pharmacological agents that are able to influence the pancreatic B-cell?

Work in our laboratory has been generously funded by project/equipment grants to M.J.D. from The British Diabetic Association, The Wellcome Trust, The Yorkshire Cancer Research Campaign, The Medical Research Council, The Nuffield Foundation, The Royal Society, The Physiological Society, Novo-Nordisk UK and the University of Sheffield Research Fund.


Received 10 November 1993

Ca\textsuperscript{2+} and pancreatic B-cell function
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Introduction
In a number of cells, including the pancreatic B-cell, cytoplasmic free Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]\textsubscript{i}) has a fundamental role in signal transduction. Over the years we have been able to demonstrate that [Ca\textsuperscript{2+}]\textsubscript{i} in the insulin-secreting B-cell is regulated by a sophisticated interplay between nutrients, hormones and neurotransmitters (Figure 1). Glucose is readily taken up by the B-cell and upon its metabolism ATP is formed. ATP will close specific ATP-regulated K\textsuperscript{+} channels and thereby trigger depolarization of the plasma membrane. This leads to opening of voltage-gated L-type Ca\textsuperscript{2+} channels, Ca\textsuperscript{2+} influx, increase in [Ca\textsuperscript{2+}], and eventually insulin release [1]. This has led to the conclusion that there is

Abbreviations used: [Ca\textsuperscript{2+}]\textsubscript{i}, cytoplasmic free Ca\textsuperscript{2+} concentration; AC, adenylate cyclase; PLC, phospholipase C; InsP\textsubscript{3}, inositol 1,4,5-trisphosphate; PKC, protein kinase C; OA, okadaic acid; ER, endoplasmic reticulum; CICR, Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release.

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A model demonstrating how Ca\textsuperscript{2+} influx through voltage-activated L-type Ca\textsuperscript{2+} channels and Ca\textsuperscript{2+} release from intracellular stores may interact in the molecular regulation of the fast [Ca\textsuperscript{2+}]\textsubscript{i} oscillations in the pancreatic B-cell.

Glucose metabolism leads to the formation of ATP. ATP closes the ATP-regulated K\textsuperscript{+} channels (K\textsubscript{ATP}), resulting in depolarization, opening of voltage-activated Ca\textsuperscript{2+} channels and increase in [Ca\textsuperscript{2+}]\textsubscript{i}. The increase in [Ca\textsuperscript{2+}]\textsubscript{i} may activate the AC- and PLC-systems. These systems are also activated by various receptor agonists acting through G-proteins, resulting in the formation of cyclic AMP (cAMP), InsP\textsubscript{3}, and DAG. DAG can also be formed directly from glucose metabolism through de novo synthesis. By activating protein kinase A (PKA), cAMP promotes phosphorylation of the voltage-gated L-type Ca\textsuperscript{2+} channel and thereby to some extent increases Ca\textsuperscript{2+} influx. DAG activates PKC, an enzyme interacting with both the voltage-activated Ca\textsuperscript{2+} channels and the plasma membrane Ca\textsuperscript{2+} pump. PKC can also directly exert feedback inhibition of the PLC system (not discussed in the present paper). InsP\textsubscript{3} mobilizes intracellularly bound Ca\textsuperscript{2+}, which in turn may promote CICR. Ca\textsuperscript{2+} originating from intracellular stores activates a low-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channel (K\textsubscript{Ca}), resulting in repolarization of the plasma membrane and closure of the voltage-activated Ca\textsuperscript{2+} channels. Interestingly, the activity of the voltage-gated Ca\textsuperscript{2+} channels can also be modulated by calmodulin-dependent protein kinases (CAM) and serine/threonine protein phosphatases (PPase). Note also that the proposed model requires that the B-cell is polarized, not only in terms of secretory granules (SG), but also voltage-gated Ca\textsuperscript{2+} channels, ER and K\textsubscript{Ca}. This means that Ca\textsuperscript{2+} influx through voltage-gated Ca\textsuperscript{2+} channels will take place in close contact with SG, thus initiating exocytosis. Ca\textsuperscript{2+} released from intracellular stores will preferentially activate K\textsubscript{Ca}, resulting in repolarization of the cell and thereby closure of the voltage-gated Ca\textsuperscript{2+} channels. It is worth noting that the phosphorylation processes, induced by, for example, PKA, PKC and inhibition of PPases, may exert their main stimulatory effects on the B-cell stimulus-secretion coupling by direct interaction with the exocytotic machinery in a manner that is not directly correlated to changes in [Ca\textsuperscript{2+}]\textsubscript{i}.

Phorbol esters

Phorbol esters

Glucose metabolism

AMP

InsP\textsubscript{3}

Ca\textsuperscript{2+}

ER

Depol

Repol

Insulin

K\textsubscript{ATP}

K\textsuperscript{+}

DAG

Ca\textsuperscript{2+}

K\textsubscript{Ca}

SG
generally a direct coupling between \([\text{Ca}^{2+}]\), and activity of the insulin secretory process. Hormones and neurotransmitters affect the B-cell through activation of receptors coupled to various effector systems. Examples of such receptor systems are adenylyl cyclase (AC) and phospholipase C (PLC). When these systems are activated cyclic AMP is formed or phosphatidylinositol 4,5-bisphosphate is hydrolysed, the latter resulting in the formation of inositol 1,4,5-trisphosphate (InsP3) and diacylglycerol [1]. In addition to affecting the voltage-gated L-type Ca\(^{2+}\) channels, cyclic AMP directly affects the exocytotic machinery; both effects are likely to be mediated by protein kinase A. InsP3 mobilizes intracellularly bound Ca\(^{2+}\), mainly from the endoplasmic reticulum (ER), and DAG activates protein kinase C (PKC). Although InsP3 increases \([\text{Ca}^{2+}]\), there is little effect on insulin release, suggesting that the trisphosphate has a role other than being primarily involved as a signal for exocytosis in the pancreatic B-cell. The physiological role of PKC is more clear. This enzyme is indeed involved as a modulator of multiple steps in the B-cell signal-transduction pathway. These steps involve the voltage-gated L-type Ca\(^{2+}\) channel, the plasma membrane Ca\(^{2+}\) pump, the PLC system and the mechanism directly regulating exocytosis [2].

It is well established that glucose stimulation of intact pancreatic islets promotes oscillations in \([\text{Ca}^{2+}]\), that are synchronized with the electrical activity [3]. Interestingly, also in isolated pancreatic B-cells, glucose stimulation induces a bursting pattern of electrical activity as well as \([\text{Ca}^{2+}]\)-oscillations [4-7]. However, in the single B-cell the oscillations in \([\text{Ca}^{2+}]\), have a frequency that is approximately one order of magnitude lower than that in the intact islet. Little is known about the molecular mechanisms regulating B-cell \([\text{Ca}^{2+}]\)-oscillations. It is likely that oscillations in \([\text{Ca}^{2+}]\), are beneficial in terms of a more efficient signal transduction and in the ability of the B-cell to avoid cytotoxic effects of this divalent cation. As yet, there is no clear experimental support for the notion that oscillations in \([\text{Ca}^{2+}]\), constitute the molecular basis for pulsatility in insulin release.

**Plasma membrane Ca\(^{2+}\) transport**
The predominant Ca\(^{2+}\) current in the B-cell is carried by L-type Ca\(^{2+}\) channels (Figure 1). The activity of these channels is regulated by protein phosphorylation either directly, of the channel protein, or of associated regulatory proteins. Phosphorylating compounds, such as Mg-ATP, cyclic AMP and the catalytic subunit of protein kinase A, slow the time-dependent run-down of Ca\(^{2+}\) currents [8]. In the pancreatic B-cell, cyclic AMP does not seem to affect peak Ca\(^{2+}\) currents, but rather the rate of inactivation, thereby increasing the net influx of the ion [9, 10].

It has been suggested that PKC has an inhibitory effect on the voltage-gated Ca\(^{2+}\) channel [11] and that stimulation of the enzyme leads to membrane depolarization and Ca\(^{2+}\) influx [12] in insulin-secreting cells. Whole-cell recordings have revealed a clear-cut increase in Ca\(^{2+}\) channel activity, subsequent to PKC activation [13]. In accordance with this, down-regulation of the enzyme results in a marked reduction of the voltage-dependent Ca\(^{2+}\) current, an effect that is associated with a delayed increase in \([\text{Ca}^{2+}]\), subsequent to glucose stimulation [14]. This implies that PKC-induced phosphorylation modulates the activity of voltage-gated L-type Ca\(^{2+}\) channels in the pancreatic B-cell (Figure 1). Glucose may influence Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels either by regulating membrane potential or by biochemically modulating the channel protein itself. The latter effect is likely to depend on DAG, formed upon glucose metabolism and then accounted for by PKC-induced phosphorylation [15-17] (Figure 1).

A number of B-cell enzymes have been shown to be activated by Ca\(^{2+}\)-calmodulin [18-20]. The calmodulin antagonist calmidazol inhibits influx of Ca\(^{2+}\) through voltage-gated L-type Ca\(^{2+}\) channels in the B-cell [21], suggesting that phosphorylation by calmodulin-dependent protein kinases is involved in the regulation of these channels (Figure 1).

With regard to possible effects of protein phosphatasas on plasma membrane Ca\(^{2+}\) transport, our interest has recently been focused on the serine/threonine protein phosphatases. Okadaic acid (OA) is a toxin that has been used extensively as a potent inhibitor of serine/threonine protein phosphatases type 1, type 2A and type 3 [22]. By using the whole-cell configuration of the patch-clamp technique, we have shown an increase in the L-type Ca\(^{2+}\) channel activity subsequent to acute application of OA to the pancreatic B-cell [23]. Moreover, our data suggest that this Ca\(^{2+}\) current is evoked at lower voltages in the presence of the phosphatase inhibitor. Therefore it seems that, in the pancreatic B-cell, serine/threonine protein phosphatases work in concert with various protein kinases in regulating the state of phosphorylation and thereby the threshold potential of the voltage-gated L-type Ca\(^{2+}\) channels (Figure 1).
Intracellular Ca\(^{2+}\) transport

In mouse pancreatic B-cells, Ins\(P_3\) releases about 30% of the Ca\(^{2+}\) sequestered into intracellular stores, most probably the ER [24]. This implies that the B-cell has both Ins\(P_3\)-sensitive, and Ins\(P_3\)-insensitive, non-mitochondrial intracellular Ca\(^{2+}\) pools. While Ca\(^{2+}\) release by Ins\(P_3\) is well established, a possible role for the trisphosphate in promoting Ca\(^{2+}\) sequestration by ER is not that clear. Interestingly, the ambient free Ca\(^{2+}\) concentration obtained in insulin-secreting RINm5F-cells, subsequent to Ins\(P_3\)-induced Ca\(^{2+}\) release, is lower than that before addition of the trisphosphate [25]. A similar effect was obtained in the presence of heparin, a blocker of Ins\(P_3\)-induced Ca\(^{2+}\) release [25]. Our data therefore suggest the presence of high-affinity Ins\(P_3\)-receptors which operate continuously in the insulin-secreting cell. High concentrations of Ins\(P_3\) will desensitize such receptors, while they are inhibited by heparin. Inositol 1,3,4,5-tetrakisphosphate did not promote Ca\(^{2+}\) sequestration in RINm5F-cells. Both thapsigargin and 2,5-di-(tert-butyl)-1,4-benzohydroquinone (tBuBHQ), two potent inhibitors of ER Ca\(^{2+}\)-ATPase, dose-dependently mobilize Ca\(^{2+}\) from intracellular pools in electropermeabilized RINm5F-cells [26]. The magnitude of Ca\(^{2+}\) increase induced by either of the two inhibitors, is always smaller than that evoked by Ins\(P_3\); and neither of them can empty the trisphosphate-sensitive pool completely. Our data are in accordance with the existence of separate uptake and release compartments in the Ins\(P_3\)-sensitive pool in insulin-secreting cells. The two Ca\(^{2+}\)-ATPase inhibitors will then release Ca\(^{2+}\), predominantly from the uptake compartment, whereas Ca\(^{2+}\) located in the release compartment requires the presence of Ins\(P_3\) for its release.

Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) represents a phenomenon whereby an increase in [Ca\(^{2+}\)], causes further release of Ca\(^{2+}\), by acting on specific receptor-operated channels located in the sarcoplasmic reticulum. The receptor for CICR is called the ryanodine receptor, because it specifically binds the plant alkaloid ryanodine [27]. Caffeine is another alkaloid which potentiates CICR [27]. Direct demonstration of CICR is difficult and therefore most studies have resorted to the use of ryanodine and caffeine to promote this process. CICR is not limited to the skeletal muscle, but rather a more general phenomenon documented in a number of different cell types [28–30]. The sarcoplasmic reticulum ryanodine receptor contains sulphhydryl groups, which upon oxidation change the conformation of the ryanodine receptor protein and thereby initiate the release of Ca\(^{2+}\) [31, 32]. Thimerosal is a sulphhydryl reagent that has been shown to sensitize CICR in non-muscle cells [33]. Interestingly, in electrophermeabilized RINm5F-cells, thimerosal released Ca\(^{2+}\) from an Ins\(P_3\)-insensitive non-mitochondrial intracellular Ca\(^{2+}\) pool in a dose-dependent manner [34]. Dithiothreitol, a reducing agent, reversed this release of Ca\(^{2+}\). Thimerosal-induced Ca\(^{2+}\) release was potentiated by caffeine, in keeping with an effect of the sulphhydryl reagent on the CICR channel. Hence, our data suggest the existence of CICR in the pancreatic B-cell as well.

Cyclic ADP ribose is a newly discovered compound that is synthesized from NAD\(^{+}\) by the ADP-ribosyl cyclases [35]. This enzyme is present in many mammalian and invertebrate tissues [36] and it has been suggested that cyclic ADP ribose may function as a general Ca\(^{2+}\)-mobilizing agent. In those cells where cyclic ADP ribose has a Ca\(^{2+}\)-mobilizing activity, it is not clear whether the compound functions as a second messenger for an extracellular stimulus or mainly serves as a cofactor for CICR, by enhancing the sensitivity of the actual receptor to Ca\(^{2+}\). It has been suggested that glucose increases the levels of cyclic ADP ribose in the pancreatic B-cell, thereby serving as an important signal for intracellular Ca\(^{2+}\)-mobilization in this cell type [37]. This suggestion was based on weak experimental evidence, obtained from studies performed on microsomes prepared from pancreatic islets. The fact that the authors of that particular study were unable to show any stimulatory effect of Ins\(P_3\), under the same experimental conditions where cyclic ADP ribose mobilized intracellular Ca\(^{2+}\), sincerely questions the physiological relevance of this signalling pathway in the pancreatic B-cell. Moreover, in a recent study, taking a more physiological approach, we were unable to demonstrate any stimulatory effect of cyclic ADP ribose on Ca\(^{2+}\) mobilization in either normal pancreatic B-cells or clonal insulin-producing RINm5F-cells, under conditions where Ins\(P_3\) always promoted Ca\(^{2+}\) release [38].

Oscillations in [Ca\(^{2+}\)]

It is well established that single B-cells and small B-cell aggregates demonstrate slow (duration 3–5 min) oscillations in [Ca\(^{2+}\)], that are dependent on influx of Ca\(^{2+}\) through the voltage-gated L-type Ca\(^{2+}\) channels. When whole pancreatic islets are dispersed into single B-cells or small B-cell clusters, there is a loss of normal cell-to-cell communication,
a loss of the complex islet-cell architecture that guarantees proper autocrine and paracrine function and also a loss of receptor agonists of neurocrine origin. This may explain why the single B-cells and small B-cell clusters do not usually show fast (duration approximately 10 s) oscillations in \([\text{Ca}^{2+}]_{i}\), which is the normal feature of whole pancreatic islets [3]. It is worth noting, however, that single B-cells or small B-cell clusters sometimes also show fast oscillations and whole pancreatic islets slow oscillations in \([\text{Ca}^{2+}]_{i}\). In both these cases the fast oscillations are then superimposed on the slow ones. It is particularly interesting to note that, under conditions where there are increased levels of intracellular cyclic AMP or \(\text{InsP}_3\), the single B-cell and small B-cell clusters demonstrate fast oscillations in \([\text{Ca}^{2+}]_{i}\), subsequent to glucose stimulation. Fast oscillations in \([\text{Ca}^{2+}]_{i}\), are also sometimes observed subsequent to stimulation of the dispersed cells with high concentrations of \(\text{K}^+\), an effect that may be secondary to an increase in \([\text{Ca}^{2+}]_{i}\), and thereby mediated by both \(\text{InsP}_3\)-induced \(\text{Ca}^{2+}\) release and CICR. A possibility that remains to be clarified is, therefore, that the slow oscillations in \([\text{Ca}^{2+}]_{i}\), observed in single B-cells or small B-cell clusters, merely reflect an experimental artefact due to the fact that there is a deprivation of the normal paracrine, neurocrine and autocrine feedback regulation.

Figure 1 shows one possible model of the complex interplay between \(\text{Ca}^{2+}\) influx, through the voltage-gated \(L\)-type \(\text{Ca}^{2+}\) channels, and \(\text{Ca}^{2+}\) release from intracellular stores, in the regulation of the glucose-induced fast oscillations in \([\text{Ca}^{2+}]_{i}\), in the B-cell. In this model, \(\text{Ca}^{2+}\) influx through the voltage-gated \(L\)-type \(\text{Ca}^{2+}\) channel is responsible for the rising phase of the \([\text{Ca}^{2+}]_{i}\), oscillation. The increase in \([\text{Ca}^{2+}]_{i}\), will, in addition to various autocrine and neurocrine receptor agonists, promote the formation of \(\text{InsP}_3\), which together with CICR induces a localized release of intracellularly bound \(\text{Ca}^{2+}\). This results in the activation of a low-conductance \(\text{Ca}^{2+}\)-activated \(\text{K}^+\) channel and, thereby, repolarization of the plasma membrane and closure of the voltage-gated \(L\)-type \(\text{Ca}^{2+}\) channels [39]. \(\text{K}^+\) currents of a similar type are evoked by hormones and neurotransmitters present in the B-cell micro-milieu and also by intracellular application of compounds mobilizing \(\text{Ca}^{2+}\) from \(\text{InsP}_3\)-sensitive \(\text{Ca}^{2+}\) stores. This suggests that the excitable pancreatic B-cell also exhibits \(\text{InsP}_3\)- and \(\text{Ca}^{2+}\)-mediated periodic increases in \([\text{Ca}^{2+}]_{i}\), associated with changes in membrane conductance. The glucose concentration is maintained high under the experimental conditions referred to and the ATP-regulated \(\text{K}^+\) channels may remain closed. The cell will therefore depolarize again, inducing the next \([\text{Ca}^{2+}]_{i}\), oscillation once the \(\text{Ca}^{2+}\)-activated \(\text{K}^+\) channels are inactivated as a result of the cell's capacity to buffer \([\text{Ca}^{2+}]_{i}\). Hence in the pancreatic B-cell, \([\text{Ca}^{2+}]_{i}\), oscillations probably represent a complex interplay between plasma membrane and intracellular \(\text{Ca}^{2+}\) transport. Another molecular mechanism that may be involved in the regulation of glucose-induced oscillations in \([\text{Ca}^{2+}]_{i}\), is oscillations in metabolism and thereby oscillations in the ATP/ADP ratio in close vicinity of the ATP-regulated \(\text{K}^+\) channels. This may result in membrane potential fluctuations. We have reasons to believe that oscillations in metabolism are indeed involved in the regulation of B-cell electrical activity and, as a result, oscillations in \([\text{Ca}^{2+}]_{i}\). The model described in Figure 1 also requires that the B-cell is polarized, not only in terms of secretory granules, but also voltage-gated \(\text{Ca}^{2+}\) channels, ER and \(\text{Ca}^{2+}\)-activated \(\text{K}^+\) channels.

**Role of \([\text{Ca}^{2+}]_{i}\), in exocytosis**

For a long time it has been known that the exocytotic process of insulin in the B-cell can be modulated to a great extent, both positively and negatively, without any major concomitant changes in \([\text{Ca}^{2+}]_{i}\). Examples of positive release signals are PKC and cyclic AMP, as convincingly demonstrated in both intact and permeabilized B-cells [40]. These signals probably evoke their effects by inducing phosphorylation of one or several sites that has a key function in regulating exocytosis. That cyclic AMP exerts most of its stimulatory effects on the B-cell stimulus-secretion coupling, by directly affecting the exocytotic machinery using protein kinase A-induced phosphorylation, was indeed confirmed in a recent study by Åmmlå et al. [41]. Moreover, a direct stimulatory role of phosphorylation was also evident from studies demonstrating that activation of PKC and inhibition of serine/threonine protein phosphatases promote exocytosis without any major effects on the B-cell handling of \(\text{Ca}^{2+}\) [23] (C. Åmmlå, L. Eliasson, K. Bokvist, P.-O. Berggren, R. E. Honkanen, Å. Sjöholm and P. Rorsman, unpublished work). We have recently shown that the potent calmodulin antagonist calmidazol, although serving as an efficient blocker of \(\text{Ca}^{2+}\) influx through the voltage-gated \(L\)-type \(\text{Ca}^{2+}\) channels (see above), has a dramatic stimulatory effect on basal insulin release [21]. In this case, insulin release did not result from unspecific permeabilization of the B-cells and was not
dependent on PKC or pertussis toxin-sensitive GTP-binding proteins.

Application of various inhibitory peptides of insulin release as well $\alpha_2$-adrenergic agonists (examples of negative release signals) dramatically suppresses exocytosis, despite stimulatory levels of $[\text{Ca}^{2+}]$. [40]. We have also shown, by measuring insulin release and $[\text{Ca}^{2+}]$, simultaneously from one single islet, that there is no direct correlation between exocytosis and $[\text{Ca}^{2+}]$, (S. Zaitsev and P.-O. Berggren, unpublished work). With this information at hand, it is tempting to speculate that $[\text{Ca}^{2+}]$ may have a permissive rather than a direct regulatory role in the B-cell stimulus-secretion coupling.

Looking at the regulation of exocytosis in more general terms, it is clear that there is no direct coupling between $[\text{Ca}^{2+}]$, and the final molecular mechanisms promoting the release process. This is not surprising in view of the very complex cellular mechanisms, including for example a number of so-called exocytotic proteins, that have been identified during recent years to be involved in regulating exocytosis in a number of various cell types.

The authors are grateful to Anita Maddock for expert secretarial assistance. The authors own research described was supported by the Swedish Medical Research Council (03X-09890, 04X-09891 and 19X-exocytosis in a number of various cell types.

Mechanisms of pancreatic B-cell dysfunction and glucose toxicity in non-insulin-dependent diabetes

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Introduction

The insulin response to food ingestion is determined by the direct actions of glucose and certain amino acids on the pancreatic B-cell together with indirect actions generated through activation of both hormonal and neural arms of the enteroinsular axis [1-3]. These signals are normally integrated at the level of the pancreatic B-cell such that insulin is secreted to appropriately regulate nutrient metabolism and glucose homeostasis. In non-insulin-dependent diabetes mellitus (NIDDM), defects in the mechanisms that regulate insulin secretion make a major contribution to the glucose intolerance and metabolic disarray associated with the disease [4-6]. Possible molecular mechanisms underlying pancreatic B-cell dysfunction in NIDDM include site-specific defects in the stimulus-secretion coupling pathway and changes in B-cell function consequent to alterations in external influences on the B-cell. The participation and interaction of the various pathways to disturbances of insulin secretion in NIDDM are considered below in the framework of our present understanding of the regulation of B-cell function and stimulus-secretion coupling.

Susceptible points in B-cell stimulus-secretion coupling

Glucose insensitivity of the pancreatic B-cell lies at the heart of defective insulin secretion in NIDDM [4-6]. Glucose is the principal regulator of B-cell function and also amplifies the insulinotropic actions of all other secretagogues, including enteroinsular hormones and neurotransmitters [7]. It follows that each site in the series of steps linking B-cell glucose recognition to insulin discharge represents a potential influential lesion that might result in defective insulin secretion, characteristic of NIDDM. The normal secretory pathway induced by glucose starts with transport of the sugar into the B-cell by the GLUT2 glucose transporter (for comprehensive coverage of the pathway see [8-11]). Phosphorylation of glucose, by glucokinase, and subsequent metabolism leads to the generation of ATP and increase of the ATP/ADP ratio. This results in closure of ATP-sensitive K+ channels (K+-ATP channels) in the B-cell membrane, depolarization, opening of voltage-dependent Ca2+ channels and Ca2+ influx. The resulting increase in cytoplasmic Ca2+ concentration ([Ca2+]i) then triggers the secretory machinery, culminating in the discharge of insulin by exocytosis. Ca2+ also activates enzymes such as adenylate cyclase (with generation of cyclic AMP) and phospholipase C (with production of inositol 1,4,5-trisphosphate and diacylglycerol, which activates protein kinase C) that serve both to displace Ca2+ from intracellular stores and to sensitize the secretory machinery to [Ca2+]i [10]. Hormonal and neural elements of the enteroinsular axis potentiate the insulin secretory process through interactions with membrane receptors that are also linked to activation of adenylate cyclase or phospholipase C [7].

Site-specific defects in B-cell dysfunction

Site-specific defects in the pancreatic B-cell have been uncovered in studies evaluating mechanisms of defective insulin secretion using islets isolated from various animal models of NIDDM [6]. Less