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Ca^{2+} Transport in Pancreatic β-Cells during Glucose Stimulation of Insulin Secretion

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ABSTRACT

The role of Ca^{2+} in the regulation of insulin secretion was evaluated using β-cell-rich pancreatic islets isolated from ob/ob-mice. The glucose stimulation of the secretory activity is supposed to result from accumulation of Ca^{2+} in the submembrane cytoplasmic space. It is likely that this process reflects the balance between increased entry of Ca^{2+} into the β-cells and an enhanced sequestration of Ca^{2+} in the organelle sinks. The proposed model can explain the cAMP potentiation of glucose-stimulated insulin release with suppression of the mitochondrial Ca^{2+} uptake. Furthermore, differences in the Ca^{2+} buffering capacity of the secretory granules may account for other characteristic features of glucose-stimulated insulin release, in particular its biphasic nature and sensitivity to suppression on withdrawal of nutrients.

INTRODUCTION

Although it is still uncertain whether the pancreatic β-cells display oscillatory volume shifts as implied in the excitable cell model proposed by Teorell (25), there is no doubt that glucose stimulation of their secretory activity is associated with prominent changes in the electrical activity involving depolarisation with the subsequent appearance of action potentials (23, 24). The rate of insulin release is correlated with the duration of the periods with these actions potentials. It can furthermore be inferred from the available electrophysiological data that the β-cell membrane possesses voltage-dependent channels for Ca^{2+}.

With its established dependence on ionic mechanisms, regulation of insulin secretion seems to be a most appropriate topic for a discussion at a symposium dedicated to Professor Teorell. In the present communication it will be demonstrated how the glucose stimulation of insulin release, and its potentiation by cAMP, can be related to alterations in the β-cell handling of Ca^{2+} resulting in enhanced concentrations of this cation in the submembrane space.

MATERIAL AND METHODS

The experiments were performed with pancreatic islets isolated from ob/ob-mice fasted overnight. The islets were either studied in static incubation systems (9) or by perifusion with a nonrecirculating medium in the parallel channels of a perifusion apparatus (6, 18). Subcellular fractions of ^{45}Ca-loaded islets were prepared by differential centrifugation under conditions minimizing calcium redistribution (19). Graphite furnace atomic absorption spectroscopy was employed for measurements of total calcium in both intact islets and subcellular islet fractions (3). When employing tracer techniques, the radioactivities were expressed in terms of μmoles of ^{45}Ca assuming the same specific radioactivities as used in the media for loading the islets. Washing the islets for 60 min with 2 mM La^{3+} made it possible to discriminate between superficial and intracellular ^{45}Ca (12, 16, 17). In the subcellular fractionation experiments, the ^{45}Ca incorporated was related to protein contents. In all other instances the different variables were expressed per islet dry weight, obtained by weighing on a quartz fiber balance.

RESULTS AND DISCUSSION

Stimulatory and inhibitory effects of Ca^{2+} on insulin release

It is well known that some extracellular Ca^{2+} is a prerequisite for the secretory response to glucose and other insulin secretagogues (10, 14). This need for extracellular Ca^{2+} may only indicate that a certain concentration of the ion is a prerequisite for the proper function of the secretory machinery. In support of the idea of a direct regulatory role for Ca^{2+}, an increase in the extracellular Ca^{2+} concentration was found to stimulate the secretory activity in the absence of glucose or other initiators of insulin release (4, 11). Fig. 1 shows the dynamics of insulin release from isolated islets after raising the Ca^{2+} concentration from 2.6 to 20.5 mM during perifusion with glucose-free medium supplemented with 5 mM theophylline. The osmotic pressure was kept constant throughout the experiments with the aid of choline chloride. It can be seen that the increase of Ca^{2+} was associated with a rapid stimulation of insulin release which then tended to fade away. Interestingly, the reduction of the concentra-

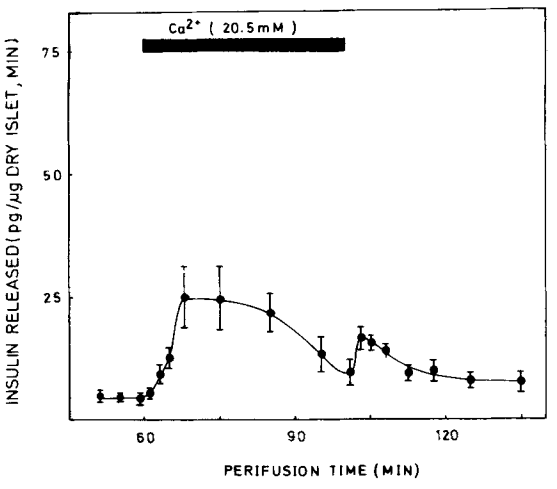

Fig. 1. Dynamics of insulin release after a temporary rise of extracellular Ca^{2+}

tion of extracellular Ca^{2+} to its original level induced a new phase of stimulated secretory rate. The release pattern observed may indicate that there exist both inhibitory and stimulatory calcium pools in the β-cells. In fact we have reasons to believe that glucose promotes the release of insulin both by increasing stimulatory Ca^{2+} in the cytoplasm and by removing inhibitory Ca^{2+} from the plasma membrane (5, 13, 26).

Transport of Ca^{2+} across the plasma membrane

An important aspect of the β-cell handling of Ca^{2+} is the effect of glucose on Ca^{2+} transport across the plasma membrane. The significance of the processes regulating the entry of Ca^{2+} is illustrated by the rapid inhibition of the glucose-stimulated insulin release seen after removal of extracellular Ca^{2+}. Early studies indicated that glucose stimulated the incorporation of ^{45}Ca into isolated islets (15, 22). However, it could not be decided at this time whether the glucose-induced retention of ^{45}Ca represented increased binding to the plasma membrane or transport into the β-cells. In the attempts to measure the intracellular uptake, advantage was taken of a La^{3+} wash technique originally proposed for studying calcium in smooth muscles. Fig. 2 shows the change in intra-

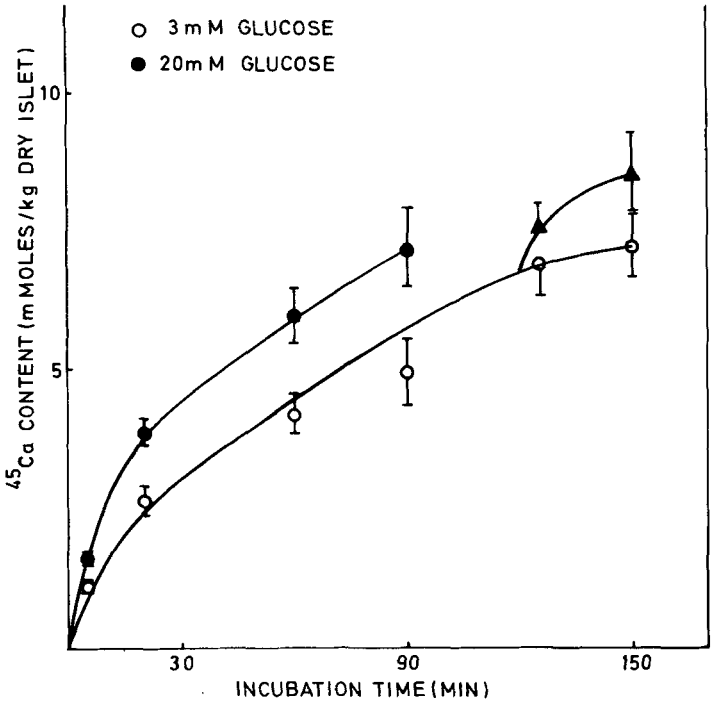

Fig. 2. Uptake of intracellular ^{45}Ca in the presence of 3 and 20 mM glucose

cellular ^{45}Ca uptake with time into islets incubated in the presence of 3 and 20 mM glucose and finally washed with 2 mM La^{3+}. A stimulatory effect of glucose was apparent already after 5 min of incubation. The solid triangles refer to an experiment in which the intracellular isotope content was first brought to near

equilibrium by incubating the islets for 120 min with ^{45}Ca in the presence of 3 mM glucose. The glucose concentration was then raised to 20 mM. This change of the glucose concentration caused the same absolute increase of the ^{45}Ca uptake as that observed when 20 mM glucose was added at the same time as the isotopic tracer. It was evident from other studies of the La^{3+}-nondisplaceable ^{45}Ca that glucose significantly stimulated a process of Ca-Ca exchange. Consequently, it can be expected that the glucose effect on La^{3+}-nondisplaceable ^{45}Ca exceeds that on total intracellular calcium. So far, we have not been able to demonstrate a statistically significant action of glucose on the islet calcium measured with graphite furnace atomic absorption spectroscopy.

It is likely that the glucose-induced depolarisation of the β-cells causes the opening of potential-dependent channels for Ca^{2+}. It is therefore far from surprising that glucose stimulates the net uptake of ^{45}Ca. However, glucose was found to have significant effects also on the efflux of ^{45}Ca. Fig. 3 shows the washout of radioactivity from islets preloaded with ^{45}Ca and subsequently peri-

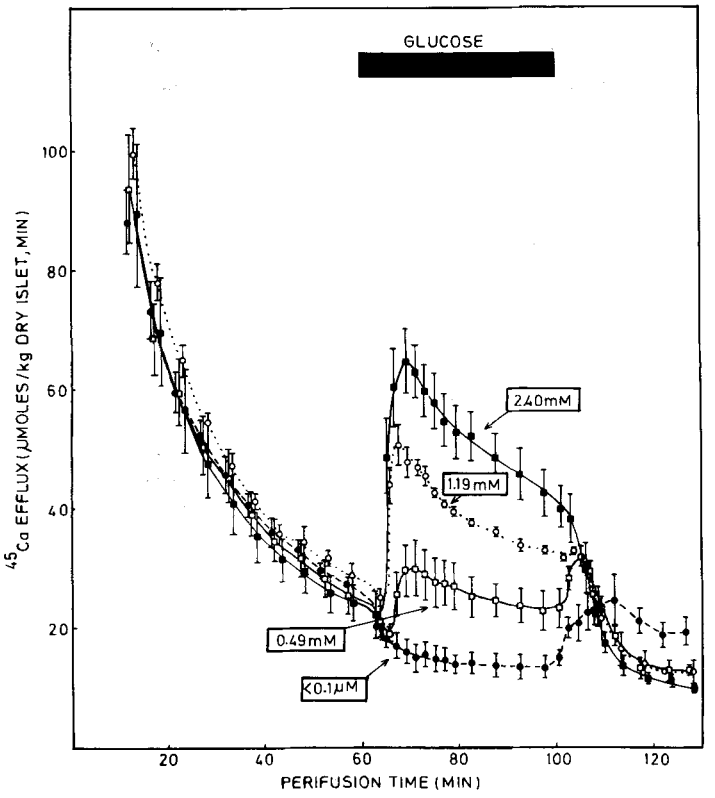

Fig. 3. Modification of ^{45}Ca efflux after introducing 20 mM glucose into perifusion media with different concentrations of Ca^{2+}

fused with media containing different concentrations of Ca^{2+}. The introduction of 20 mM glucose into the perifusion medium resulted in both inhibitory and stimulatory effects on the ^{45}Ca efflux. In the presence of moderate concentrations of extracellular Ca^{2+} a period of transient inhibition was followed by a

more prominent stimulatory phase. The stimulatory action can not be explained by loss of calcium with the secretory granules during exocytosis (6). With the demonstration that the effect required the presence of glucose during the ^{45}Ca loading (6, 13, 14), it seems likely that the stimulation of ^{45}Ca efflux reflects an exchange with the non-radioactive Ca^{2+} entering the β-cells. This idea is supported by the fact that glucose has only an inhibitory effect on ^{45}Ca efflux during perifusion with Ca^{2+}-deficient medium as indicated in Fig. 3.

The glucose-induced inhibition of ^{45}Ca efflux was seen even after extending the radioactive loading period to 7 days. This means that the inhibition cannot simply be due to preferential mobilisation of calcium stores with low specific radioactivity. Experimental situations resulting in stimulated net uptake of ^{45}Ca and insulin release are not always associated with inhibition of ^{45}Ca efflux from preloaded islets. Among various conditions tested only the removal of Na^{+} imitated all these effects of glucose. The absence of inhibited ^{45}Ca efflux under various conditions of stimulated entry of Ca^{2+} suggests that glucose inhibition is essentially accounted for by mechanisms other than stimulated re-entry of extruded ^{45}Ca. It is strongly suggested from the concentration dependence of the glucose effects (2, 21) that it is the stimulated entry of Ca^{2+} rather than an inhibition of its efflux which represents the major determinant for the functionally important intracellular Ca^{2+}.

Calcium buffering by intracellular organelles

Although an increased net uptake of ^{45}Ca was usually associated with stimulated insulin release these processes were not strictly correlated. Prolonged starvation resulted for example in an enhanced incorporation of ^{45}Ca despite a concomitant suppression of the glucose-stimulated insulin release. In view of the differences encountered it seems likely that also alterations in the Ca^{2+} buffering of the organelles represent an important mechanism for modulating the critical pool of cytoplasmic Ca^{2+}. In the attempts to elucidate this matter, the subcellular organelles were isolated under conditions which minimized the redistribution of calcium. Fig. 4 shows the amounts of radioactivity in the cell organelles, related to their protein contents, after loading the islets for 120 min with ^{45}Ca in the absence and presence of 20 mM glucose. The exposure to glucose resulted in a significant increase of the ^{45}Ca incorporation both into the mitochondria and the secretory granules. It was evident from other studies (7, 8) that the glucose stimulation of the mitochondrial uptake of ^{45}Ca was depressed after raising the β-cell content of cAMP, a condition associated with a potentiation of the glucose-induced insulin release.

A comparison of the amounts of ^{45}Ca in the organelles with the total calcium contents as recorded by graphite furnace atomic absorption spectroscopy indicated that readily exchangeable calcium accounted for no less than 30 % of the

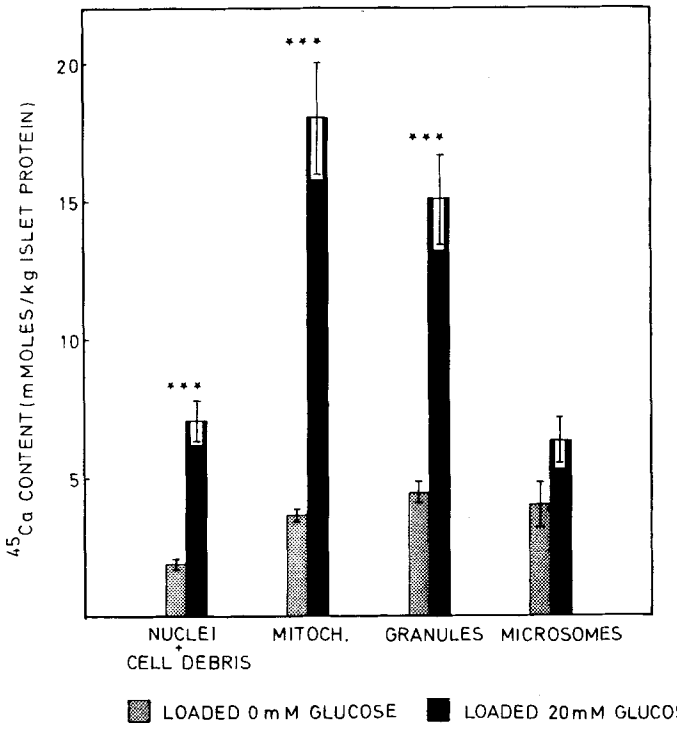

Fig. 4. Distribution of ^{45}Ca among subcellular islet fractions after 120 min of incubation in the presence and absence of 20 mM glucose

calcium present in the secretory granules. With the demonstration that the secretory granules contain substantial amounts of exchangeable calcium, subject to glucose regulation, it seems likely that also this organelle participates in the regulation of the functionally important intracellular Ca^{2+}. Using the fluorescent probe 9-aminoacridine it has been possible to demonstrate that the interior of the granule sac is maintained at a low pH (1). The secretory granules may consequently incorporate Ca^{2+} in exchange for protons. It is possible that Ca^{2+} must reach a certain concentration in the granule sac before the granule content can be extruded by exocytosis. Furthermore, the location of many secretory granules in the cell periphery makes them particularly suitable for regulating the stimulatory pool of cytoplasmic Ca^{2+} which is supposed to exist in the immediate submembrane space.

A model for glucose regulation of the functionally important Ca^{2+}

A schematic illustration of our present ideas of how glucose affects the functionally important Ca^{2+} in the pancreatic β-cells is given in Fig. 5. The grey area to the left represents the plasma membrane. The stimulatory action of glucose on insulin release is tentatively related both to mobilisation of inhibitory Ca^{2+} in the plasma membrane and to accumulation of cytoplasmic Ca^{2+} in the submembrane space. The basic idea is that the permeability of the plasma membrane for monovalent ions, essentially K^+, is regulated by the redox equilibrium between sulphydryl groups and disulphide bridges in a regulatory membrane

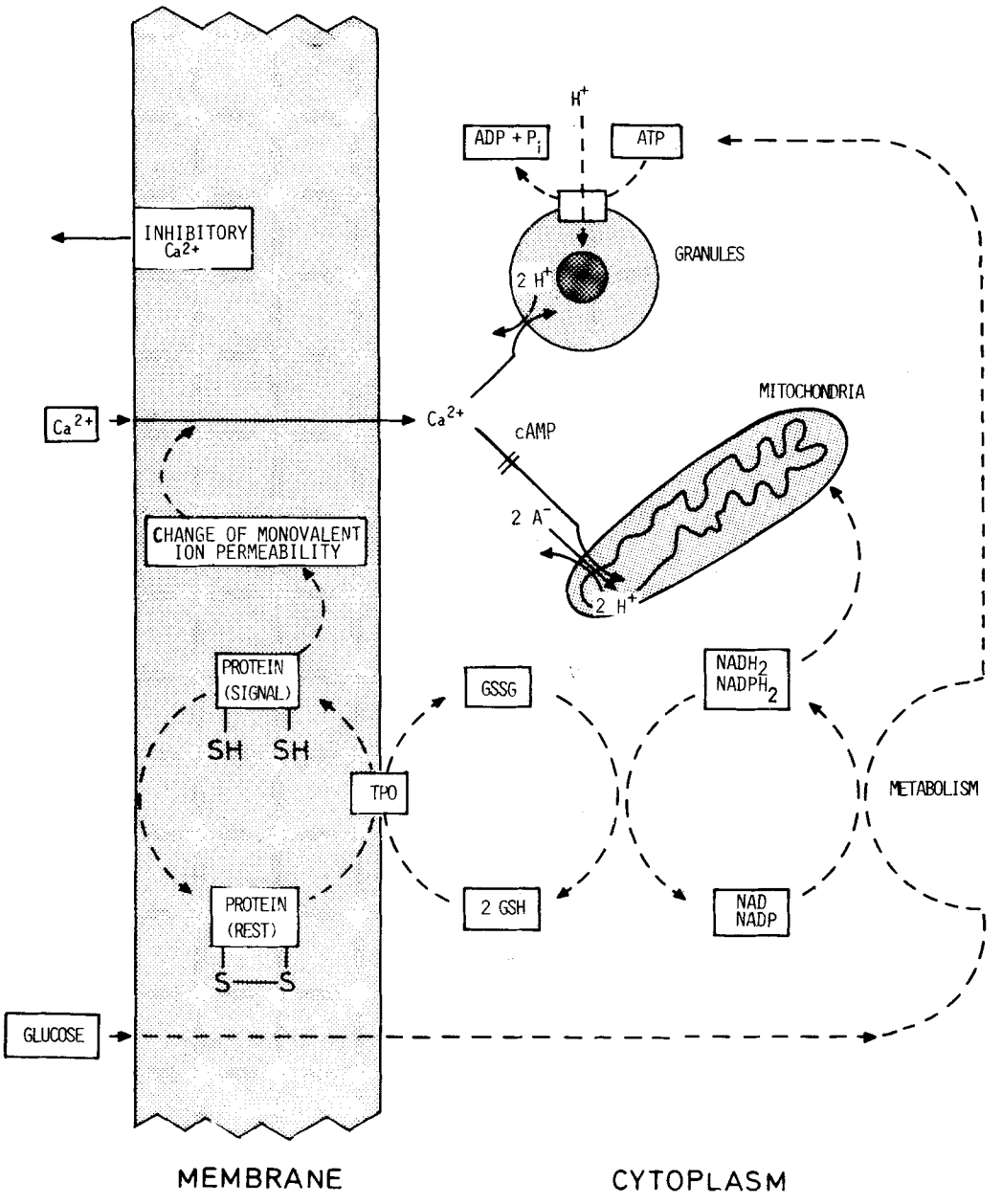

Fig. 5. Model illustrating Ca^{2+} movements in the β-cell induced by glucose

protein. The metabolism of glucose produces reducing equivalents which, by shifting the equilibrium away from the disulphide bridge conformation, results in a depolarisation of the β-cells. The depolarisation makes it possible for Ca^{2+} to enter into the β-cell through potential-dependent channels. Another effect of the reduced pyridine nucleotides might be to increase the net accumulation

of calcium in mitochondria in analogy to what has been described for other cell types (20). It is evident from the model that the glucose effect on the stimulatory pool of cytoplasmic Ca^{2+} reflects the balance between stimulated entry of Ca^{2+} and its removal by the mitochondria and the secretory granules. Accordingly, the potentiation of the glucose-induced insulin release by cAMP can be explained with a suppression of the glucose-stimulated uptake of Ca^{2+} into the mitochondria.

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