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Review

The pancreatic beta-cell as a fuel sensor: an electrophysiologist's viewpoint*

P. Rorsman

Department of Islet Cell Physiology, Novo Nordisk A/S, Copenhagen, Denmark

Summary The pancreatic beta cell serves as the fuel sensor of the entire body and controls, via secretion of the hypoglycaemic hormone insulin, the blood glucose concentrations within narrow limits by regulation of glucose uptake and release. During the last 30 years, a combination of biochemical and ultrastructural approaches has resulted in dramatic progress in the understanding of the processes by which glucose and other nutrients modulate the release of insulin. The beta cells have also been investigated using electrophysiological techniques and were thus found to be electrically excitable and to undergo complex changes in their membrane potential when exposed to glucose and other stimulators of secretion. The application of the patch-clamp technique to the pancreatic islet preparations has revolutionized the understanding of how bioelectrical processes participate in the fuel-sensing of the beta cell. An important achievement was the identification of an ATP-sensitive K^+-channel as the resting and glucose-sensitive membrane conductance of the beta cell. This channel also constitutes the target of the hypoglycaemic sulphonylureas: a group of compounds which have been used successfully in the treatment of insulin-dependent diabetes mellitus for several decades. [Diabetologia (1997) 40: 487–495]

Keywords Ion channels, ATP, insulin, exocytosis, pancreas.

The central role of ions in the control of cellular excitability has been recognized for more than a century and dates back to the pioneering work of Ringer [1]. For a long time, electrical excitability was a property believed to be confined to a small group of highly sophisticated cells such as nerve and muscle cells in which the role and need of electrical signalling was obvious. However, during the 1960s and 1970s it became obvious that a number of endocrine cells share this capacity and that they utilize changes in their membrane potential to transduce changes in their environment to acceleration of hormone secretion [2, 3].

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Corresponding author: Dr. P. Rorsman, Department of Islet Cell Physiology, Novo Nordisk A/S, Fruebjergvej 3, DK-2100 Copenhagen, Denmark

Abbreviations: K-ATP channel, ATP-sensitive potassium channel; PKA, protein kinase A.

The pancreatic beta cell is electrically excitable

In 1968 Dean and Matthews [3] provided the first evidence for glucose-stimulated electrical activity in the pancreatic beta cell. The salient features of this electrical activity are summarized in Figure 1. In the absence of glucose, or at substimulatory glucose concentrations (< 7–8 mmol/l), the membrane potential of the beta cell is negative (–60 mV). Following elevation of glucose to insulin-releasing concentrations, the beta cell depolarizes and once the cell becomes sufficiently depolarized (i.e. exceeds the "threshold potential"), electrical activity is generated. This electrical activity consists of slow oscillations in membrane potential between a depolarized plateau, on which action potentials are superimposed (active

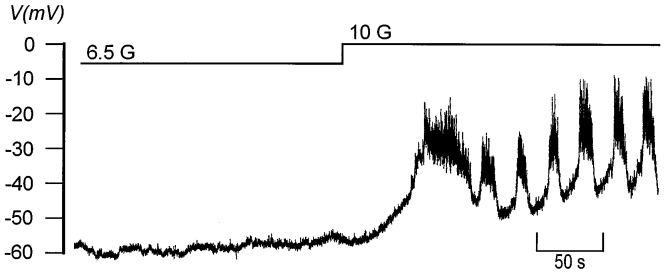

Fig. 1. The membrane potential (V) of a single beta cell within an intact pancreatic islet recorded in the presence of 6.5 and 10 mM glucose as indicated by the staircase

phase), and repolarized electrically silent intervals. The beta cell responds to glucose in a graded fashion: the fraction active phase increases as the glucose concentration is raised until electrical activity becomes continuous at concentrations above 20 mmol/l. The induction of electrical activity is a key event in the sequence of events that culminates in the release of insulin and it has been possible to demonstrate that the periods of electrical activity coincide with pulsatile insulin secretion [4]. More extensive accounts of the beta cell electrical activity have been published and interested readers are referred to these for a detailed description [5, 6].

ATP-sensitive K^+-channels: the biophysical basis for the fuel-sensing of the beta cell

Recordings using intracellular electrodes impaled into the beta cell in intact pancreatic islets have been invaluable in determining the effects of insulin-releasing agents on the membrane potential and electrical activity of the beta cell (reviewed in [5]). However, because it was not possible to control the membrane potential of the preparation using this technique, the identity and characteristics of the ion channels underlying the electrical activity could not be determined. Such analyses had to await the application of the patch-clamp technique to pancreatic islet cells [7]. This technique, the features of which relevant to the study of pancreatic beta cells have been described at length elsewhere [6], permits the recording under voltage-clamp conditions (i.e. the membrane potential is kept constant irrespective of any activation of membrane currents) of both the minute *single-channel* and the *whole-cell* currents; the latter reflecting the summed activity of all the ion channels in the entire plasma membrane. The introduction of the patch-clamp technique revolutionized electrophysiology and in just a few years transformed it from an "art", practised in only a few laboratories, into a standard technique of cell physiology. Using the various recording modes of the patch-clamp technique it became possible to demonstrate that the glucose-sensitive resting conductance of the beta cell is due to the activity of K^+-channels which are inhibited by intracellular ATP (the K-ATP channel) [7–11]. The regulation of the K-ATP channel is extremely complex but there is now agreement that changes in the cytoplasmic ATP/ADP-ratio represents an important determinant of channel activity [11, 12]. In parallel with the work on the K-ATP channel, the voltage-dependent membrane currents participating in the generation of the beta cell action potential were characterized. In mouse beta cells, which represent the "classic" preparation for electrophysiological experiments, the depolarizing phase of the action potential is attributable to the activation of voltage-gated Ca^{2+}-channels which are sensitive to dihydropyridines such as nifedipine (L-type Ca^{2+}-channels) [13, 14]. The repolarization of the action potential principally results from the opening of voltage-dependent K^+-channel with a time course of activation which is delayed relative that of the Ca^{2+}-channels (hence *delayed* rectifying K^+-current) [13, 15, 16].

The K-ATP channel: a "target" of the hypoglycaemic sulphonylureas

Following the identification of the K-ATP channel as the glucose-sensitive membrane conductance of the beta cell it was proposed that this channel also represents the target of the hypoglycaemic sulphonylureas, compounds which have been used in the treatment of non-insulin-dependent diabetes for several decades. With the aid of the patch-clamp technique it was possible to show that this was indeed the case and that therapeutic concentrations of the sulphonylureas produce a concentration-dependent inhibition of the K-ATP channel [17–19]. The exact nature of the interactions between the sulphonylureas and the K-ATP channel could not be resolved in the first patch-clamp experiments, but the observation that they remained inhibitory in isolated membrane patches enabled the conclusion that their effect is *not* secondary to interference with beta cell metabolism. Some of the sulphonylureas were found to be very potent inhibitors of the K-ATP channel. For example, glibenclamide was effective at nanomolar concentrations [19]. By using the sulphonylureas as ligands it was thereby possible to purify and eventually to clone the sulphonylurea receptor. Thanks to these efforts we now know that the K-ATP channel is a complex of a 145 kDa sulphonylurea receptor (SUR; [20]) and an inward rectifier K^+-channel protein (KIR6.2; [21, 22]). Hopefully this novel molecular information can be exploited in current and future endeavours to develop new and more tissue-selective antidiabetic compounds. In this context it is pertinent that K-ATP channels in different tissues

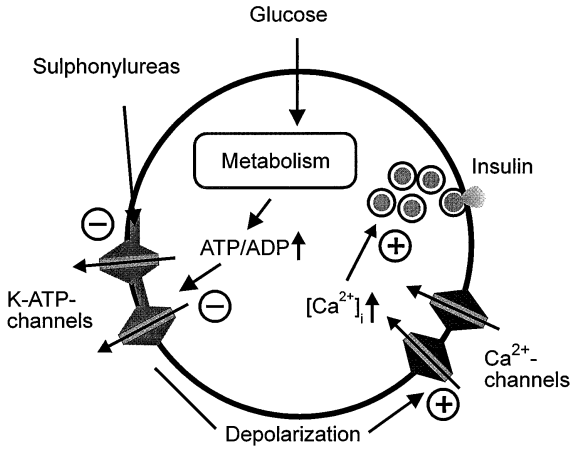

Fig. 2. Schematic model for the stimulus-secretion coupling of the pancreatic beta cell. Glucose produces, via its metabolism, an increased cytoplasmic ATP/ADP-ratio which inhibits the K-ATP channels. This results in membrane depolarization and the opening of voltage-dependent Ca^{2+}-channels, an increase in $[Ca^{2+}]_i$ and the initiation of exocytosis of the insulin-containing secretory granules. Also indicated in the model is the site of action of the hypoglycaemic sulphonylureas which inhibit the K-ATP channels by a direct effect which is not dependent on glucose metabolism

have distinct molecular composition. For example, the K-ATP channels in cardiac and beta cells contain distinct isoforms of SUR (denoted SUR2 and SUR1, respectively) [23]. This accounts for the different pharmacological properties of cardiac and beta cell K-ATP channels. It is not known which SUR is present in the K-ATP channels in smooth muscle cells (e.g. those in the blood vessels) but it is likely to represent yet another isoform of SUR as neither SUR1 nor SUR2 is expressed in smooth muscle preparations.

A model for glucose-stimulated insulin secretion

A simple model for the stimulus-secretion coupling of the pancreatic beta cell is shown in Figure 2. In the absence of glucose, the cytoplasmic ATP/ADP ratio is low and the K-ATP channels are active. Each beta cell is equipped with thousands of K-ATP channels and their summed activity effectively clamps the beta-cell membrane potential at the K^+ equilibrium potential which (with the K^+-gradients existing over the beta-cell membrane) is around –70 mV. Ion channels other than the K-ATP channels are present, and perhaps active even in the absence of glucose, but they are too few to influence the membrane potential as long as the K-ATP channels remain active. When the beta cell is exposed to glucose, the associated acceleration of glucose metabolism leads to a rise in the cytoplasmic ATP/ADP-ratio and the K-ATP channels close. Once they are almost completely inhibited (>90%), the remaining K-ATP conductance is unable to balance the depolarizing influence of (tonically active?) background conductance(s) and the beta cell depolarizes. When this depolarization is large enough, voltage-gated Ca^{2+}-channels become activated in a feed-forward manner (i.e. the initial depolarization up to the threshold potential causes the opening of a few voltage-gated Ca^{2+}-channels which in turn causes a bigger depolarization and the opening of additional Ca^{2+}-channels with resultant further depolarization, etc), thus accounting for the upstroke of the beta-cell action potential. The associated Ca^{2+}-influx causes a transient elevation of the cytoplasmic Ca^{2+}-concentration ($[Ca^{2+}]_i$) [24] which, via a series of poorly defined reactions, culminates in the exocytosis of the insulin-containing granules.

The unique feature of the pancreatic beta cell, essential for its ability to serve as the body's fuel sensor, is the presence of the K-ATP channels. As discussed above, the activity of these channels sets the membrane potential of the beta cell and thus determines its electrical and secretory activities. If the beta cell had not been equipped with K-ATP channels, it would have been tonically active and constantly releasing its insulin into the circulation regardless of the glucose concentration. Indeed, these are precisely the characteristics of beta-cells isolated from patients with persistent hyperinsulinaemic hypoglycaemia of infancy [25]. This rare hereditary disease is linked to mutations in SUR [26], which is part of the K-ATP channel complex (see above), and thus results in the formation of non-functional K-ATP channels. The fact that K-ATP channels play such an important role in the stimulus-secretion coupling of the beta cell does not exclude, however, that metabolic regulation of more distal processes also contributes to the overall fuel-sensing of the beta cell. For example, there is evidence suggesting that glucose metabolism may control both the functional state of the voltage-gated Ca^{2+}-channels [27] (thus determining the amount of Ca^{2+}-entry and the extent of Ca^{2+}-induced exocytosis) as well as the insulin secretory process itself [28]. The objective of beta-cell electrical activity is to generate the intracellular signal that initiates the exocytosis of the insulin-containing granules. In the remainder of this review I shall therefore discuss the control of exocytosis in the insulin-secreting beta cell and consider various modulatory mechanisms.

Capacitance measurements of insulin secretion

Elucidation of the fundamental properties of exocytosis requires a means to record secretion in single cells with high temporal (millisecond) resolution. Unfortunately, none of the traditional biochemical

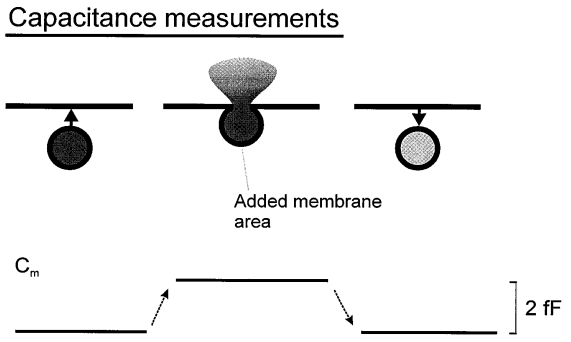

Fig. 3. Principle for capacitance measurements of exocytosis. The exocytosis of a secretory granule (left and centre) results in the incorporation of the granular membrane with the plasma membrane. The added membrane causes an increase in cell surface area which can be detected as an increase in cell capacitance (C_m), an electrical parameter of the cell which is proportionally related to the surface area (A; i.e. $C_m \propto A$). The specific membrane capacitance is 10 fF/μm^2. The surface area of a secretory granule can be estimated as 0.2 μm^2 assuming spherical geometry and a granule diameter of 0.25 μm [30]. The fusion of a single granule accordingly results in a capacitance increase of 2 fF. When a granule is subsequently retrieved by endocytosis, a corresponding decrease in cell capacitance can be recorded (right)

approaches for measuring insulin release have achieved this resolution. We have therefore been obliged to monitor changes in cell capacitance as an indicator of exocytosis. The membrane capacitance (C) is an electrical property of the cell which is proportionally related to the cell surface area. When the insulin-containing granules undergo exocytosis, their membranes are incorporated into the plasma membrane (Fig. 3). The resulting increase in cell surface area can be monitored as an increase in cell capacitance using electrophysiological techniques [29]. The major advantages of capacitance measurements over more traditional approaches to study secretion are: 1) the measurements can be carried out in single cells with millisecond resolution; 2) the experiments are carried out in a voltage-clamped preparation: i.e. any effects of a compound on membrane conductances and membrane potential will not influence the results; and 3) large proteins such as antibodies can be applied intracellularly by including them into the pipette solution which replaces the cytoplasm when utilizing the whole-cell recording mode of the patch-clamp technique. The specific capacitance of biological membranes (including those of the plasma membrane and the secretory granules) is ≈ 10 fF/μm^2. With a diameter of 250 nm [30], each beta-cell granule can be estimated to add 0.2 μm^2 of membrane area which corresponds to 2 fF of capacitance. Capacitance increases with this unitary amplitude have been recorded [31] and may accordingly reflect single exocytotic events (i.e. the fusion of an individual granule with the plasma membrane). It must be kept in mind that recordings of cell capacitance do not involve measurements of insulin secretion as such but changes in a physical property of the cell which hopefully reflect exocytosis. To ascertain that this is indeed the case we have combined capacitance measurements with fluorimetric and/or electrochemical detection of insulin secretion [32]. So far we have encountered no discrepancies between the capacitance measurements and secretion detected by the alternative methods making it reasonable to conclude that an increase in cell capacitance can be equated to exocytosis. The conclusion is reinforced by the observations that insulin secretion and exocytosis as reported by the capacitance measurements exhibit the same Ca^{2+}-dependence [32], are equally affected by cooling [33] and similarly modulated by hormones and neurotransmitters [34, 35]. Capacitance measurements finally offer the unique possibility of studying the retrieval of the secreted membranes by endocytosis. In this case, membrane retrieval results in a decreased membrane surface area and thus a reduction of the cell capacitance [36].

Exocytosis in the beta cell is rapid

Using capacitance measurements it was possible to demonstrate that the latency between the opening of the Ca^{2+}-channels and the onset of exocytosis is less than 50 ms. This is shorter than the time required for $[Ca^{2+}]_i$ to equilibrate within the beta cell which suggests that the Ca^{2+}-channels, the secretory granules and the release sites are situated in the near vicinity of each other. During the first 50 ms the rate of capacitance increase approached 1 pF/s. Using the conversion factor of 2 fF/granule this can be converted to an exocytotic rate of 500 granules/s. Since a beta cell contains 13000 granules [30], this value corresponds to a release rate of 4% of the total granule number being released per second! Clearly, exocytosis can only continue at this high rate for a very limited period and during protracted stimulations exocytosis proceeds at much slower average rates. This decline in the exocytotic rate reflects the gradual depletion of the pool of granules which is immediately available for release ("the readily releasable pool"). Once this pool has been depleted other processes, such as the refilling of the readily releasable pool (see below), become rate-limiting to exocytosis.

It should be made clear that the short latency (< 50 ms) observed in the capacitance measurements does not imply that the release process (including the dissolution of the Zn^{2+}-insulin crystal) is completed within this time span: the latency is simply a reflection of the time required for the granular membrane to fuse with the plasma membrane and thus be detected by the capacitance measurements. Even so the above considerations illustrate the high speed and capacity of the exocytotic machinery in the beta cell.

Modulation of Ca^{2+}-induced exocytosis

Exocytosis in the beta cell is clearly Ca^{2+}-dependent but Ca^{2+} should perhaps be regarded as an initiator rather than a determinant of exocytosis. This is suggested by the observation that the amplitude of the exocytotic responses depends to a greater extent on the activity of protein kinases and phosphatases than the actual $[Ca^{2+}]_i$. For example, agents which increase cytoplasmic cyclic AMP levels, such as glucagon and glucagon-like peptide-1, potentiate glucose-stimulated insulin secretion by a protein kinase A (PKA)-dependent mechanism almost 10-fold without much affecting Ca^{2+}-influx or $[Ca^{2+}]$ [34, unpublished data]. In the case of glucagon-like peptide-1 (GLP-1), no stimulation of exocytosis was observed in the absence of glucose suggesting that ATP derived by glucose metabolism is required for the PKA-dependent phosphorylation. Our capacitance measurements indicate that this stimulation principally results from PKA accelerating granule mobilization from the reserve pool into the readily releasable pool thus increasing the size of the latter over fivefold. When exocytosis is subsequently initiated by elevation of $[Ca^{2+}]_i$, a greater number of granules are available for release. We have previously postulated that cAMP acts by "sensitizing" the secretory machinery [34]. However, the fact that the relationship between $[Ca^{2+}]_i$ and exocytosis remains the same in the absence and presence of cAMP is hard to reconcile with such a concept and an increased size of the readily releasable pool may produce effects on exocytosis which at first glance are difficult to distinguish from a sensitizing mechanism.

The molecular mechanism by which PKA accelerates granule mobilization remains obscure. In nerve endings, PKA is known to exert a similar action as in the beta cell by phosphorylation of the protein synapsin-1 which controls the interactions between the vesicles and the cytoskeleton. Synapsin-1 is not expressed in pancreatic beta cells but a synapsin-1-like protein, which may fulfill the function of synapsin-I, has recently been characterized in insulin-secreting cells [37].

Ca^{2+}-induced exocytosis is also enhanced by agents which activate protein kinase C, such as ACh and the phorbol ester 4-β-phorbol-12-β-myristate-13-α-acetate (PMA) [35]. In general, conditions which promote protein phosphorylation lead to enhancement of secretion. Conversely one would expect that agents which produce the activation of protein phosphatases inhibit exocytosis. Indeed, this seems to be the mechanism by which the inhibitory hormones and neurotransmitters somatostatin, galanin and adrenaline suppress glucose-stimulated insulin secretion. The action of these compounds is mediated by activation of an inhibitory (pertussis toxin-sensitive) G-protein and culminates in the activation of the protein phosphatase calcineurin [38].

Hypoglycaemic sulphonylureas stimulate insulin secretion by interaction with exocytotic machinery

Perhaps the most surprising finding that has emanated from the capacitance measurements is that the sulphonylureas, in addition to closing the K-ATP channels, also stimulate insulin secretion by direct interaction with the exocytotic machinery [39] (but see [40] for conflicting data). Such an effect is not easily detected in ordinary assays of insulin secretion as the stimulation of secretion resulting from the closure of the K-ATP effectively obscures any contribution of a late mechanism. Using capacitance measurements it became possible to separate the two effects as the membrane potential was voltage-clamped and thus held constant irrespective of K-ATP channel activity. An effect of the sulphonylureas on exocytosis would also be consistent with the ultrastructural and biochemical evidence indicating that as much as 90% of the sulphonylurea-binding in the beta cell is intracellular and localized to the secretory granules [41, 42].

The observations that the sulphonylureas interfere with exocytosis, possibly by binding to granular sulphonylurea receptors, raises several interesting questions: 1) Is the granular sulphonylurea receptor the same as the 145 kDa SUR which is part of the K-ATP channel? 2) Do the granular sulphonylurea receptors couple to ion channels in the granule membrane. Sulphonylurea-sensitive membrane currents have been demonstrated in pancreatic zymogen granules and have been proposed to control the fusion process [43]. It is attractive to speculate that the sulphonylureas modulate exocytosis in the beta cell by interference with similar conductances in the insulin-containing granules. In this context it is of interest that the 145 kDa sulphonylurea receptor has been reported to promiscuously couple to K^+-channel proteins other than KIR6.2 and it is therefore possible that it may also associate with other channel proteins [44]; 3) What is the *physiological* role of the granular sulphonylurea receptors and do they participate in "normal" Ca^{2+}-induced exocytosis? The answers to these questions are clearly central to the understanding of how the sulphonylureas stimulate exocytosis in the beta cell. However the significance of these results may not be limited to the understanding of the control of insulin secretion. The sulphonylureas have been postulated to enhance glucose uptake in fat cells [45, 46]; an effect which seems as controversial [47] as the effect on exocytosis in the beta cell [40]. Glucose uptake in fat cells involves the insertion of the glucose transporters into the plasma membrane by exocytosis of their intracellular storage vesicles [48, 49]. Since exocytosis in various cell types appears to involve the same molecular processes it is attractive to speculate that the mechanism we have described in the pancreatic beta cell is also operational in other cells (such as

adipocytes) and accounts for some of the reported extrapancreatic actions of the sulphonylureas.

Metabolic regulation of exocytosis

In this review I emphasize the metabolic regulation of the beta cell. I have already described how ATP, via regulation of the K-ATP channels, controls the membrane potential and thereby Ca^{2+}-influx and Ca^{2+}-induced exocytosis. However, there is evidence suggesting that ATP also controls insulin secretion in a more direct way. Experiments on permeabilized insulin-secreting cells have indicated that withdrawal of ATP from the cytoplasm results in 90% inhibition of exocytosis [50]. Moreover, glucose exerts a strong stimulatory action (particularly at late times) in cells which are already maximally depolarized by high extracellular K^+, i.e. under conditions where the sugar is unable to act via depolarization and elevation of $[Ca^{2+}]_i$ [28]. Collectively these observations indicate that access to cytoplasmic ATP (or another glucose metabolite) is somehow rate-limiting to exocytosis. We have applied capacitance measurements in conjunction with photorelease of caged Ca^{2+} from its caged "precursor" Ca^{2+}/NP-EGTA to test this hypothesis. A representative experiment is shown in Figure 4. Here $[Ca^{2+}]_i$ was elevated in two different cells which were dialysed with an ATP-containing or an ATP-free solution. Whereas elevation of $[Ca^{2+}]_i$ in the presence of ATP produced a biphasic stimulation of exocytosis (seen as rapid initial increase in cell capacitance followed by a sustained second slower phase), no change in cell capacitance was observed in the absence of ATP. If anything, the response in the latter cell consisted of a transient *decrease* in cell capacitance which may reflect endocytosis of granules that had been inserted in the plasma membrane during the period required for the wash-in of the Ca^{2+}/NP-EGTA complex and the concomitant wash-out of the endogenous ATP. Ca^{2+}-induced exocytosis in the beta cell is clearly highly dependent on access to cytoplasmic ATP. In this respect the pancreatic beta cell differs from other neuroendocrine cells. In both chromaffin and pituitary cells, large exocytotic responses can be obtained long after complete wash-out of ATP [51]. As I shall try to explain below, this does *not* necessarily imply that the biochemical regulation of exocytosis in the beta cell differs from that in the other cell types in any fundamental way.

As alluded to above, both ultrastructural and functional studies have suggested that the granules in endocrine cells exist in pools of different "releasability" [52]. A small fraction of the total granule population is immediately available for release and accordingly designated as "the readily releasable pool". These granules are probably located just beneath the

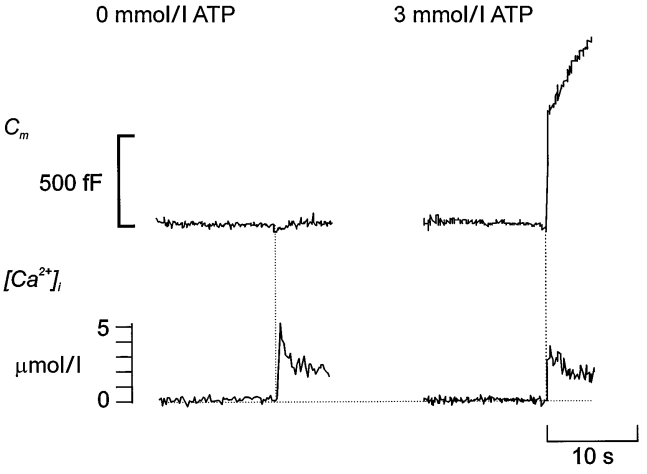

Fig. 4. Exocytosis is ATP-dependent. Exocytosis (monitored as an increase in cell capacitance; C_m) was elicited by photorelease of Ca^{2+} from its caged precursor Ca^{2+}/NP-EGTA which had been preloaded into the cell. Upon irradiation with ultraviolet light, the affinity of NP-EGTA for Ca^{2+} is dramatically reduced resulting in the "release" of Ca^{2+}. Photolysis was effected as indicated by the dotted vertical lines in the presence (right) and absence of ATP (left). Note the failure of Ca^{2+} to elicit exocytosis in the absence of ATP. The capacitance increase in the presence of ATP ($\approx$ 1000 fF) corresponds to the release of 500 secretory granules

membrane and are the first to undergo exocytosis when $[Ca^{2+}]_i$ is elevated. The vast majority of granules are not immediately available for release and presumably located further away from the plasma membrane. These granules need to be "mobilized" into the readily releasable pool before they can be released and are referred to as the "reserve pool". The "mobilization" of the granules may involve either their physical translocation within the cell, a chemical modification of the granules (such that the release probability is increased) or both.

According to current biochemical models of exocytosis, hydrolysis of ATP is required in the chemical modification of the granules which precedes exocytosis ("priming"). When $[Ca^{2+}]_i$ subsequently rises to exocytotic levels, the primed granules (and only those) can be released in a process which does not require any *further* consumption of ATP. However, the pool of primed/energized granules is of limited size and once these granules have been released the pool needs to be replenished in an ATP-dependent way by mobilization of granules from the reserve pool. A simple explanation to the apparent greater ATP-dependence of exocytosis in the beta cell than in the other neuroendocrine cells is therefore that the insulin-secreting cell contains fewer primed granules. In the pituitary cell, the size of this pool has been estimated both functionally and by electron microscopy. Both methods suggest that the primed pool comprises $\approx$ 4000 granules. This is considerably higher than the corresponding number in the pancreatic beta

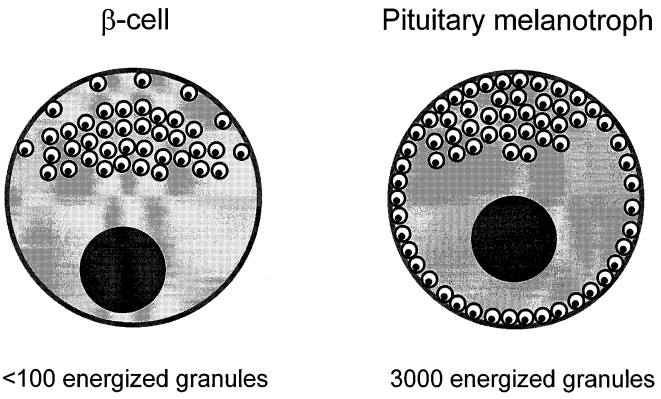

Fig. 5. Comparison of the number of primed granules in the beta cell and in a pituitary cell. The pituitary cell contains a much larger number pool of primed (energized) granules, which are located just beneath the plasma membrane and that can be rapidly released in a seemingly ATP-independent fashion when $[Ca^{2+}]_i$ is elevated, than the beta cell

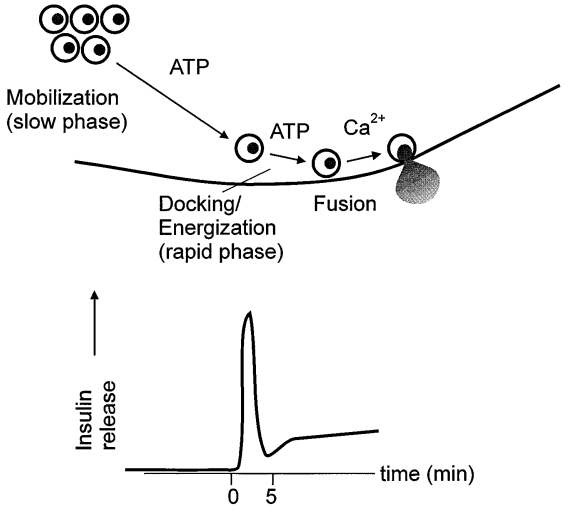

Fig. 6. Biphasic glucose-stimulated insulin secretion can be explained as the release of distinct pools of granules. The first phase of insulin secretion can be accounted for by the release of readily releasable (primed/docked) granules which are located immediately below the plasma membrane. The second slower phase results from the time- and ATP-dependent mobilization of granules situated further away from the plasma membrane

cell. Based on our capacitance measurements, we estimate that only 15–50 granules (Fig. 5), or 0.1–0.3 % of the total granule population (≈ 13 000 [30]), exist in the "primed" state and are capable of being released in an ATP-independent fashion. Since the number of energized granules in the beta cell is only ≈ 1 % of that in the pituitary cell, it is not surprising that insulin secretion exhibits a higher ATP dependence. It seems possible that this represents an important functional adaptation as keeping the number of energized granules low provides the beta cell with the means of rapidly adjusting its secretory capacity to the metabolic state.

Comparison with insulin secretion

A rapid elevation of $[Ca^{2+}]_i$ produces a biphasic stimulation of exocytosis consisting of an initial rapid increase in cell capacitance followed by a second slower phase (Fig. 4). This biphasic response can be interpreted as the release of different pools of granules. Whereas the first rapid phase is likely to correspond to the release of granules situated immediately beneath the membrane (the readily releasable and/or primed pool), the second slower phase reflects the mobilization of the granules from the reserve pool. The biphasic increase in cell capacitance is clearly reminiscent of the biphasic nature of glucose-stimulated insulin secretion [53] and it is attractive to speculate that it can be explained in similar terms (Fig. 6). In fact, the size of the readily releasable pool which can be released in an ATP-independent fashion compares favourably with the number of granules that can be estimated to undergo exocytosis during the first phase of glucose-stimulated insulin secretion (L. Eliasson, P. Rorsman, unpublished data). Using photorelease of ATP from a caged precursor we believe it has been possible to estimate the time required for a granule to pass from the reserve pool into the readily releasable pool and to be released. We consistently observed a delay of ≈ 10 s between the application of ATP and the onset of secretion under experimental conditions that ensure that the primed/readily releasable pool was previously depleted. The long latency argues that the process of mobilization involves physical translocation, and not just chemical modification, of the granules.

Glucose metabolism, ATP and diabetes

In this review I have attempted to illustrate how glucose metabolism, via changes in the cytoplasmic ATP concentration, exerts its control of insulin secretion. The action of ATP is exerted at several levels. First, it controls the size of the readily releasable pool of granules by regulating the rate of granule mobilization/priming. Secondly, ATP generated by glucose metabolism determines the amplitude of Ca^{2+}-evoked secretion via protein kinase A and C-dependent phosphorylation of exocytosis-regulating proteins. Thirdly, glucose metabolism modulates the activity of the voltage-dependent Ca^{2+}-channels and thus Ca^{2+}-entry and Ca^{2+}-induced secretion. Finally, by controlling the activity of the ATP-sensitive K^+-channel, ATP regulates the membrane potential of the beta cell and thereby the electrical and secretory activities. Because ATP exerts so many regulatory functions it is not surprising that conditions which interfere with the ability of the beta cell to generate ATP have marked effects on its secretory capacity. For example, it has been reported that increased

activity of ATP-consuming substrate cycles of glucose metabolism (e.g. glucose → glucose 6-phosphate → glucose; reactions catalysed by glucokinase and glucose 6-phosphatase, respectively) is an early sign of human non-insulin-dependent diabetes [54, 55]. Such a defect will not only interfere with the ability of glucose to depolarize the beta cell but also, as outlined above, reduce the refilling of the readily releasable pool thus possibly accounting for absence of a first phase of glucose-stimulated insulin secretion in these patients [56]. Finally, it deserves pointing out that currently available pharmacological principles for the treatment of non-insulin-dependent diabetes, such as the sulphonylureas, only rectify the inability of glucose to close the K-ATP channel but fail to correct the other ATP-dependent steps and these accordingly require alternative therapeutic approaches.

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Note added in proof. Recently Isomoto et al. (J Biol Chem 271: 24321-24324, 1996) describe an isoform of the cardiac sulphonylurea receptor (SUR2B). This isoform is present in smooth muscle cells and, when it is coexpressed with KIR6.2, forms K-ATP channels with the pharmacological properties of the smooth muscle type of the channel.

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