THE INFLUX OF Ca^{2+} INTO PANCREATIC β -CELLS AND ITS REGULATION BY GLUCOSE

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ABSTRACT

Estimates of the rate of Ca^{2+} entry into cells with ^{45}Ca require correction for the radioactivity bound to the cell surface. The use of La^{3+} for displacing superficially bound ^{45}Ca in the virtual absence of transmembrane fluxes made it possible to measure the unidirectional influx component for Ca^{2+} in suspended aggregates of cells prepared from β -cell-rich pancreatic islets isolated from *ob/ob*-mice. Whereas depolarization with K^+ induced an almost immediate increase in the entry of Ca^{2+} , a corresponding stimulation with glucose required more than 60 sec. After prolonged exposure to 20 mM glucose the rate of the Ca^{2+} entry was equivalent to $1.9 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, which is twice the basal influx. Evidently, the glucose stimulation of the influx of Ca^{2+} results in a subsequent increase also of the outward transport of the ion. It can be concluded from the rapid entry of Ca^{2+} that the turnover rate for intracellular calcium exceeds 5% per min in the stimulated β -cell.

The radioactive isotope ^{45}Ca has been widely employed for studying the Ca^{2+} movements across the plasma membrane of the pancreatic β -cells. In a number of experiments the rate of Ca^{2+} influx has been estimated from measurements of the short-term uptake of ^{45}Ca by isolated islets employing tritiated mannitol (15) or sucrose (5, 20, 27) to monitor the volume of the extracellular space. However, this approach is far from ideal in being influenced by differences in the diffusion rates of the radioactively labelled indicators. Even more important, no correction was made for the proportionately large amounts of ^{45}Ca bound to the exterior of the cells and the capsule surrounding the islets.

Previous studies have established that La^{3+} is able to displace superficially bound Ca^{2+} and suppress its transmembrane fluxes. So far washing with La^{3+} has been found to be useful for estimation of the exchangeable intracellular calcium in both isolated islets (10, 17) and dispersed cells (25) when measuring ^{45}Ca uptake under apparent isotopic steady state conditions. The present communication de-

scribes the use of La^{3+} for analyses of the unidirectional influx component in Ca^{2+} transport across the plasma membrane. By measuring the short-term uptake of ^{45}Ca by suspended aggregates of β -cells subsequently centrifuged through oil into a solution of La^{3+} , it was demonstrated that the Ca^{2+} entry into the β -cells indeed represents a very rapid process markedly stimulated by glucose.

MATERIALS AND METHODS

Chemicals and Media

Reagents of analytical grade and deionized water were used. The silicone oils AR 20 and AR 200 were provided by Wacker-Chemie GmbH, Munich, F.R.G. Tissue culture medium RPMI 1640 and heat-inactivated fetal bovine serum were obtained from Gibco, Paisley, Renfrewshire, U.K. Collagenase (EC 3.4.24.3) and $^{45}\text{CaCl}_2$ were products of Boehringer Mannheim GmbH, Mannheim, F.R.G. and New England Nuclear Chemicals GmbH, Dreieich, F.R.G., respectively. Packard Instrument Co., Downers Grove, IL,

supplied Soluene^R-350 and Scintillator 299TM.

Culture of β -cells was performed in RPMI 1640 medium supplemented with 10% serum, 100 IU/ml penicillin, 100 μ g/ml streptomycin and 50 μ g/ml gentamycin. The basal medium used when preparing the β -cell suspension and studying the uptake of ^{45}Ca was buffered with Hepes and physiologically balanced in cations with Cl^- as the sole anion (9).

Preparation of β -Cell Aggregates

Adult *ob/ob*-mice were taken from a non-inbred colony and used as a source of pancreatic islets containing more than 90% β -cells (8). Between 200 and 400 islets were isolated from each animal by collagenase, and a suspension of cells was prepared by shaking in basal medium deficient in Ca^{2+} essentially as described by Lernmark (19). With this procedure the cells remained in pauci-cellular aggregates. The suspension was centrifuged through 7 cm of the basal medium supplemented with 4% (w/v) albumin to remove damaged cells. The cells were then resuspended in 8 ml of serum-containing tissue culture medium. The suspension was kept in this medium for 24 h at 37°C with gentle shaking in a 50 ml Nunclon flask (A/S Nunc, Kamstrup, Denmark) in an atmosphere of 5% CO_2 in air. After brief hand-shaking to disrupt large aggregates followed by washing and centrifugation through 4% (w/v) albumin, the pellet was resuspended in an albumin-free basal medium lacking glucose. Phase contrast microscopy revealed that the resulting preparation consisted essentially of aggregates containing 5–50 cells.

Studies of ^{45}Ca Uptake

The incubations were carried out at 37°C in poly-ethylene test tubes (volume, 0.4 ml) containing 175 μ l of a mixture of silicone oils (10 vol AR 20 and 6 vol AR 200) layered on 100 μ l of basal medium also containing 1 mM LaCl_3 and enough sucrose (30 mg/ml) to attain the required density. To prevent precipitation of $\text{La}_2(\text{CO}_3)_3$ the medium was prepared immediately before each experiment, the pH being adjusted by the addition of NaOH protected from exposure to CO_2 . At zero time 50 μ l samples of the cell suspension were added to equal volumes of basal medium, containing ^{45}Ca and test substances, layered on the silicone oils. After incubation for 15 sec to 5 min

the cell aggregates were separated from the radioactive solution by centrifugation (Microfuge 152, Beckman-Spinco, 30 sec at 9,500 g) through the water-impermeable barrier of silicone oils into the La^{3+} solution. Immediately after centrifugation the test tubes were placed on ice. By cutting off the bottoms of the tubes it was possible to obtain samples of the La^{3+} -containing medium and to remove the pelleted cells. After freeze-drying and weighing on a quartz fiber balance, the pellet (5–50 μ g) was resuspended and dissolved in Soluene^R-350. Samples of the medium employed in the loading of the islets were used as external standards for liquid scintillation measurements of ^{45}Ca in the pellet and the La^{3+} -containing wash medium. In no case did the ^{45}Ca concentration in the La^{3+} -wash medium exceed 1% of that in the loading medium.

It was checked that radioactivity was not transferred to the La^{3+} -wash medium, when performing the experiments without cells. In other control experiments the cells were exposed to ^{45}Ca in the presence of 1 mM La^{3+} . The amounts of radioactivity associated with the cells after different periods of incubation in the La^{3+} -containing medium and subsequent centrifugation through the silicone oils are shown in Table 1. Irrespective of the loading time the radioactivity corresponded to 0.2–0.3 mmol/kg dry weight. Similar values were obtained when glucose and other depolarizing test substances were included in the incubation medium (not shown).

Table 1 Amounts of Radioactivity Associated with the β -Cells after Incubation for Different Periods of Time with ^{45}Ca in the Presence of 1 mM La^{3+}

Incubation time (min)	^{45}Ca content (mmol/kg dry weight)
0.5	0.23 ± 0.01
1.0	0.26 ± 0.05
2.0	0.25 ± 0.02
5.0	0.29 ± 0.04

After 24 h of storage in a tissue culture medium, aggregates of β -cells were centrifuged through albumin and resuspended in a glucose-free medium containing 1.28 mM Ca^{2+} . After 15 min of preincubation at 37°C, the uptake of ^{45}Ca was measured in a similar type of medium supplemented with 1 mM La^{3+} . Exposure to ^{45}Ca for 30 sec up to 5 min was followed by centrifugation of the cell aggregates through silicone oils into a solution of La^{3+} . The ^{45}Ca content is expressed as mmol calcium with the same specific radioactivity as in the incubation medium. Mean values $\pm$ SE for 4–5 experiments

Evaluation of Results

The radioactivity was expressed as mmol calcium with the same specific radioactivity as in the loading medium. The intracellular Ca²⁺ uptake was defined as the amount of La³⁺-nondisplaceable ⁴⁵Ca incorporated per kg dry weight after subtraction of the radioactivity associated with the cells when 1 mM La³⁺ was present during the loading procedure.

RESULTS

The intracellular uptake of ⁴⁵Ca after administration of 20 mM glucose or raising the extracellular concentration of K⁺ is shown in Fig. 1. In both cases the uptake was significantly

stimulated in comparison with control islets. The action of K⁺ was prompt, resulting in more than 3-fold increase within 30 sec. However, the effect of glucose was delayed. Accordingly, there was no stimulation of the uptake of La³⁺-nondisplaceable ⁴⁵Ca during the first 60 sec after exposure to the sugar.

Fig. 2 illustrates how previous exposure to glucose affects its stimulation of the entry of Ca²⁺. In addition to demonstrating the latency of the glucose effect when the cells were pre-incubated in the absence of the sugar, it is evident that prolonged exposure to glucose results in a substantial increase in the entry of Ca²⁺. By including observation periods as short as 15 sec, estimates could be made of the unidirectional influx component. This was

Fig. 1 Effects of glucose and excessive K⁺ on the intracellular uptake of ⁴⁵Ca by pancreatic β -cells. After 24 h of storage in a tissue culture medium, aggregates of β -cells from *ob/ob*-mice were centrifuged through albumin and resuspended in a glucose-free medium containing 1.28 mM Ca²⁺. Subsequent preincubation for 15 min at 37°C was followed by measurement of the ⁴⁵Ca uptake in a similar type of medium supplemented either with 20 mM glucose (panel A) or 25 mM KCl (panel B). In both cases basal uptake in the absence of additives is indicated as a dotted line. Exposure to ⁴⁵Ca for 30 sec to 2 min was followed by centrifugation of the cell aggregates through silicone oils into a solution of La³⁺. The radioactivity retained after washing for 30 min with La³⁺ is expressed as mmol calcium with the same specific radioactivity as in the incubation medium. Mean values $\pm$ SE for 4-5 experiments

Fig. 2 Modifying effects of glucose on its subsequent stimulation of the intracellular uptake of ^{45}Ca by pancreatic β -cells. After 24 h of storage in a tissue culture medium, aggregates of β -cells from *ob/ob*-mice were centrifuged through albumin and resuspended in a medium containing 1.28 mM Ca^{2+} supplemented or not supplemented with 20 mM glucose. Subsequent preincubation for 120 min at 37°C was followed by measurement of the ^{45}Ca uptake in a similar type of medium supplemented with 20 mM glucose. The basal uptake observed when glucose was absent during the preincubation and the subsequent exposure to ^{45}Ca is indicated as a dotted line. Exposure to ^{45}Ca for 15 sec to 5 min was followed by centrifugation of the cell aggregates through silicone oils into a solution of La^{3+} . The radioactivity retained after washing for 30 min with La^{3+} is expressed as mmol calcium with same specific radioactivity as in the incubation medium. Mean values $\pm$ SE for 5–11 experiments

found to be $1.9 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for cells continuously exposed to glucose, implying a doubling of the basal influx rate. Glucose was more efficient in promoting intracellular incorporation of ^{45}Ca after the first 2 min, when the β -cells had been preincubated in the absence of the sugar. Consequently, there were no differences in the cellular contents of radioactivity related to the preincubation with glucose 5 min after exposure to ^{45}Ca .

DISCUSSION

Previous studies on isolated pancreatic islets

have indicated the advantage of using La^{3+} for discriminating between ^{45}Ca bound to the exterior of the cells and that incorporated intracellularly (10, 17). The La^{3+} -displaceable pool of superficial ^{45}Ca was rapidly exchangeable, constituting a substantial part of the total amount of ^{45}Ca associated with the islets (12, 18). Although binding to the capsule surrounding the islets originally resulted in an overestimate of the ^{45}Ca associated with the exterior of the cells (14), the amounts displaced by La^{3+} from *ob/ob*-mouse islets lacking capsule were calculated up to as much as 2–3 mmol per kg dry weight (4). This value is

equivalent to the amounts of ⁴⁵Ca found in the present study to be incorporated intracellularly during 5 min of stimulation with glucose. Consequently, estimates of the rate of Ca²⁺ entry into the β -cells require correction for the amounts of radioactivity bound to the cell surface.

The time course for the uptake of La³⁺-nondisplaceable ⁴⁵Ca was essentially linear for up to 30 sec. An observation period of 15 sec should therefore provide a measure of the unidirectional influx of Ca²⁺. Since it can be expected that the time required for Ca²⁺ to diffuse into the central parts of intact islets is considerably longer (16), the present studies were performed with small aggregates of cells. Although single dispersed cells are more ideal with respect to the diffusion times for the isotope, such preparations suffer from a poor secretory response to glucose (19, 21) despite a normal metabolism of the sugar (7). The advantage of using small aggregates of β -cells is that they respond adequately to glucose both with regard to increased cytoplasmic Ca²⁺ (23) and stimulation of insulin release (22).

Whereas depolarization with K⁺ induced a prompt increase in the entry of Ca²⁺, the corresponding stimulation with glucose required more than 60 sec. A similar latency in the glucose effect has been described when studying ⁴⁵Ca uptake in intact islets using sucrose as an extracellular space marker (5, 20, 27), and it has also its counterpart in the time needed for increasing cytoplasmic Ca²⁺ (23) and stimulating insulin release (3, 6, 26). Studies of the initial uptake of ⁴⁵Ca after prolonged exposure to glucose indicated a rate of Ca²⁺ influx of 1.9 mmol·kg⁻¹·min⁻¹, i.e. twice the basal value. This rate compares favourably with recent estimates of the influx based on patch clamp analyses of Ca²⁺ currents (24). The pronounced stimulatory action of glucose on the influx of Ca²⁺ has no correspondence with regard to net increase of the element (1, 2). It can therefore be concluded that the glucose stimulation of the entry of Ca²⁺ is associated with a subsequent increase of the outward transport of the ion. The total amounts of intracellular calcium in the pancreatic β -cells of the *ob/ob*-mice have been estimated to about 30 mmol·kg⁻¹ (1, 2). The turnover rate for calcium consequently exceeds 5% per min in the stimulated β -cell.

Glucose modifies the Ca²⁺ movements in the pancreatic β -cells not only by affecting the

transport across the plasma membrane but also by promoting the sequestration of the ion in intracellular stores (11, 13, 14). The latter process can be expected to be reflected in the incorporation pattern for ⁴⁵Ca during prolonged exposure to the sugar. It was therefore of interest to note that glucose was more efficient in promoting incorporation after the first 2 min when the β -cells had been preincubated for 120 min in the absence of glucose. This effect may be accounted for by enhanced sequestration of calcium due to previous depletion of the element in the glucose-sensitive intracellular stores.

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