularly suitable for quantitative studies of CICR, which takes the form of transient rises of $[Ca^{2+}]_i$ in discrete locations in the cytoplasm [108]. Moreover, these indicators act as mobile buffers that bind the triggering Ca^{2+} with high affinity and snatch it away from the site of action [109]. In this respect, lower affinity brighter indicators like fluo-3 which can be used at lower concentrations are less of a problem. The global increase of $[Ca^{2+}]_i$ that one sees in a β-cell upon stimulation by glucose plus incretin hormones (e.g., GLP-1) is a net result of Ca^{2+} that enters through the plasma membrane and Ca^{2+} that is released from the stores by the process of CICR (provided the conditions for engaging CICR mechanism are in place). However, direct visualization of the CICR component may be difficult because of cell-wide increase of $[Ca^{2+}]_i$. One trick we employed was to use Sr^{2+} instead of Ca^{2+} as the trigger and exploited the differences in the fluorescence properties of Ca^{2+}- and Sr^{2+}-bound fluo-3. By this way one can show Sr^{2+}-induced Ca^{2+} release and assume that it is equivalent to CICR [110]. Another trick is to use verapamil which reduces the probability of opening of the L-type VGCCs and thereby reduces their contribution to the $[Ca^{2+}]_i$ increase. This enables better visualization of the $[Ca^{2+}]_i$ increase that is attributable to CICR. The rationale of such approach is based on the facts that verapamil does not reduce the amplitude of the single channel current; it reduces only the frequency of the triggering events but not their effectiveness in eliciting CICR [111]. In the experiment illustrated in Fig. 11.1, we stimulated a human β-cell first by 30 mM KCl which resulted in an increase of $[Ca^{2+}]_i$ to ~400 nM. We then applied verapamil which reduced the $[Ca^{2+}]_i$ to the baseline. We then washed away KCl and added instead glucose plus GLP-1. Glucose depolarized the β-cells but the expected sustained $[Ca^{2+}]_i$ increase was absent because of verapamil. Nevertheless, the L-type VGCC-mediated trigger events (which were now less frequent because of verapamil), did elicit large $[Ca^{2+}]_i$ transients by activating CICR. These $[Ca^{2+}]_i$ transients are too large to be explained by Ca^{2+} entry through the L-type VGCCs per se. These are due to synchronous activation of RyRs in clusters. In this protocol glucose facilitates CICR by increasing the ER Ca^{2+} content and by providing ATP and fructose 1,6 diphosphate, all of which sensitizes the RyRs. GLP-1 was included in this protocol since it facilitates CICR by PKA-dependent phosphorylation of the RyRs [77, 112]. In addition,

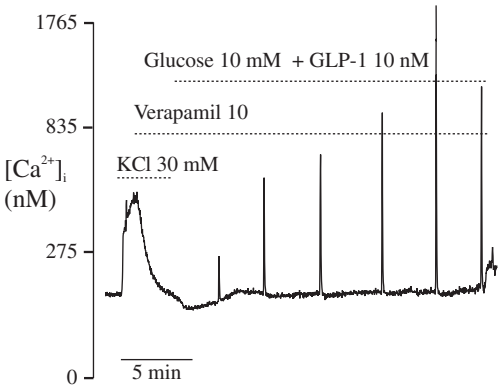

Fig. 11.1 CICR in human β-cells. $[Ca^{2+}]_i$ was measured by microfluorometry in fura-2-loaded single human β-cells. The cell was depolarized by KCl (25 mM) which increased $[Ca^{2+}]_i$. Verapamil (10 μM) was then added which lowered $[Ca^{2+}]_i$ to the baseline. (For rationale of using verapamil, please see the text and the references.) KCl was then removed and the cell was activated by glucose (10 mM) plus GLP-1 (10 nM). This protocol allowed visualization of CICR in the form of large Ca^{2+} transients

cAMP-regulated guanine nucleotide exchange factors (Epac) can also activate CICR via RyRs in human β-cells [113].

One important function of CICR in the β-cells is that it amplifies Ca^{2+}-dependent exocytosis [114, 115]. Secretory vesicle-associated RyRs are thought to play a role in exocytosis by increasing local Ca^{2+} concentration [78]. It may be noted that stimulation of β-cells by glucose alone (without cAMP-elevating agents) does not engage RyRs and thus glucose-induced insulin-secretion from human β-cells is not sensitive to inhibition or stimulation by ryanodine specially when protocols for use-dependent inhibition of RyRs by ryanodine are not employed [80]. CICR takes the form of large local Ca^{2+} transients and their function depends on the subcellular location of the transients. One possibility is that a large Ca^{2+} transient caused by CICR repolarizes plasma membrane potential by activating Kca channels. Thus a CICR event can end a burst of electrical activity and bring back the membrane potential from plateau depolarization to the baseline repolarized state and thereby increase the frequency of membrane potential oscillations. This view is supported by the observations that β-cells of $SERCA3^{-/-}$ mice as well as thapsigargin-treated β-cells (both of which would be unable to trigger CICR) spend a higher proportion of time in depolarized state and have lower frequency of membrane potential oscillation [91]. One may speculate that at early stages of development of type 2 diabetes, β-cell failure can be predominantly a depolarization failure or a repolarization failure. This view is akin to two forms of heart failure where one can have predominantly systolic failure or predominantly diastolic failure. Repolarization failure of β-cells (failure of β-cells to "relax") will lead to hyperinsulinemia and disturb the pulsatility of insulin secretion, all too well-known features of early stages of diabetes. In terms of Ca^{2+} signaling, such repolarization failure can be attributed to failure of CICR,

which can in principle be corrected by GLP-1 an established therapeutic agent for type 2 diabetes (see chapter by Leung and Cheng in this book).

11.12 $[Ca^{2+}]_i$ Oscillation in β-Cells

In the normal human body, β-cells are stimulated by glucose, the concentration of which oscillates at ~4 min interval. However, in most experiments, β-cells are stimulated by a constantly elevated concentration of glucose. In the normal human body β-cells are supplied with glucose (and other nutrients, hormones) through a rich network of capillaries; in most in vitro experiments, glucose is not delivered to the islet cells through capillaries. As mentioned earlier, human islets secrete insulin in the form of pulses at ~5 min intervals both in the fasting and in the fed states. One would expect that $[Ca^{2+}]_i$ in the human islets would change in the form of oscillations with one $[Ca^{2+}]_i$ peak every ~5 min; $[Ca^{2+}]_i$ would return to the baseline in between the peaks. This expectation is based on the observations made in mouse islets, where glucose induces baseline $[Ca^{2+}]_i$ oscillations and corresponding pulses of insulin secretion [116]. However, stimulation of human islets by glucose shows many types of $[Ca^{2+}]_i$ responses [117]. In many islets, $[Ca^{2+}]_i$ is increased and remains persistently elevated, and in others there are some high frequency sinusoidal oscillations of $[Ca^{2+}]_i$ on top of the $[Ca^{2+}]_i$ plateau [117–121]. Such sinusoidal oscillations of $[Ca^{2+}]_i$ on top of a $[Ca^{2+}]_i$ plateau have been described also in islets obtained from a subject with impaired glucose tolerance [119]. As early as in 1992, Misler et al. wrote: "four of 11 islets showed little or no response to 10 mM glucose while still responding to 20 μM tolbutamide. The pattern of glucose response of glucose-sensitive islets was also variable. Four islets displayed glucose-induced oscillations superimposed on a plateau. Two islets displayed a slow rise to a plateau without oscillations. The remaining islets showed an increasing frequency of short transients on an unchanging baseline; these transients ultimately coalesced into a prominent spike-like rise" [122]. Note that these are not bad islets; in fact these are islets of such good quality that they could be used for transplantation into human body for the cure of diabetes. Investigators know that stimulation of human islets by glucose often leads to persistent elevations of $[Ca^{2+}]_i$, rather than baseline oscillations of $[Ca^{2+}]_i$. To increase chances of obtaining oscillatory changes in $[Ca^{2+}]_i$, investigators sometimes replace extracellular Ca^{2+} by Sr^{2+} [123]. This maneuver yields nicer oscillatory changes in $[Sr^{2+}]_i$ and pulsatile insulin secretion from human islets [123]. But again, nature has chosen Ca^{2+} and not Sr^{2+} for signaling.

Some islet researchers assume that normal human islets should respond by $[Ca^{2+}]_i$ increase in the form of baseline $[Ca^{2+}]_i$ oscillations and that persistent $[Ca^{2+}]_i$ elevation is a sign of subtle damage to the islets or suboptimal experimental conditions [123]. At first sight, this seems to be a fair argument: for instance, some Ca^{2+} laboratories receive islets from a human islet isolation facility located next door; others receive islets via transatlantic flights. Ca^{2+} measurement techniques that use UV light and fura-2 acetoxymethyl esters (or similar probes) can damage

islets whose metabolism is often stunned and whose microcirculation and neural connections are lost. In fact, many individual islets obtained from normal subjects do not show any Ca^{2+} response at all to any stimulus [119]. Investigators select, consciously or subconsciously, the experiments that show nice $[Ca^{2+}]_i$ oscillations (because the islets that do not show oscillations are presumed to be the bad ones). In fact, they select the very islet that they choose to examine. There are up to several millions of islets in a human pancreas and they differ in their sizes, structures, and cellular make-up (see Chapter by In't Veld and Marichal in this book). They look different even to the naked eyes and under the microscope. Some look like "nice" encapsulated islets and others look like small aggregates of loosely associated cells, both types being normal. Investigators choose the "nice" ones for their experiments but still get different kinds of $[Ca^{2+}]_i$ responses. It is noteworthy that most such studies did not employ any cAMP-elevating agents, making CICR impossible.

$[Ca^{2+}]_i$ responses of single human β-cells to glucose are also extremely heterogeneous. Nevertheless, when single human β-cells are stimulated by glucose (in the absence of other nutrients, hormones, or neurotransmitters), many of them do respond by $[Ca^{2+}]_i$ changes in the form of slow oscillations, whereby $[Ca^{2+}]_i$ reaches to peaks every 2–5 min and then return to the baseline. Some investigators show that when $[Ca^{2+}]_i$ oscillations occur in one human β-cell, the neighboring β-cells in an aggregate or in an islet show $[Ca^{2+}]_i$ oscillation in a synchronized manner [117, 123]. This is due to coupling between β-cells via gap junctions made of connexin36 [116, 124]. Other investigators report that synchrony of $[Ca^{2+}]_i$ oscillation between groups of β-cells occur in mouse islets but not in human islets [4, 118]. Experiments using expressed fluorescent vesicle cargo proteins and total internal reflection fluorescence microscopy show that stimulation of single human β-cells by glucose gives rise to bursts of insulin vesicle secretion (at intervals of 15–45 s) that coincides with transient increase of $[Ca^{2+}]_i$ [125]. However, it needs to be pointed out that glucose-induced baseline $[Ca^{2+}]_i$ oscillations in single β-cells that we are talking about occur only in Petri dishes and are unlikely to occur in vivo. In vivo, hormones (e.g., glucagon and incretins) and amino acids (e.g., glycine and many others) are likely to transform the oscillatory $[Ca^{2+}]_i$ changes to a persistent elevation of $[Ca^{2+}]_i$ [121]. Thus, in the human β-cells and islets, persistent increase of $[Ca^{2+}]_i$ in response to glucose is a rule rather than exception. The underlying cause of glucose-induced baseline $[Ca^{2+}]_i$ oscillations in β-cells is thought to be the electrical bursts (clusters of large amplitude brief action potentials; one burst accounting for one episode of $[Ca^{2+}]_i$ increase). Study of β-cells from large mammals (e.g., dogs), however, shows that bursts occur only during the initial period of stimulation by glucose. In the later part of stimulation, bursts disappear; instead, there is sustained plateau depolarization to −35 to −20 mV and sustained increase of $[Ca^{2+}]_i$ to 500–1000 nM which causes tonic exocytosis [126]. Furthermore, at least some studies claim that insulin secretion is pulsatile even when $[Ca^{2+}]_i$ is stably elevated [127, 128]. It should be noted that stimulation of β-cells by glucose increases concentration of many molecules in the β-cells in an oscillatory manner (e.g., ATP [129] and cAMP [11]). Of these, oscillations of $[Ca^{2+}]_i$ are the easiest one to record and have, therefore, been adopted for modeling studies. It is thus not

surprising that pulsatility of insulin secretion from human islets in vivo has been modeled based on data obtained from in vitro experiments done on mice islets (see chapter by Bertram et al. in this book). This is in spite of the fact that the kind of electrical bursts and baseline $[Ca^{2+}]_i$ oscillations that occur in mouse islets have not been reproducibly demonstrated in human islets. This is not because of scarcity of human islets. In fact, during recent years it has become easier to obtain human islets for basic researches [130]. At present it appears that human islets show a wide variety of electrical activities and patterns of $[Ca^{2+}]_i$ changes which cannot explain the pulsatile insulin secretion into the human portal vein. Other less obvious factors that are unrelated to $[Ca^{2+}]_i$ oscillations, e.g., islet-liver interaction, may well constitute part of the mechanisms that determine pulsatile insulin secretion into the portal vein under normal conditions [131].

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