β-Cell cytoplasmic Ca^{2+} balance as a determinant for glucose-stimulated insulin release

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Summary. The introduction of new techniques and the access to clonal lines of insulin-secreting cells have enabled re-evaluation of glucose effects on Ca^{2+} movements in pancreatic β cells. It became evident that glucose, in addition to stimulating the entry of Ca^{2+}, also promotes active sequestration of the ion in intracellular stores and its extrusion from the β cells. The balance between these processes will determine the activity of Ca^{2+} in the cytoplasm and consequently the rate of insulin release. With the demonstration that glucose can not only increase but also lower cytoplasmic Ca^{2+}, it follows that exposure to the sugar under certain conditions results in a paradoxical inhibition of insulin release. In diabetic patients this may be manifest as prompt reduction of circulating concentrations of insulin and C-peptide after an intravenous injection of glucose. The concept of the dual action of glucose might aid in explaining a number of poorly understood phenomena, such as the induction of rhythmic oscillations of the membrane potential of β cells and the fact that their secretory response is improved by prolonged exposure to glucose and after priming with the sugar.

Key words: Glucose, cytoplasmic Ca^{2+} balance, β cells, insulin release, depolarization, intracellular Ca^{2+} sequestration, diabetes.

Oskar Minkowski provided the first definite proof of a relationship between the pancreatic gland and diabetes. When receiving the Minkowski award 1969, I felt that no topic would be more appropriate for the prize lecture than the pancreatic islets. The last part of this lecture was devoted to the presentation of in vitro techniques for exploring the mechanisms for glucose regulation of insulin secretion, employing β cell-rich pancreatic islets isolated from ob/ob-mice [1]. On this occasion, interest was focused on how the β cells recognized glucose as a secretory stimulus. However, after the chance observation that metabolism of the sugar might be linked to ion permeability of the plasma membrane by changes in thiol/disulphide balance in a regulatory membrane protein [2], most of the subsequent research has been concerned with how glucose affects the β cell's handling of Ca^{2+}.

At the meeting of Minkowski prize winners in Capri in 1976 evidence was provided that, in addition to the stimulatory cytoplasmic pool of Ca^{2+}, there also exists inhibitory calcium in the plasma membrane [3]. Since then, the introduction of new techniques and the access to clonal lines of insulin-secreting cells have enabled considerable expansion of our knowledge of Ca^{2+} movement in the pancreatic β cell, culminating in the discovery that glucose has dual actions on cytoplasmic Ca^{2+} activity. In addition to presenting evidence for glucose initiation of Ca^{2+} movement with opposing effects on insulin release, it will be shown how the concept of cytoplasmic Ca^{2+} balance could aid in explaining the rhythmic oscillations of membrane potential and other phenomena resulting from exposure of pancreatic β cells to glucose.

Influx of Ca^{2+}

After the original observations that glucose promotes the retention of ^{45}Ca in islets from rats [4] and ob/ob-mice [5], the La^{3+} wash technique made it possible to demonstrate that the radioactivity incorporated in response to glucose is located intracellularly [6]. The observation of a substantial islet uptake of ^{45}Ca, non-displaceable by La^{3+}, does not necessarily imply that glucose also increases intracellular calcium content. As a matter of fact, we have reason to believe that a major effect of glucose is to stimulate intracellular Ca^{2+}–Ca^{2+} exchange. Both analysis of islets from rats using a fluorometric technique [7] and of islets from ob/ob-mice employing electrothermal atomic absorption spectroscopy [8] failed to demonstrate a glucose-induced increase of the total amounts of calcium. Neither was it possible to confirm reports [9] about pronounced acute glucose effects on the ^{45}Ca content of islets loaded to isotopic equilibrium [10]. However, even if most of the glucose-stimulated long-term uptake of ^{45}Ca can be explained
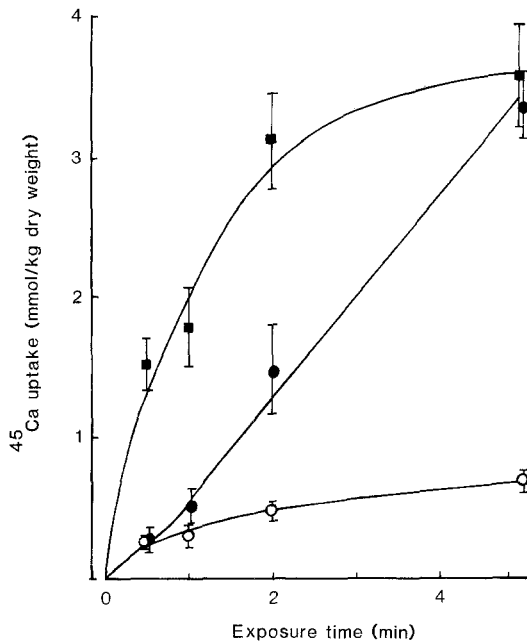
Fig. 1. Effects of glucose and excessive K⁺ on the intracellular uptake of ⁴⁵Ca into pancreatic β cells. Aggregates of cells were prepared from β-cell-rich pancreatic islets of *ob/ob*-mice and incubated for different periods of time with ⁴⁵Ca (1.28 mmol/l) in a Hepes-buffered medium without additives (○) or supplemented with glucose (20 mmol/1; ●) or KCl (25 mmol/l; ■). After exposure to ⁴⁵Ca, the cell aggregates were centrifuged through oil into a cold medium containing La³⁺ (1 mmol/1). Mean values ± SEM for four to five experiments

in terms of increased turnover of intracellular calcium pools, there is no longer any doubt that exposure to the sugar can also result in an increased net uptake of Ca²⁺ into the β cells. In *ob/ob*-mouse islets, maintained at isotopic equilibrium, a modest (14%) increase in the amounts of La³⁺-non-displaceable ⁴⁵Ca was observed 30 min after raising the glucose concentration from 5.5 to 20 mmol/1 [10]. Moreover, it has been possible to demonstrate a glucose-stimulated net uptake of Ca²⁺ both in *ob/ob*-mouse islets [11] and in insulin-secreting clonal RINm5F cells [12] by monitoring alterations of the Ca²⁺ concentration in a suspension medium containing µmol/1 concentrations of the cation. The glucose-promoted intracellular net uptake of Ca²⁺ is not necessarily due to increased entry of the ion, but may also reflect a reduced outward transport. One way to discriminate between these alternatives is to measure the unidirectional influx of ⁴⁵Ca in short-term experiments. Several authors have performed this analysis by incubating the islets with ⁴⁵Ca in the presence of extracellular space markers [5, 13–15]. In an attempt to reduce the problems related to the diffusion of ⁴⁵Ca into the extracellular islet space and its surface binding, the technique was recently modified to allow the measurement of uptake of the isotope by suspended β cells subsequently washed with La³⁺ (N. Wesslén and B. Hellman: unpublished data). Figure 1 presents results obtained with this approach. It is evident that glucose is a potent

stimulator of the entry of Ca²⁺ into the β cells, although the effect is delayed in comparison with that obtained after depolarization with K⁺.

Efflux of Ca²⁺

The extrusion of Ca²⁺ from pancreatic β cells is mediated both by Na⁺-Ca²⁺ exchange diffusion [16–18] and a calmodulin-activated transport system based on a high-affinity ATPase [19]. The exploration of the Ca²⁺ efflux mechanism has been considerably aided by measuring the washout of radioactivity from islets loaded with ⁴⁵Ca. Using this approach, glucose has been found to have both stimulatory and inhibitory effects on ⁴⁵Ca efflux (Fig. 2). The dose-response relationship for the stimulatory component mimics that of insulin release in being sigmoidal with a half-maximal response at 7–9 mmol/l [20, 21]. The enhanced ⁴⁵Ca efflux is also similar to insulin release in being associated with depolarization of the β cells and requiring the presence of extracellular Ca²⁺. However, loss of calcium with the secretory granules during exocytosis can only contribute to a minor extent to the observed increase of ⁴⁵Ca efflux [22]. Neither does the stimulated ⁴⁵Ca efflux reflect a specific action of glucose in mobilizing intracellular calcium stores. Other depolarizing agents promoting the entry of Ca²⁺ initiate an even greater stimulation of ⁴⁵Ca efflux. We are therefore left with the alternative that the stimulatory component of ⁴⁵Ca efflux is essentially due to displacement of ⁴⁵Ca from intracellular binding sites following increased entry of non-radioactive Ca²⁺ [20, 21, 23]. This explanation is in accordance with data, already alluded to, indicating that a major effect of glucose is to increase the turnover of intracellular calcium. Evidently glucose promotion of the intracellular ⁴⁰Ca-⁴⁵Ca exchange is sufficient to overcome the competitive inhibition of the outward transport of ⁴⁵Ca exerted by the entering ⁴⁰Ca. Isolated pancreatic islets are not unique in responding with a stimulated ⁴⁵Ca efflux, when exposed to agents which increase the entry of non-radioactive Ca²⁺. Similar effects have been observed during perifusion studies with the posterior pituitary and adrenal medulla [24].

In accordance with the idea that stimulation of ⁴⁵Ca efflux results from increased entry of Ca²⁺, a lowering of the extracellular concentration of Ca²⁺ unmasked the inhibitory component of glucose action (Fig. 2). When the extracellular concentration of Ca²⁺ was < 0.1 µmol/l, only inhibition was observed. The inhibitory component not only precedes the stimulatory phase but also disappears more rapidly on omission of glucose. Thus, at moderately reduced concentrations of Ca²⁺, the result is a substantial "off-induced" increase of ⁴⁵Ca efflux (Fig. 2 B). Glucose has been found to inhibit the efflux of ⁴⁵Ca during perifusion with a Ca²⁺-deficient medium also when the islets are loaded to isotopic equilibrium. The observed inhibition cannot
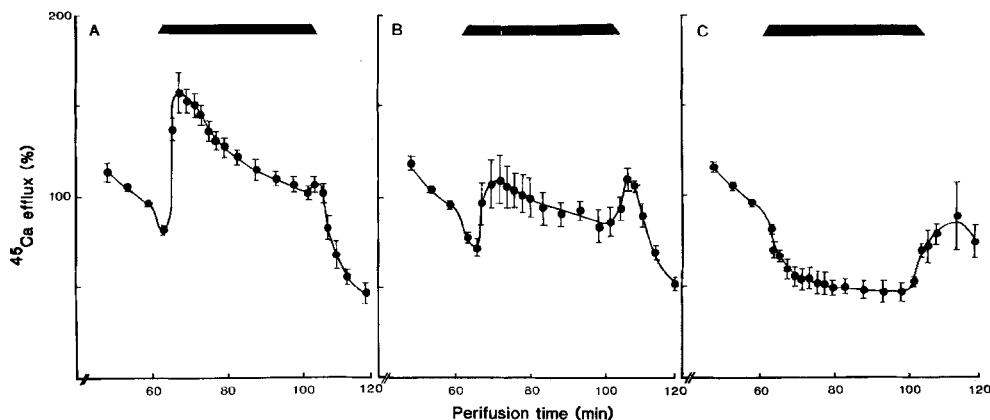
Fig. 2. Effects of glucose on ⁴⁵Ca efflux from *ob/ob*-mouse islets perifused with different concentrations of Ca²⁺. The islets were loaded with ⁴⁵Ca in the presence of glucose (20 mmol/l) and perifused with media containing (A) 1.2 mmol/l, (B) 0.5 mmol/l or (C) < 0.1 μmol/l Ca²⁺. The lowest concentration of Ca²⁺ was obtained by including EGTA (0.5 mmol/l) in the medium. Glucose (20 mmol/l) was introduced into the perifusion medium during the period indicated by the horizontal bar. The data are given as a percentage of the average ⁴⁵Ca efflux during the 10-min period preceding the introduction of glucose. Mean values ± SEM for four experiments

consequently be explained by preferential mobilization of calcium stores with low specific radioactivity.

Although it is generally agreed that inhibition of ⁴⁵Ca efflux reflects a true decrease in the rate of Ca²⁺ efflux, divergent opinions have been expressed about the mechanisms involved. Apart from the original concept that glucose interferes with the active extrusion of Ca²⁺ from the β cells [15, 20], the impaired efflux may result from trapping of the cation in cellular organelles [22]. It is essential for the understanding of glucose regulation of insulin release to discriminate between these alternatives, because of their opposite effects on the cytoplasmic concentration of Ca²⁺. Although islets have been reported to contain Ca²⁺-activated ATPase activity subject to glucose inhibition [25], the relevance of this observation for the outward transport of Ca²⁺ remains to be proven. We have no reason for believing that glucose inhibits Ca²⁺ efflux by increasing Na⁺ activity in the pancreatic β cells. Instead, it has been postulated that glucose inhibition of the Ca²⁺ efflux reflects an increased production of H⁺, competing with Ca²⁺ for exit by Na⁺-Ca²⁺ counter transport [20, 26, 27]. Observations in our laboratory led us to doubt that glucose interferes directly with the system for active extrusion of Ca²⁺. It is, for example, difficult to reconcile such an effect with the finding that inhibition of the ⁴⁵Ca efflux obtained in the presence of a low concentration of glucose (4 mmol/l) remains relatively unaffected by alterations of the magnitude and even direction of the Ca²⁺ gradient across the plasma membrane [28]. Moreover, glucose was found to be equally effective in inhibiting ⁴⁵Ca efflux in the absence of Na⁺, when the loss of intracellular K⁺ was prevented by replacing Na⁺ by K⁺ [29].

In addition to disproving the arguments for a direct inhibitory action of glucose on the outward transport of Ca²⁺, we have reasons for believing that this process instead will be stimulated following glucose-mediated entry of Ca²⁺. So far, it has not been possible to measure the unidirectional efflux component during stimulated influx of the ion. However, when taking into account

that the sugar considerably enhances the entry of Ca²⁺ (Fig. 1) without much increasing the intracellular content of the element, it can be anticipated that the efflux of Ca²⁺ is raised. Indeed, it is likely that the rate of the outward transport for Ca²⁺ represents a complex function of cytoplasmic Ca²⁺ activity, as has been postulated for other cells [30].
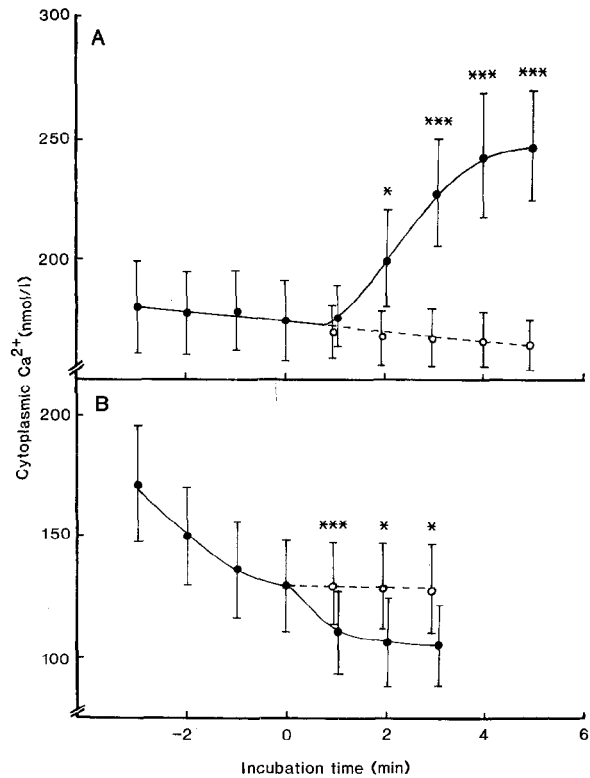
Sequestration of Ca²⁺ into intracellular stores

If glucose-induced suppression of Ca²⁺ efflux reflects reduction of the cytoplasmic concentration after organelle uptake, it should be possible to demonstrate a Ca²⁺ incorporation into the organelles different from that following depolarization. Experiments with labelling of the organelles in situ in the β-cell-rich pancreatic islets from *ob/ob*-mice indicated that mitochondria account for a considerable part of the ⁴⁵Ca taken up in response to glucose [8, 31, 32]. In the intact cells the mitochondrial net uptake of ⁴⁵Ca was activated even at 4 mmol/l glucose, a concentration insufficient for opening the voltage-dependent Ca² channels in the plasma membrane. Moreover, the effect of glucose in retaining ⁴⁵Ca in the mitochondria could not be mimicked by introducing depolarizing concentrations of K⁺ in a medium deficient in Ca²⁺. Since procedures for raising intracellular Na⁺ activity have been found to be effective for mobilizing the ⁴⁵Ca incorporated in response to glucose [21, 33], it is pertinent to note that the concentration of Na⁺ is an important denominator for the net accumulation of Ca²⁺ in mitochondria isolated both from normal islets [34] and a rat insulinoma [35, 36]. The β-cell mitochondria can be expected to be particularly active in buffering Ca²⁺ under conditions of raised cytoplasmic concentrations of this ion. The set point for Ca²⁺ buffering by isolated insulinoma mitochondria has been estimated as 0.9 μmol/l [35], a concentration five to ninefold that of the cytoplasm in the unstimulated β cell.

Glucose, but not excessive K⁺, promoted the net uptake of Ca²⁺ into insulin-producing cells, when the
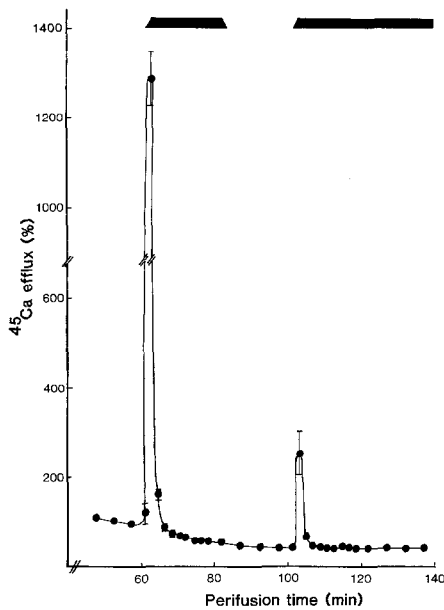
Fig. 3. Effects of carbamylcholine on ^{45}Ca efflux during perifusion with glucose-containing medium deficient in Ca^{2+}. The islets were loaded with ^{45}Ca in the presence of glucose (20 mmol/l) and perifused with a Ca^{2+}-deficient medium supplemented with EGTA (0.5 mmol/l) and glucose (20 mmol/l). Carbamylcholine (100 μmol/l) was introduced during the periods indicated by the horizontal bars. The data are given as percentage of the average ^{45}Ca efflux during the 10-min period preceding the first introduction of carbamylcholine. Mean values ± SEM for four experiments are shown

Fig. 4A and B. Effects of glucose on the cytoplasmic Ca^{2+} activity in suspended β cells. Isolated cells were prepared from β-cell-rich pancreatic islets of ob/ob-mice and loaded with the fluorescent indicator quin-2. At zero time glucose (20 mmol/l) was added to a Hepes-buffered medium containing **A** 1.20 or **B** 0.20 mmol/l Ca^{2+}. Open symbols and dotted lines indicate the Ca^{2+} activities expected without modifications of the media. Mean values ± SEM for 11 (**A**) and four (**B**) experiments respectively. Statistically significant differences obtained with the modifications of the media are indicated by $*p < 0.05$ and $***p < 0.001$. Reproduced from Rorsman et al. [39]

ion was present in concentrations of 10–20 μmol/l [11, 12]. This observation has encouraged the search for non-mitochondrial uptake of Ca^{2+} stimulated by glucose. During perifusion of islets from ob/ob-mice with a Ca^{2+}-deficient medium, muscarinic receptor stimulation with carbamylcholine resulted in an immediate mobilization of ^{45}Ca, provided that the islets had been exposed to glucose (B. Hellman and E. Gylfe, unpublished data). This might indicate high affinity accumulation of Ca^{2+} in the carbamylcholine-sensitive pool, implying that the pool was also filled during perifusion with the Ca^{2+}-deficient medium. It was consequently possible to induce a second peak of stimulated ^{45}Ca efflux, when carbamylcholine was re-introduced into the perifusion medium supplemented with glucose (Fig. 3). Muscarinic stimulation of the polyphosphoinositide breakdown may initiate a prompt mobilization of a separate pool of calcium in the pancreatic β cells by a number of mechanisms. The rapid response is, for example, compatible with both release of calcium bound to the inner face of the plasma membrane and mobilization of calcium from the endoplasmic reticulum mediated by inositol 1,4,5-triphosphate. In support for participation of the endoplasmic reticulum, the carbamylcholine ef-

fect was found to depend on uptake of Ca^{2+} promoted by K^+ but not by Na^+.

Cytoplasmic Ca^{2+} activity

With the introduction of the fluorescence indicator quin-2, it has been possible to measure directly the cytoplasmic Ca^{2+} activity associated with alterations of insulin release. Basal Ca^{2+} activity was found to be 100–180 nmol/l in clonal tumour β cells [37, 38] and normal β cells from ob/ob-mice [39], and it increased substantially during stimulation of insulin release. The effect of glucose on normal β cells is shown in Figure 4. It is evident that, in addition to raising cytoplasmic Ca^{2+}, the sugar also has the ability to lower the activity of this ion. The latter effect became apparent when preventing the glucose-mediated entry of Ca^{2+} by either lowering the extracellular concentration of the ion or blocking the potential-dependent Ca^{2+} channels with D-600.

The observations described so far make it possible to summarize the principal actions of glucose on the cytoplasmic Ca^{2+} activity as shown in Table 1. It is evi-

Table 1. Principal actions of glucose on Ca^{2+} movement in pancreatic β cells

Property studied	Action of glucose	Latency of effect (s)	Threshold concentration for the effect of glucose (mmol/l)	Alteration of cytoplasmic Ca^{2+} activity
Entry of Ca^{2+}	Stimulation	60	5	Increase
Intracellular sequestration of Ca^{2+}	Stimulation	10–20	0	Decrease
Outward transport of Ca^{2+}	Stimulation	>60	5	Decrease

Glucose concentration (mmol/l)
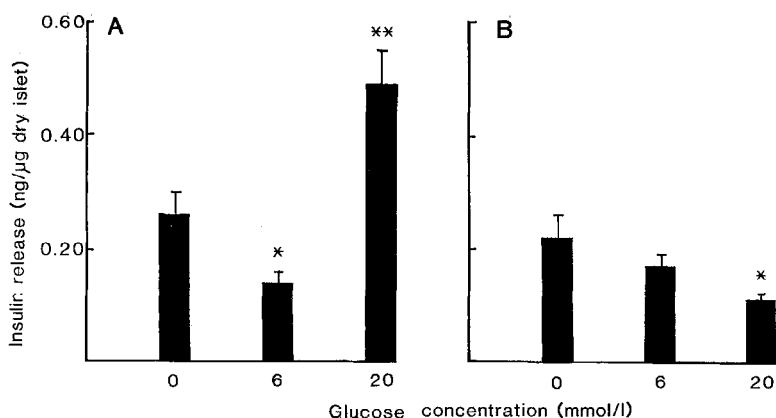
Fig. 5 A and B. Insulin secretory response to different concentrations of glucose in the absence (A) or presence (B) of D-600. Islets. from *ob/ob*-mice were cultured for 3 days in Ca^{2+}-deficient RPMI 1640 medium supplemented with glucose (1 mmol/l), 10% serum and EGTA (0.2 mmol/l). After culture the islets were exposed to different concentrations of glucose during 60 min of incubation in the same type of medium containing Ca^{2+} (0.42 mmol/l) and albumin (1 mg/ml). Culture and subsequent incubations of the islets were performed either in the (A) absence or (B) presence of D-600 (50 μmol/l). The amounts of insulin released during the incubation period are presented as mean values ± SEM for 10 experiments. Statistically significant differences from control media lacking glucose are indicated by *$p < 0.02$ and **$p < 0.005$

dent that the available data call for a reconsideration of previous ideas that Ca^{2+} mobilization from intracellular stores is a factor in glucose-stimulated insulin release [15, 36] and that the sugar inhibits the outward transport from the pancreatic β cells [15, 20]. Since glucose apparently has the opposite effect, its action on cytoplasmic Ca^{2+} activity instead will reflect the balance between increased entry of Ca^{2+} and the enhanced removal of the ion from the cytoplasm by intracellular trapping and outward transport. It is important to note that glucose stimulation of these three important Ca^{2+} movements occurs with different latency (Table 1). As suggested from the ^{45}Ca efflux studies, and in accordance with the measurements of cytoplasmic Ca^{2+} activity, the intracellular sequestration of Ca^{2+} can be expected to be manifest earlier and at lower concentrations of glucose than those which facilitate Ca^{2+} influx with subsequently increased outward transport.

Inhibition of insulin release

Glucose is the major physiological stimulator of insulin release. However, it follows from its ability to lower the cytoplasmic Ca^{2+} activity that, under certain conditions, the sugar may also inhibit insulin release. Indeed, we have demonstrated an apparently paradoxical inhibition of insulin release in a number of experimental situations. Although it was not commented on at the time, glucose inhibition of insulin release was already observed in a Ca^{2+}-deficient medium 10 years ago [40].

In view of the small effects of suppressing an already low cytoplasmic Ca^{2+} activity, it is not surprising that the inhibitory component in glucose action on insulin release has essentially escaped notice. When searching for experimental procedures to demonstrate an in-

hibitory action of glucose on insulin release, it was considered important, therefore, not only to counteract the glucose-mediated entry of Ca^{2+} into the β cells, but also to perform the studies under conditions in which the cytoplasmic Ca^{2+} activity was raised. In this way, glucose was found to inhibit the release of insulin obtained by Na^+ mobilization of intracellular Ca^{2+} [33]. The inhibitory component in the glucose action on insulin release could also be clearly demonstrated after increasing the Ca^{2+} permeability of the β cells by prior culture of the islets in a Ca^{2+}-deficient medium [41]. Some of the results obtained when using this approach are shown in Figure 5. Thus glucose (6 mmol/l) significantly inhibited insulin release, whereas a maximally depolarizing concentration of the sugar was stimulatory (Fig. 5 A). The addition of D-600, a blocker of the voltage-dependent Ca^{2+} channels, modified the secretory response to glucose (Fig. 5 B). In the latter case glucose became inhibitory even at a concentration of 20 mmol/l. The presence of D-600 had consequently transformed a stimulatory action of glucose into inhibition, an observation reinforcing the idea that entry of Ca^{2+} through the voltage-dependent channels is the mechanism initiating insulin release.

Functional significance of the cytoplasmic Ca^{2+} balance

The discovery of opposing actions of glucose on cytoplasmic Ca^{2+} activity might aid the understanding of several unresolved questions related to the function of the islets in normal and diabetic organisms. Some important phenomena supposedly depending on the promotion of the intracellular sequestration of Ca^{2+} by glucose are given in Table 2.

Since lowering cytoplasmic Ca^{2+} activity has already been suggested as a decisive factor in glucose-induced oscillations of membrane potential [42–44], it is noteworthy that in the present model the time-average effect of high concentrations of glucose is to increase cytoplasmic Ca^{2+} activity. However, for glucose concentrations up to 5 mmol/l, it is implicit from the concept of Ca^{2+} balance that there will only be suppression of cytoplasmic Ca^{2+} activity. With the acceptance of a Ca^{2+}-dependent K$^+$ conductance of the β cell plasma membrane [42–44], it is easy to understand how a reduction of cytoplasmic Ca^{2+} resulting from intracellular buffering will initiate depolarization. Glucose concentrations above 5 mmol/l further increase the Ca$^+$ buffering, resulting in additional depolarization with bursts of action potentials related to the opening of voltage-dependent Ca^{2+}-channels. Lowering the cytoplasmic

Table 2. Phenomena postulated to be related to glucose promotion of intracellular sequestration of Ca^{2+}
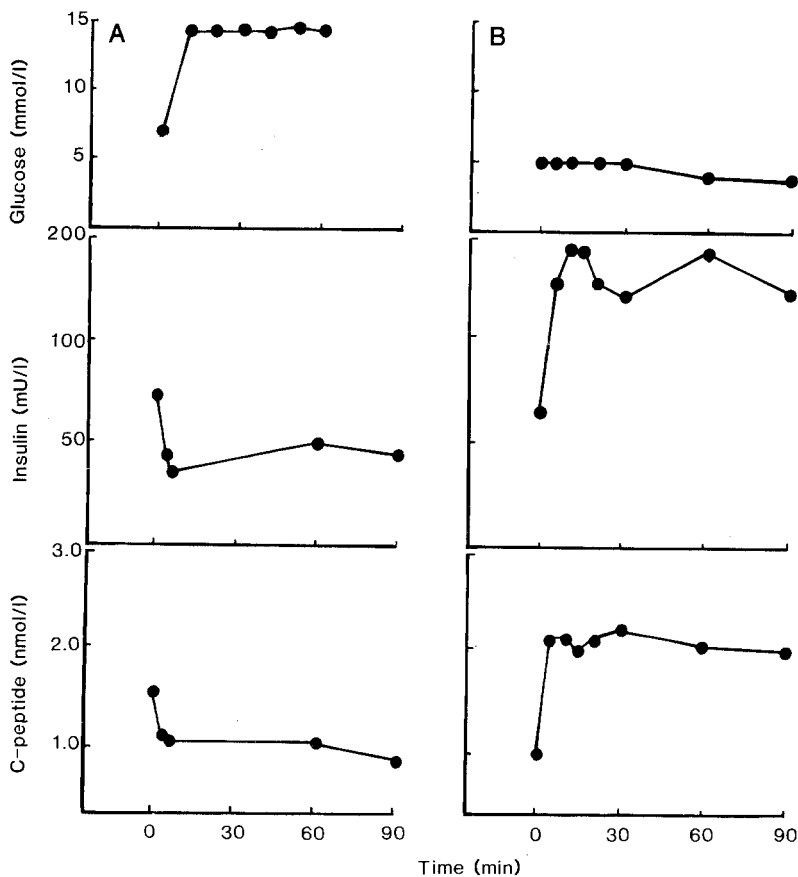
Depolarization induced by Ca^{2+}-regulated K$^+$ permeability with subsequent influx of Ca^{2+} into pancreatic β cells.

Appearance of a progressively increasing second phase of insulin release.

Potentiation of glucose-stimulated insulin release after priming with the sugar

Demonstration of a paradoxical glucose inhibition of insulin release in certain cases of defective β cell function.

Glucose-induced inhibition of glucagon release from pancreatic α cells.

Ca^{2+} activity to below the resting level will consequently be a phenomenon that only occurs during a short period preceding the burst. When the glucose concentration is raised further, there is a concomitant increase of the sequestration and outward transport of Ca^{2+}, resulting in prolongation of the bursts. A continuous burst pattern emerges at maximally stimulating concentrations of glucose, when the processes for elimination of Ca^{2+} from the cytoplasm balance the influx. Even if the time-average Ca^{2+} activity reaches a maximum in the latter situation, the highest peak activities occur at concentrations of glucose which stimulate secretion submaximally. The fact that regulation of K$^+$ conductance by Ca^{2+} can in itself explain the depolarizing effect of glucose by no means excludes the existence of additional mechanisms by which the sugar affects the membrane potential. Recent patch clamp studies have demonstrated that the β cells also possess K$^+$-selective channels, which are rapidly and reversibly blocked by cytoplasmic ATP [45, 46].

The intracellular buffering of Ca^{2+} can be expected to be less pronounced with time due to a limited capacity for sequestration. The concept of a dual action of glucose might thus explain its ability to induce an increasing second phase of insulin release and the fact that the secretory response is improved after priming with the sugar. The cyclic burst pattern of the electrical response persists during prolonged exposure to glucose, a condition supposed to be associated with saturation of the intracellular buffering system. It is likely therefore that the calmodulin-dependent plasma membrane

Fig. 6 A and B. Effects of intravenous (A) glucose (300 mg/kg body weight) and (B) tolbutamide (1 mg) on the serum levels of glucose, insulin and C-peptide in a 14-year-old girl with mild diabetes. Reproduced from Hellman et al. [50]

Ca^{2+}-ATPase is deactivated more slowly than the cytoplasmic Ca^{2+} activity decreases. Such a mechanism should lead to reduction of cytoplasmic Ca^{2+} below the resting activity with subsequent initiation of depolarization. When glucose is omitted, release of Ca^{2+} from the sequestrating organelles prevents a rapid decrease of the cytoplasmic Ca^{2+} activity so that there is time for the plasma membrane Ca^{2+} ATPase to adapt to the new situation.

The effect of glucose in promoting intracellular sequestration can be changed to be more easily recognized under conditions, when metabolism of the sugar is unable to initiate the depolarization of β cells. The observation of a glucose-induced inhibition of insulin release in itself suggests a defect in β cells which may be significant for their ability to counteract diabetes. It is evident from recent studies in our laboratory (unpublished data) that the normal insulin secretory response to glucose can be altered into an inhibitory action in the presence of diazoxide, a compound supposed to counteract the depolarizing action of glucose [47]. Further evidence that impairment of β cell function may be apparent as a paradoxical inhibition of insulin release has been obtained in studies of the perfused pancreas from rats made diabetic with alloxan (W. Grill: personal communication).

Whereas glucose-stimulated entry of Ca^{2+} can be regarded as specific for pancreatic β cells, the promotion of intracellular sequestration of Ca^{2+} by the sugar might be a phenomenon of more general relevance. When rapidly metabolized, glucose also potentially lowers the cytoplasmic Ca^{2+} activity in other cells. It is still an open question whether such a mechanism accounts for the inhibitory action of glucose on the release of glucagon from pancreatic α cells. If this is the case, the excessive glucagon secretion in diabetes can be explained by saturation of the intracellular buffering system for calcium following prolonged exposure to raised concentrations of glucose.

Cytoplasmic Ca^{2+} balance in human diabetes

A paradoxical glucose inhibition of insulin release can be regarded as an indicator of defective β cell function (Table 2). It is, therefore, not surprising that intravenous glucose infusions have been reported to result in a temporary reduction of circulating concentrations of insulin in several diabetic patients [48–50]. This largely neglected phenomenon was demonstrated recently in a patient with mild diabetes, in whom the glucose-induced depression of serum insulin was not only pronounced and long-standing, but also possible to induce after an oral load [50]. Figure 6 shows the results of intravenous injections of glucose and tolbutamide in this patient. The falling values for insulin and C-peptide are compatible with half-lives for these hormones of only 4–5 and 8–9 min, respectively. This suggests that during an initial phase glucose induces an almost complete inhibition of secretory activity in pancreatic β cells.

In the light of the available data, it cannot be ruled out that glucose-induced suppression of circulating insulin is due to endogenous production of a non-adrenergic inhibitor of secretion, such as somatostatin. However, at least in certain cases of diabetes, it seems likely that the reduction of serum insulin reflects glucose-induced inhibition of insulin release mediated by lowering of cytoplasmic Ca^{2+} in analogy to the observations made in the experimental studies. Although sulphonylurea compounds are known to promote the release of somatostatin [51], tolbutamide was found to be a potent stimulator of insulin release in the patient demonstrated in Figure 6. Sulphonylureas are depolarizing agents, which promote the entry of Ca^{2+} by opening the voltage-dependent channels in the β cell membrane [21, 24, 52]. The patient could, therefore, be an example of a situation, where the inhibitory component in the glucose action on insulin release was unmasked due to interference with the depolarizing action of the sugar.

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