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Significance of ionic fluxes and changes in membrane potential for stimulus-secretion coupling in pancreatic B-cells

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Key words. Pancreatic B-cells; ions; insulin release; membrane potential.

Introduction

It has been known for more than fifteen years that ions play a crucial role in the stimulus-secretion coupling in pancreatic B-cells. Their importance was first demonstrated by the influence of extracellular cations on insulin release[35, 91], by the stimulatory effect of glucose on $^{42}K^+$ uptake by islet cells[57], and by the appearance of electrical activity in B-cells stimulated with insulin secretagogues[23]. Since then, numerous investigations have addressed various aspects of the complex role of ions in the B-cell function. Many of them have already been reviewed in articles dealing with ionic fluxes in islets and with the importance of ions for insulin release[37, 42, 64, 71, 72, 74, 109, 111], or with the regulation of the membrane potential in B-cells[2, 76, 77, 82, 86, 87].

This report is an attempt to summarize and to integrate our current knowledge of the mechanisms controlling ionic fluxes in islet cells and membrane potential in B-cells. Emphasis will be put on certain aspects of the question that were studied recently, that were not discussed previously or that remain controversial. The significance of these phenomena for insulin release will also be considered. The review, however, will be almost totally restricted to the events occurring during glucose stimulation and will not deal with Ca^{2+} fluxes, that are discussed in another chapter of this series.

1. Membrane potential of B-cells or of non-B-cells?

Despite their limitations and possible artifacts, high resistance microelectrodes are the most sensitive and reliable means to measure the membrane potential of islet cells. Most, if not all laboratories currently use a technique of long-lasting impalements in single cells of partially microdissected islets[89]. The electrical activity induced by glucose in certain islet cells is so typical that it is now used to identify insulin-secreting B-cells. To what extent, however, are we sure to record the membrane potential of B-cells? The confidence of electrophysiologists rests on three types of considerations: statistical, anatomical and functional. First, the probability to impale B-cells is the highest simply because they constitute 70–80% of the mouse islet. Second, PP-cells are very rare in the splenic part of the pancreas that is used for the experiments and the recordings are usually

not made on the surface of the islet, where A- and D-cells form a thin layer. Third, under numerous experimental conditions, good correlations have been found between the temporal and quantitative changes in electrical activity and in insulin release[25,27,80,81,89,96,107]. For D-cells, however, only the statistical and anatomical criteria apply. One cannot rule out that occasionally the membrane potential is measured in a cell that is not recognized as a D-cell.

Impalements of identified non-B-cells are very scarce. It has been reported[60] that A-cells in monolayer cultures derived from newborn rat pancreas generate action potentials upon electrical stimulation. The physiological significance of this observation remains unclear since B-cells from the same preparation were not spontaneously active in the presence of glucose.

2. *Characteristics of the changes in membrane potential induced by glucose in B-cells*

In the absence of glucose or in the presence of a low concentration of the sugar, the membrane potential of B-cells is stable and usually comprised between −60 and −70 mV[47,80,85]. Higher values up to −90 mV are occasionally recorded.

Stimulation with glucose depolarizes the B-cell membrane and triggers a typical electrical activity[27,80,89], the characteristics of which are illustrated in figure 1 (upper panel). Raising the glucose level from 3 to 15 mM is followed by an initial depolarization of 10–15 mV, which brings the membrane potential to a threshold at which the electrical activity starts. This latter exhibits a biphasic pattern[81]. A first long phase of continuous spike activity is followed by a partial repolarization without spikes and finally by the development of slow waves. Each *slow wave* (fig. 2) is characterized[80] by a fast depolarization from a *threshold potential* (V_t) to a *plateau potential* (V_p), onto which spike activity is superimposed. At the end of the burst of spikes, the membrane repolarizes to a level (*repolarization potential* – V_r) slightly more negative than the threshold potential.

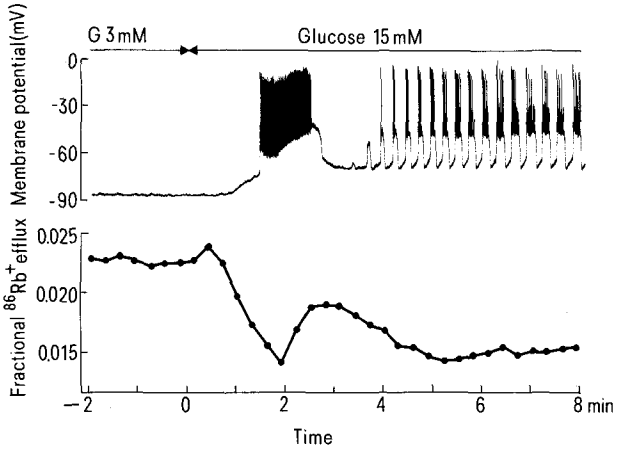

Figure 1. Effects of a rise of glucose concentration from 3 to 15 mM, on the membrane potential of a single mouse B-cell (upper panel) and on $^{86}Rb^+$ efflux from a batch of 60 mouse islets (lower panel). The two representative experiments were performed in different perifusion systems.

During the *interval,* the membrane slowly depolarizes *(prepotential)* until the threshold is reached again.

The depolarization between the resting potential and the threshold potential occurs when the concentration of glucose is raised to 6–7 mM[47,85]. At this latter concentration slow waves are present in about 30% of the cells[9,47,51]. If the concentration of the sugar is increased further, the absolute values of the membrane potential are little affected, but the slow waves with activity lengthen, whereas the intervals shorten[9,80,89]. Finally, when the glucose level reaches approximately 18 mM, the B-cell membrane remains persistently depolarized at the plateau potential and exhibits a continuous spike activity. It is thus evident that each B-cell can adapt the magnitude of its response to the prevailing concentration of glucose. If the intensity of the electrical activity is expressed as the *fraction of plateau phase* (i.e. the fraction of time spent at the plateau level with spike activity), its increase with the concentration of glucose is characterized by a sigmoidal relationship[85,89]. In earlier experiments[25] a sigmoidal relationship was found between the concentration of glucose and the number of active cells. It was considered as evidence for marked differences in the sensitivity of individual B-cells to glucose. However, these results were obtained with short repetitive impalements and it is obvious that cells may then erroneously be considered inactive if the recording is made between two phases of activity. As the concentration of glucose is raised, the slow waves lengthen and the probability to impale an apparently inactive cell decreases. There is no dispute, however, that B-cells may exhibit different sensitivities to glucose in a range of glucose concentrations between 5 and 8 mM[9,80,85].

The slow waves pattern is not specific for glucose. It has also been recorded in B-cells stimulated by glyceraldehyde[27], by leucine[46], by ketoisocaproate[46,58], by the leucine analogue BCH[46], by 3-phenylpyruvate[82] or by low concentrations of tolbutamide[48].

a) *The resting membrane potential*

Although the resting potential of B-cells is mainly determined by a high K^+ permeability of the plasma membrane, it is less negative than the equilibrium potential for K[8,84]. This may be due to the depolarizing effect of a basal influx of Na^+[79,102], and possibly also of an electrogenic Na/Ca exchange[83]. On the other hand, the activity of an electrogenic sodium pump tends to hyperpolarize the membrane by a few mV[47].

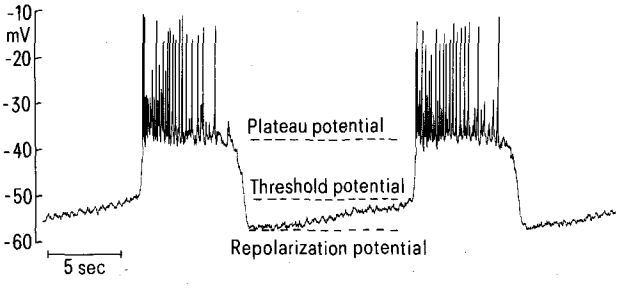

Figure 2. Slow waves of membrane potential induced by 10 mM glucose in a single mouse B-cell.

b) The initial depolarization

There seems to be general agreement that the depolarization between the resting and the threshold potential is brought about by a decrease in K^+ permeability of the plasma membrane. This conclusion is based on many convergent findings discussed elsewhere[87]. Among them, the principal observations are: a) that the rate of K^+ efflux from islet cells is mainly decreased by glucose concentrations between 3 and 6 mM[39], and b) that this depolarization is associated with an increase in the input resistance of the B-cell membrane[8].

A square-wave increase in the concentration of glucose is followed by a biphasic decrease in the efflux rate of $^{86}Rb^+$ (used as tracer for K^+) from perifused islets (fig. 1, lower panel). This biphasic pattern had escaped notice in early experiments and was first clearly demonstrated by Sehlin and Freinkel[112]. It is tempting to suggest that a biphasic change in K^+ permeability of the B-cell membrane contributes to the biphasic pattern of electrical activity and eventually of insulin release, but such a proposal awaits experimental support.

It is well established that the metabolism of glucose by B-cells is necessary for the sugar to decrease the rate of K^+ ($^{86}Rb^+$) efflux[13, 39, 41] and to induce depolarization and electrical activity[27]. However, the coupling factor between metabolic events and K^+ permeability of the plasma membrane remains elusive. A number of possibilities have been envisaged. First, several lines of evidence support the role of a more reduced redox state in the cytoplasm[41, 69, 73]. However, the increase in cytosolic NADPH/NADP ratio that occurs in mouse islets when the concentration of glucose is raised from 3 to 7 mM is only one fourth of that occurring when glucose is raised to 20 mM (C. J. Hedeskov, personal communication). Second, the role of an increased generation of H^+ by glucose metabolism is particularly emphasized by Pace and coworkers[98], but is thought to be of secondary importance by others[15, 43]. Third, the role of cyclic AMP can be ruled out since dibutyryl cAMP and activators of adenylate cyclase have no or very little effect on K^+ efflux and B-cell membrane potential at non stimulatory concentrations of glucose[41, 51, 54]. Fourth, it has been suggested that glucose could lower the concentration of cytoplasmic Ca^{2+} and thereby decrease the activation of Ca-sensitive K-channels[2, 100], present in B-cells[3, 40, 75]. This hypothesis has received some support from the recent demonstration[104] that, in islet tumor cells at least, 4 mM glucose reduces the cytosolic Ca^{2+} activity estimated by the fluorescence of the Ca^{2+} indicator quin-2.

c) The slow waves

There remains some debate concerning the ionic mechanisms underlying the slow waves of membrane potential (fig. 2) triggered by glucose concentrations between 7 and 18 mM. Elucidation of these mechanisms is essential for our understanding of the effects of glucose in B-cells, since the major change caused by an increase in the concentration of the sugar is a lengthening of these slow waves.

Experiments with Na^+-free solutions or with tetrodotoxin have clearly established that an inward Na^+ current plays no important role in the depolarization to the plateau[79, 86, 89, 102, 117].

Two main hypotheses, based on voltage-dependent[20, 85, 89] or non-voltage-dependent[8, 100] changes in membrane permeability have been formulated to explain the depolarization phase of the slow waves. The first hypothesis ascribes it to an increase in Ca^{2+} conductance. It rests on the observations that the slow waves are abolished by Ca^{2+} omission[89] and by Ca channel-blockers[78, 85, 88, 101], whereas their amplitude increases when the concentration of extracellular Ca^{2+} is raised[4, 85, 88, 101]. The second hypothesis ascribes it to a rapid and marked decrease in K^+ conductance. The cornerstone of this proposal is the measurement of the highest membrane resistance at the onset of the depolarization[100].

Several objections have been or can be raised against each of these hypotheses. First, it has been argued[2] that an influx of Ca^{2+} is not absolutely necessary for glucose to depolarize the B-cell membrane to the plateau level. This argument is based on the observation that in the presence of Ca channel-blockers the membrane potential does not always[49] stay at the level of the silent intervals, but often slowly decreases and eventually reaches the plateau level[85, 87, 101]. However, the objection[2] neglects the possible direct inhibition of K channels by these agents[66]. It also fails to explain why, in the absence of Ca^{2+} influx, a rapid depolarization to the plateau never occurs, as one would still expect if this latter were merely due to inactivation of K^+ conductance. Second, it has been shown that current injection through the recording electrode is unable to alter the rhythm of the slow waves[6]. However, a stronger electrical stimulus passed through a suction pipette completely interrupts and resets that rhythm[20], showing that the slow waves are voltage-dependent. Third, Atwater and coworkers[6] recently acknowledged that in many cells, a drop in input resistance, and not an increase, is simultaneous with the onset of depolarization. In conclusion, the debate remains opened, but the hypothesis of an increased Ca^{2+} conductance is no longer dismissed by previous defenders of the alternative hypothesis[6].

There is fair agreement that the repolarization phase at the end of the slow waves is, at least in part, due to an increase in K^+ permeability[3, 52, 100]. This latter may result from activation of Ca-sensitive K channels by Ca^{2+} ions that have entered the cell during the slow wave and the spikes[3, 40, 100], but also from activation of voltage-dependent K channels[20, 100]. An inactivation of Ca channels is also possible.

The role originally attributed[7, 80] to an electrogenic sodium pump has been somewhat exaggerated or misunderstood. We have recently discussed in detail the possible contribution of the sodium pump to the slow waves[47]. The ability of ouabain to induce slow waves in the presence of threshold concentrations of glucose (6–7 mM) simply shows that the small depolarization resulting from the pump blockade can trigger the process, at least transiently. It does not mean that under physiological conditions the depolarization phase of the slow waves is due to an inhibition of the electrogenic sodium pump. It is also clear that activation of the pump is not an absolute prerequisite for the repolarization phase of the slow waves[47, 100]. However, even if the

experimental evidence suggests that the slow waves are not due to the cyclic activity of the pump, it does not allow one to exclude that its functioning is important for normal slow waves to appear under physiological conditions[47, 83, 117].

d) The prepotential

The prepotential is the progressive depolarization (fig. 2) that precedes, during the interval, the fast depolarization of the slow wave. Measurements of membrane resistance suggest that it is due to the gradual decrease of an outward K^+ current[8, 100]. It has been speculated that this fall in K^+ conductance results from the progressive lowering of cytoplasmic free Ca^{2+} and hence from a lesser activation of Ca-sensitive K channels[2, 100]. It could also involve a time and voltage-dependent inactivation of other K channels. Furthermore, without denying the role of a decrease in K^+ conductance, we feel it premature to exclude the participation of other currents (in particular a Ca^{2+} current) during this phase. Thus, the slope of the prepotential is markedly increased when cyclic AMP concentration in islet cells is raised[51, 54] or when the sodium pump is inhibited[47], two conditions not thought to decrease cytoplasmic free Ca^{2+}.

e) The spikes

The spikes appearing on the plateau of the slow waves (fig. 2) are currently thought to be slow Ca^{2+} action potentials[26, 88, 101], occurring through channels where Na^+ ions can probably act as partial agonists and antagonists[26, 101]. Their repolarization phase involves an activation of K channels[101], but inactivation of Ca channels cannot be ruled out.

3. Regulation of the electrical activity in B-cells

With the ionic mechanisms described above in mind, we can now try to answer the key question: how does glucose lengthen the slow waves?

Whatever the possible action of the sugar on the Na-K ATPase, one can exclude that its effects on the slow wave duration is due to an inhibition of the sodium pump for a simple reason: under steady state, ouabain is without effect on the slow wave duration, and the increase in electrical activity, that it causes, is entirely accounted for by the shortening of the intervals[47]. On the other hand, it is possible that glucose somehow delays or prevents inactivation of Ca channels. Cyclic AMP may be involved in this process, but the effect of the sugar on the slow waves cannot be ascribed solely to the increase in the concentration of the nucleotide in B-cells[51]. A third possibility is that high concentrations of glucose prevent activation of Ca-sensitive K channels. Two hypotheses have been put forward. The first hypothesis suggests that glucose lowers the sensitivity of these channels to Ca^{2+}[40]. This could be due to a change in the cytosolic redox potential (see above), or to an increased generation of protons that would antagonize the activation of the channels by Ca^{2+}[30, 98]. This latter mechanism would imply a localized acidification of the cytoplasm despite the overall increase in pH brought about by glucose in islet cells[28, 70]. The hypothesis that glucose protects K channels from activation by Ca^{2+} has been challenged by Lebrun and coworkers[68], who conclude, on the contrary, that glucose increases Ca-dependent K-permeability. Even if the true effect of glucose and its underlying mechanism remain controverted, the argument suffers from an important weakness: all these experiments[68] have been carried out under conditions (1 mM $CaCl_2$) where no slow waves are present[88, 101]. The second hypothesis suggests that increasing concentrations of glucose increase the capacity of the B-cell to buffer Ca^{2+} entering during the slow waves and the spikes[2, 100]. This would delay the moment at which the cation reaches a cytoplasmic concentration sufficiently high to activate K channels. Such a mechanism apparently precludes that a rise in cytoplasmic free Ca^{2+} serves as primary trigger for insulin release[119] except if one postulates that the exocytotic process has a higher sensitivity to Ca^{2+} than the K channels. However, this would then make it difficult to explain the initial depolarization from the resting potential (where there occurs little insulin release) to the threshold potential (where insulin release is stimulated). Other objections against the hypothesis have been raised elsewhere[71]. In conclusion, elucidation of the mechanisms whereby glucose modulates the duration of the slow waves still awaits further investigation.

Another important regulatory mechanism has been characterized recently. Raising the concentration of cyclic AMP in B-cells potentiates the electrical activity triggered by glucose, but the changes brought about by the nucleotide differ from those resulting from a rise in the concentration of the sugar[50, 51, 54]. Cyclic AMP markedly accelerates the depolarization during the prepotential: this results in a shortening of the intervals and an increase in the frequency of the slow waves, with little change in their duration. Acetylcholine also augments glucose-induced electrical activity by decreasing the amplitude of the repolarization phase of the slow waves and by increasing the rhythm of these latter[18, 33]. The mechanism of this effect is still unclear and does not seem to be mediated by cyclic AMP[33]. The existence of distinct mechanisms to control the electrical properties of the B-cell membrane may be particularly well suited to ensure fine modulation of the effects of a primary stimulus (glucose) by a potentiator (gastrointestinal hormones, neurotransmitters), acting via cyclic AMP or not.

4. Significance of the electrical activity in B-cells for insulin release

Until recently, attention was mainly focused on the correlations existing between insulin release by perifused islets or the perfused pancreas and electrical activity in single B-cells[25, 27, 80, 81, 85, 89, 96, 107]. This naturally led to the conclusion that the electrical events occurring at the level of the B-cell membrane play an essential triggering role in the stimulus-secretion coupling, even if emphasis was put more on the spikes[25] or on the slow waves[80, 89]. However, the devil's advocate can argue that the corre-

lations are good, because certain of these electrical events are the consequence of exocytosis. To answer that objection, dissociations between both events must be sought.

First, insulin release can be stimulated without electrical activity being present in B-cells, when triggering Ca^{2+} may be mobilized from intracellular stores[49,51]. This is illustrated by figure 3, which shows that, in the presence of 3 mM glucose, 1 mM isobutylmethylxanthine increases insulin release (A), depolarizes the B-cell membrane (B), but does not induce spike activity. Figure 3 also shows that, in the presence of 7 mM glucose, 10 mM theophylline increases insulin release (C), but hyperpolarizes the B-cell membrane (D). The mechanisms underlying these opposite changes in membrane potential are discussed elsewhere[51].

Second, insulin release can be potentiated without electrical activity in B-cells being increased to the same extent. This is observed when cyclic AMP concentration is raised in islet cells[51] and may be due to an amplification of the effectiveness of Ca^{2+} at intracellular sites and/or its mobilization from cellular organelles.

Third, insulin release can be inhibited without electrical activity being suppressed in B-cells. This type of dissociation occurs when extracellular pH is lowered or when anion fluxes are blocked[98]. These experimental conditions may interfere with the latest step of the stimulus-secretion coupling, the exocytotic process itself, by preventing the chemiosmotic lysis of the granules. A similar dissociation is observed when the tempertature of the perifusion medium is decreased to 27 °C[5]. Concentrations of adrenaline known to abolish insulin release also fail to suppress glucose-induced electrical activity in B-cells[19,106].

It can thus be safely concluded that no change in the membrane potential of B-cells, measured with the conventional microelectrode technique, is the mere consequence of insulin release. Conversely, the dissociations described above strengthen the previous suggestions that the electrical events play a causal role in the stimulation of release[25,80,89]. The proposal must be restricted, however, to stimuli, the insulinotropic effect of which depends on extracellular Ca^{2+}, and hence on Ca^{2+} influx[51]. In addition, the depolarization of the B-cell membrane per se does not seem to be a prerequisite for the exocytosis, even if a permissive role cannot be excluded. It is possible that insulin release is pulsatile as long as the triggering signal remains intermittent (slow waves with bursts of spikes). If such is the case, the two phenomena are likely to be out of phase since the concentration of free Ca^{2+} in the cytoplasm is probably highest at the end of the slow wave, when the membrane repolarizes to the silent level. Convincing evidence for periodic release by individual B-cells is still lacking. Furthermore, simultaneous measurement of membrane potential and insulin release has shown that the functional event largely outlasts the electrical signal[107]. Certain B-cells stimulated with a constant concentration of glucose also display regular oscillations, with a period of 4–5 min, in the intensity of their electrical activity[17,53]. Their link with the oscillations in insulin release measured in the perfused pancreas[115] is still unsettled.

5. *Cationic fluxes in islet cells*

a) *Potassium fluxes*

Since the pilot work of Sehlin and Täljedal[113], K^+ fluxes in islet cells have been the subject of numerous studies. Several of these have already been discussed in the preceding sections, and reviews on this specific subject have appeared[42,71]. In this paragraph, we only wish to draw the reader's attention to two particular problems. It is often difficult, or even impossible to interpret K^+ ($^{86}Rb^+$) efflux studies adequately, if the changes in membrane potential occurring under the same conditions are not known. A decrease in $^{86}Rb^+$ efflux from islet cells may be measured, when the experimental condition causes depolarization (e.g. glucose) or hyperpolarization of the B-cell membrane (e.g. omission of K from a glucose-free medium)[47]. On the other hand, an increase in $^{86}Rb^+$ efflux may be measured, when the experimental condition causes depolarization (e.g. ouabain or arginine)[46,47] or hyperpolarization of the B-cell

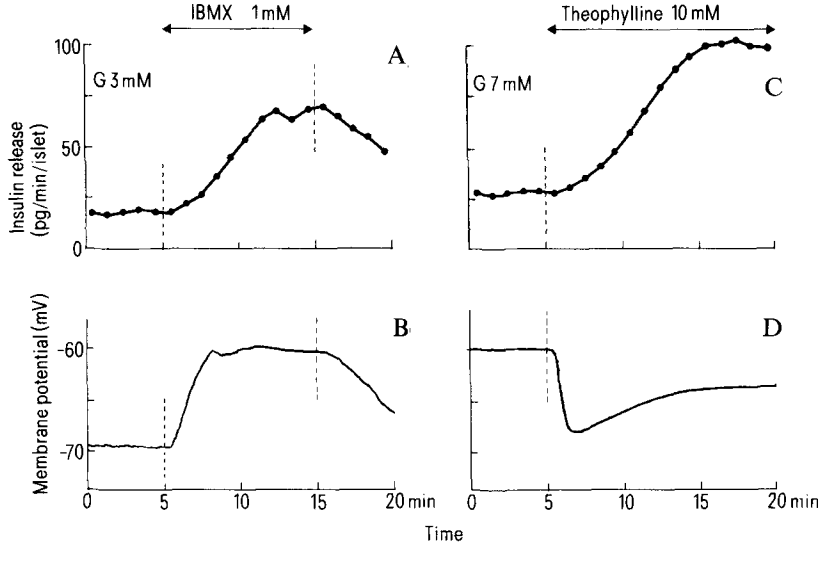

Figure 3. Effects of isobutylmethylxanthine (IBMX) and theophylline on insulin release by batches of 40 mouse islets (A and C) and on the membrane potential of single mouse B-cells (B and D). The concentration of glucose (G) was 3 mM (A and B) or 7 mM (C and D). Representative experiments are shown.

membrane (e.g. diazoxide)[48]. All depends on whether a change in K^+ fluxes permeability causes the change in membrane potential or is the consequence of this latter. Furthermore, the true changes in K^+ permeability may be underestimated if the changes in membrane potential are not taken into consideration[22]. It should also be realized that changes in $^{86}Rb^+$ efflux may occur without modifications of the membrane potential. Electrically neutral K^+ fluxes (coupled to the cotransport of an anion or the countertransport of a cation) may exist in islet cells[98,116]. As in other tissues[29], they could play a role in the regulation of cell volume.

Studies using energy dispersive X-ray analysis have shown that K content of B-cells transiently increases upon stimulation with glucose[62]. This increase may result from the reduced efflux of the cation (and the persistence of the sodium pump activity) but should, by no means, be considered as the cause of the depolarization. Under steady state stimulation with glucose, no change or a slight decrease in K content of B-cells was estimated[62]. By contrast, atomic absorption spectroscopy[12] or $^{42}K^+$ uptake measurements[12,41] suggest that the sugar increases K content in islet cells. The reason for the discrepancy is unknown and since neither technique discriminates between bound and free ions, it remains unclear whether glucose causes any change in the activity of K^+ in the cytoplasm.

Future investigations in this area will have to precise the modalities and the sites of K^+ movements in islet cells. It will be important to define to what extent measurements of $^{42}K^+$ uptake reflect the activity of the sodium pump or passive processes, and to determine whether insulin secretagogues influence K compartmentalization in B-cells.

b) Sodium fluxes

Measurements of $^{22}Na^+$ fluxes in islet cells have yielded controversial results. One study[63], carried out with rat islets, suggests that glucose accelerates Na^+ influx, but, because of an even greater acceleration of efflux, decreases Na net uptake. Another study[34], carried out with mouse islets, suggests that glucose has no effect on Na^+ influx, but increases Na net uptake. Furthermore, omission of extracellular Ca^{2+} accelerates $^{22}Na^+$ efflux from mouse islets[113], but not from rat islets[63]. It may seem doubtful that such discrepancies are due to species differences, but this cannot be entirely ruled out. Thus, in the same laboratory, veratridine was found to depolarize the membrane of rat B-cells[93], but not of mouse B-cells[117].

Both radioisotopic studies[34,63] estimated an intracellular Na concentration of about 75–100 mM in non-stimulated islet cells. This is much higher than the Na^+ activity of 30 mM, indirectly estimated from measurements of the membrane potential[8,84]. It is therefore possible that Na is compartmentalized or partially bound in B-cells.

The inhibitory effect of ouabain[63,113], shows that Na^+ efflux from islet cells is mediated by a sodium pump, but other unidentified systems have also been considered[63]. A detailed discussion of the properties of the sodium pump in B-cells may be found elsewhere[47]. Na^+ influx in islet cells may proceed through different specific pathways. The stimulation of $^{22}Na^+$ influx by veratridine and scorpion toxin and its inhibition by tetrodotoxin suggest the existence, in rat islet cells, of Na channels analogous to those present in nerve cells[103]. That they are also present in mouse B-cells (even if they only play a minor role) is suggested by the changes in electrical activity produced by high concentrations of tetrodotoxin[86,117]. The Na/Ca exchange and a Na/H exchange are two other possibilities for Na^+ entry. The evidence for the presence of the non-electrogenic Na/H exchange in B-cells is still indirect[30,98]; it is based on alterations of glucose-induced electrical activity in B-cells by amiloride, a drug known to inhibit such an exchange process in other tissues.

Two lines of evidence suggest that the role of Na^+ ions in the stimulus-secretion coupling is only indirect: tetrodotoxin has little[103] or no effect[92,93] on glucose-induced insulin release, and omission of extracellular Na^+ only causes a delayed inhibition[36,65]. It is still unclear to what extent this latter results from alterations in Ca handling[119], changes in pH[98], inhibition of the sodium pump[86] or alterations in glucose metabolism[38] in B-cells.

c) Magnesium fluxes

Mg^{2+} may play a modulatory role in the release of insulin[21], through complex interactions with Ca^{2+} at the level of the plasma membrane[4,10,61] and also at intracellular sites controlling exocytosis[97].

Atomic absorption spectrometry has shown that total islet Mg is fairly stable and not affected by glucose[10]. A more detailed study of Mg^{2+} fluxes could be carried out recently with radioactive $^{28}Mg^{2+}$. In non-stimulated islet cells, $^{28}Mg^{2+}$ efflux is activated by extracellular Mg^{2+} and Na^+, through mechanisms that may correspond to Mg/Mg and Na/Mg exchanges[56]. Glucose, but not 3-O-methylglucose, stimulates $^{28}Mg^{2+}$ uptake; it also accelerates $^{28}Mg^{2+}$ efflux in the presence of extracellular Mg^{2+}, but decreases the efflux rate in the absence of extracellular Mg^{2+}[55]. Further experiments are necessary to determine whether these Mg^{2+} fluxes contribute to the changes in B-cell membrane potential induced by glucose, to establish whether they result in variations in free cellular Mg^{2+} and to elucidate their significance for the stimulus-secretion coupling.

6. Anionic fluxes in islet cells

a) Chloride fluxes

Measurements of $^{36}Cl^-$ fluxes suggest that the anion is actively accumulated in islet cells and that an increase in the permeability of the B-cell membrane to Cl^- contributes to the depolarizing effect of glucose[108]. This interpretation is not supported by electrophysiological studies, which all agree that Cl^- is passively distributed across the membrane of B-cells[26,30].

The mechanism responsible for the increase in Cl^- efflux during stimulation by glucose[108] and other insulin secretagogues[110] remains obscure. It cannot be exclusively due to the activation of a HCO_3/Cl exchange, since it also occurs in HCO_3^--free solutions[108]. Part of the ap-

parent uptake of $^{36}Cl^-$ may be accounted for by a Cl/Cl exchange[109]. Its inhibition by furosemide[110] is suggestive of the existence in islet cells of a cotransport system for Na/K/Cl, although Cl^- uptake is not impaired when either Na^+ or K^+ alone is omitted from the incubation medium.

The exact role of Cl^- ions in the process of insulin release is difficult to define precisely, because the anion may exert direct or indirect effects at different stages of the stimulus-secretion coupling. Experiments with islets, the membrane of which has been rendered permeable by high voltage discharges, suggest that Cl^- might inhibit the secretory process at intracellular sites[97,120]. On the other hand, substitution of isethionate or another impermeant anion for extracellular Cl^-, inhibits glucose-induced insulin release[95,108,114,116], but does not impair the effect of all secretagogues[116]. It is therefore unlikely that Cl^- ions are only involved in the chemiosmotic lysis[99] of insulin granules undergoing exocytosis. Ca^{2+} fluxes in islet cells are indeed altered in the absence of Cl^-[116]. It is possible that certain changes in the B-cell function occurring in low Cl^- solutions result from modifications of intracellular pH[30,98,116].

b) Bicarbonate fluxes

Several years ago, it was reported[44,45] that a sufficient concentration of extracellular HCO_3^- is necessary for glucose to stimulate Ca^{2+} uptake and insulin release by isolated islets. These observations have been confirmed[67], but the underlying mechanisms remain obscure.

Recent studies[28] using [^{14}C]-HCO_3^- suggest that glucose accelerates HCO_3^- influx in islet cells. Unfortunately, the experimental conditions do not permit to exclude that part of the apparent uptake of the tracer is, in fact, due to labelled CO_2. Evidence that the uptake can be inhibited by blockers of the anion transport system would considerably strengthen the hypothesis. Such an increased influx of HCO_3^- may reflect activation of a HCO_3/Cl exchange process by H^+ produced during glucose metabolism. It may contribute to the rise in cellular pH that occurs during glucose stimulation[28,70], but is not the sole mechanism of this rise, since this latter persists in HCO_3^--free solutions[70]. The increase in glucose-induced electrical activity (lengthening of slow waves) recorded upon withdrawal of extracellular HCO_3^- has been ascribed to a decrease in B-cell pH that would be secondary to the arrest of the HCO_3/Cl exchange[30,98]. However, because HCO_3^--free solutions also lack CO_2, the pH of islet cells does not decrease, but increases under these conditions[67,70]. Finally, it is possible that the electrophysiological recordings have been too short. Thus, the changes in insulin release resulting from manipulations of the HCO_3^- concentration in the medium are of slow onset and of slow reversibility[44,45].

c) Phosphate fluxes

Numerous studies, discussed elsewhere[31,72,111], have attempted to elucidate the mechanisms and significance of the 'Phosphate flush', an early, transient release of inorganic phosphate occurring in islet cells upon stimulation by insulin secretagogues[32]. It can be dissociated from insulin release and is currently considered either as an integral early step in the recognition of nutrient secretagogues by B-cells[31] or as the consequence of metabolic events evoked by these secretagogues[16]. The phosphate flush induced by glucose is paralleled by a marked, sustained and poorly reversible decrease in the content of inorganic phosphate in islet cells[14,118]. Surprisingly, however, energy dispersive X-ray analysis suggests that during glucose stimulation total phosphorus decreases only transiently and then increases[62].

No change in the membrane potential of B-cells has ever been associated with the phosphate flush. In this respect, it is important to draw attention to an observation, the interest of which may have escaped notice. The phosphate flush is not prevented by inhibitors of anion transport, which not only abolish the acceleration of Cl^- efflux triggered by glucose, but also decrease the basal rate of phosphate efflux[1].

7. Species specificity of ionic fluxes and changes in B-cell membrane potential

The effects of insulin secretagogues on ionic fluxes have been studied in islets of normal rats or mice of different strains. Except for Na^+ fluxes (see above), no fundamental difference in the handling of monovalent ions has been observed between islets from the two species. In our experience, however, quantitative differences may exist under certain experimental conditions.

Since the initial report[23] that islet cells are electrically active, the partially microdissected mouse islet has remained the preparation of choice to study the membrane potential of B-cells. Practically all our knowledge of the electrical properties of the insulin-secreting cells has been obtained in the albino mouse. Rat islets have also been used immediately after collagenase isolation[96], or after several days of culture, when the cells already form monolayers[94]. With a technique of short impalements, it was observed that most of these rat islet cells had a relatively low membrane protential, but their membrane depolarized and exhibited electrical activity upon stimulation by glucose. However, the spikes occurred in bursts on top of slow waves of membrane potential only rarely[94]. A more recent study[59] using impalements of longer duration in rat islets cultured for five days suggests that the slow wave pattern of electrical activity may also be the characteristic response of the rat B-cell to glucose. Quantitative differences are evident from the electrical activity recorded in mouse B-cells, but it is unclear whether they are due to the culture conditions or to a true species difference. The membrane potential of guinea-pig islet cells has also been measured[24], but neither its sensitivity to glucose nor the presence of electrical activity were reported. Recent preliminary studies[105] indicate that in hyperinsulinemic ob/ob mice the membrane potential of B-cells is markedly altered. In spontaneously diabetic db/db mice, abnormal regulation of both K^+ permeability[11] and membrane potential of B-cells[90] have been reported. From this brief survey there appears to be a need for a detailed study of the electrical properties of B-cells in other species than the albino mouse. In the meantime it

should no longer be tacitly assumed that the electrical activity is the same in all species. In particular, comparison of ionic fluxes measured in rat islets with changes in membrane potential of mouse B-cells should only be made with caution.

Conclusions

This brief review has tried to shed some light on the mechanisms and significance of the changes in membrane potential and in ionic fluxes occurring in B-cells upon glucose stimulation. There is now strong evidence that, under physiological conditions at least, these electrical events – and the underlying modifications of ionic permeabilities and fluxes – play a causal role in the stimulation of insulin release. It also seems clear that certain accompanying ionic fluxes have no direct stimulatory role, but may be important in maintaining cellular homeostasis. Recent experimental evidence has also shown that the electrical activity in B-cells is not an all-or-none stereotypic response. Not only can its intensity be adjusted to the magnitude of the stimulus, but its characteristics can also be modulated by potentiators. Our knowledge of the stimulus-secretion coupling has markedly progressed over the past few years, but elucidation of several important steps remains a challenging goal. There is no doubt that parallel measurements of insulin release, of ionic fluxes and of membrane potential in B-cells will still contribute to that understanding.

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Cytosolic free Ca^{2+} in insulin secreting cells and its regulation by isolated organelles

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Summary. The role of Ca^{2+} in secretagogue-induced insulin release is documented not only by the measurements of ^{45}Ca fluxes in pancreatic islets, but also, by direct monitoring of cytosolic free Ca^{2+}, $[Ca^{2+}]_i$. As demonstrated, using the fluorescent indicator quin 2, glyceraldehyde, carbamylcholine and alanine raise $[Ca^{2+}]_i$ in the insulin secreting cell line RINm5F, whereas glucose has a similar effect in pancreatic islet cells. The regulation of cellular Ca^{2+} homeostasis by organelles from a rat insulinoma, was investigated with a Ca^{2+} selective electrode. The results suggest that both the endoplasmic reticulum and the mitochondria participate in this regulation, albeit at different Ca^{2+} concentrations. By contrast, the secretory granules do not appear to be involved in the short-term regulation of $[Ca^{2+}]_i$. Evidence is presented that inositol 1,4,5-trisphosphate, which is shown to mobilize Ca^{2+} from the endoplasmic reticulum, is acting as an intracellular mediator in the stimulation of insulin release.
Key words. Pancreatic B-cell; insulin secretion; cytosolic free Ca^{2+}.

Introduction

Ca^{2+} plays an important role in cell activation in general[11,25] and in stimulus-secretion coupling in the B-cell in particular[30,60]. The large body of evidence emphasizing the importance of Ca^{2+} in the regulation of insulin release from the pancreatic B-cell was reviewed in depth previously[100]. Although overwhelming, the evidence remained circumstantial that cytosolic free Ca^{2+}