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DUAL EFFECT OF GLUCOSE ON CYTOPLASMIC Ca^{2+} IN SINGLE PANCREATIC β-CELLS

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The cytoplasmic Ca^{2+} concentration (Ca^{2+}_i) was measured in single pancreatic β-cells from ob/ob-mice using the fluorescent indicator fura-2. Raising the glucose concentration from 3 to 20 mM resulted in 25 % initial lowering of Ca^{2+}_i, followed by 250 % rise above the basal level of 49 ± 3 nM. Tolbutamide (100 μM) was as effective as glucose in increasing Ca^{2+}_i, although its action was more rapid and not preceded by any reduction. The results support the concept that stimulated removal of Ca^{2+} from the cytoplasm is an essential part of the physiological glucose effect on the pancreatic β-cells.

In recent years considerable progress has been made in the understanding of how glucose affects the Ca^{2+} movements in the pancreatic β-cells. Evidence has been provided that glucose not only stimulates the entry of Ca^{2+} but also promotes the active sequestration of the ion in intracellular stores and its extrusion from the β-cells (1-3). Since the balance between these processes determines the cytoplasmic concentration of Ca^{2+} (Ca^{2+}_i), it is not unexpected that the sugar may under certain conditions also inhibit insulin release (2-4). The exploration of how glucose affects Ca^{2+}_i was considerably facilitated when fluorescent Ca^{2+} indicators became available in the form of membrane-permeable esters. Using the indicator quin-2 Rorsman et al (5) demonstrated with aggregates of cells prepared from ob/ob-mouse islets that glucose stimulation of insulin release is associated with a rise of cytoplasmic Ca^{2+}. However, when suppressing the entry of Ca^{2+} either by lowering its extracellular concentration or by adding a blocker of the potential-dependent Ca^{2+} channels the net effect of glucose was to lower Ca^{2+}_i.

With fura-2, a representative for the second generation of the fluorescent Ca^{2+} indicators, we have recently been able to make more detailed observations of how glucose affects Ca^{2+}_i in suspensions of cells from ob/ob-mouse islets (3,6). It was observed that the glucose-induced reduction of Ca^{2+}_i is not restricted to conditions of prevented entry of Ca^{2+} but actually represents a phenomenon preceding the increase obtained with the opening of the voltage-dependent channels. It might be

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argued that the opposite effects of glucose on cytoplasmic Ca^{2+} observed in suspensions of islet cells simply reflect the different reactions of the β and α_2-cells to the sugar stimulus (3). The present microfluorometric study excludes this possibility in providing direct evidence for a dual action of glucose on $Ca^{2+}{}_i$ of single pancreatic β-cells.

MATERIALS AND METHODS

Islets of Langerhans were isolated from the splenic part of the pancreas by collagenase digestion using 10 months old ob/ob-mice taken from a non-inbred colony (7). Single cells were prepared by shaking in a Ca^{2+}-deficient medium (8). After suspension in RPMI 1640 medium supplemented with 10 % fetal calf serum, 100 IU/ml penicillin, 100 µg/ml streptomycin and 60 µg/ml gentamycin the cells were allowed to attach during 8-48 hours to circular 25 mm cover glasses coated with fibronectin. Further experimental handling was performed with a basal medium physiologically balanced in cations with Cl^- as the sole anion (9). After rinsing, the cells were loaded with fura-2 during 40 min of incubation in 0.5 µM fura-2 tetraacetoxymethylester (Molecular Probes, Eugene, OR).

The cover glasses with the fura-2 loaded cells were rinsed and used as the bottom of an open chamber designed for microscopic work (10). Two injection needles were inserted on opposite sides of the silicone rubber wall of the chamber (volume 100 µl) and connected to a two-channel peristaltic pump allowing perifusion at a rate of 1.0 ml/min. The rapidity of medium exchange in the chamber is shown in Fig. 1. The chamber was placed on the stage of an inverted microscope (Nikon, Diaphot) within a climate box maintained at 37° C by an air-stream incubator. The microscope was equipped for epifluorescence microfluorometry with 100 W Hg light source, quartz illumination optics and a 100 X UV-fluorite objective. The excitation wavelength was altered manually by changing between 340 and 360 nm interference filters (half-

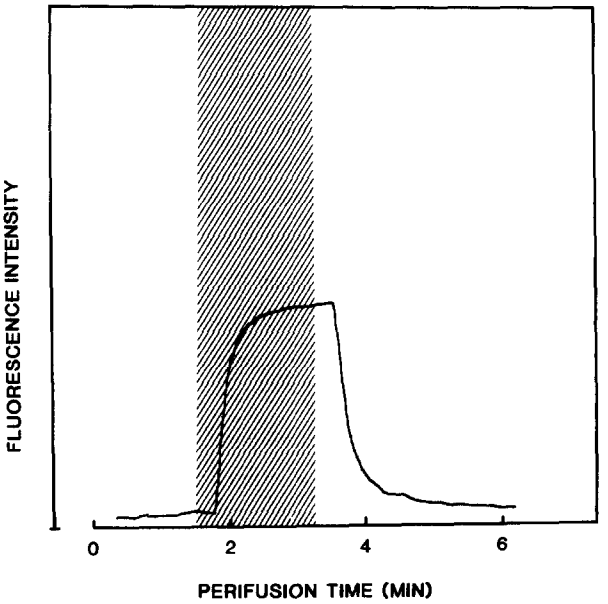

Fig. 1. Characterization of the perifusion system. The K^+ salt of fura-2 was included in a concentration of 1 µM in the perifusion medium during the period indicated by the shaded area. The fluorescence intensity of the perifusate was continously recorded in the perifusion chamber with the microscope fluorometer.

-bandwith 1 nm). Excitation intensity was adjusted with neutral density filters. Emitted light was collected through a 510 nm interference filter (half-bandwith 30 nm) and measured with a Nikon DC photometer P1. Single β-cells were centered within the cell-sized measuring field of the microfluorometer. The Ca^{2+}-dependent fluorescence excited at 340 nm was monitored continously with frequent checks of the fluorescence excited at 360 nm. Autofluorescence was negligible. The 340/360 nm fluorescence ratio was used for calculating Ca^{2+}_i with a previously described standard procedure (11) using a K_d for the Ca^{2+}-fura-2 complex of 231 nm (12). Results are presented as means ± S.E. for measurements on one cell in each animal. Statistical significances were judged from the differences between paired data using Student's t-test.

Selection of β-cells for the analyses was facilitated by the fact that these cells represent more than 90 % of the cells in the islets of the ob/ob-mice (7). The identification was based on the large size and low nuclear/cytoplasmic volume ratio compared to the α_1 and α_2-cells. Fluorescent staining by introducing 25 μM acridine orange into the perifusion medium containing 20 mM glucose (13) made it possible to check the cytological characterization at the end of the experiments.

RESULTS

The basal cytoplasmic Ca^{2+} concentration of single pancreatic β-cells exposed to 3 mM glucose and 1.28 mM Ca^{2+} was 49 ± 3 nm (n=14). A rise of the glucose concentration to 20 mM resulted in a dual effect on Ca^{2+}_i. Although the sensitivity to glucose varied considerably, the type of response obtained was always the same (Fig. 2). After an initial lowering of Ca^{2+}_i, there was an increase starting after 1-2 min and reaching levels considerably exceeding those in 3 mM glucose. The stimulatory phase was characterized by an introductory peak followed by stabilization of the elevated Ca^{2+}_i at

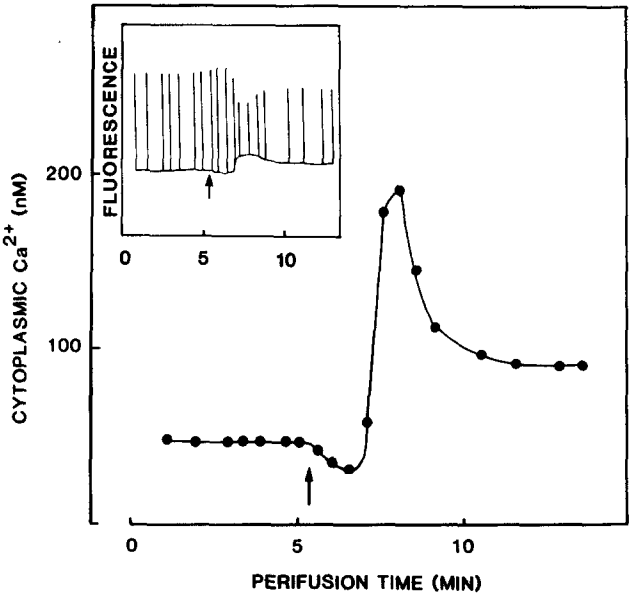

Fig. 2. Cytoplasmic Ca^{2+} activity in a mouse β-cell after raising glucose from 3 to 20 mM. The curve illustrates the dual action of glucose on Ca^{2+}_i in a single β-cell loaded with fura-2 after culture overnight. The inset shows the original fluorescence trace with excitation at 340 nm and frequent checks at 360 nm (peaks) used for the ratiometric calculation of Ca^{2+}_i. Arrows indicate the time at which the glucose concentration was increased.

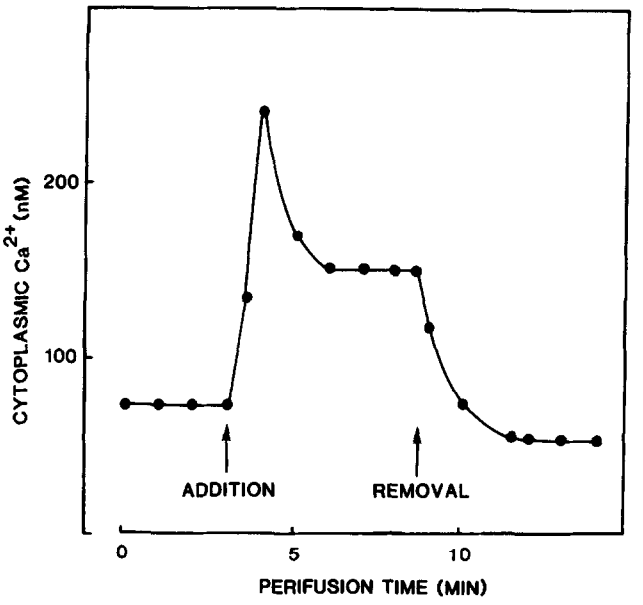

Fig. 3. Cytoplasmic Ca^{2+} activity in a mouse β-cell exposed to 100 μM tolbutamide in the presence of 3 mM glucose. The curve illustrates the rapid increase of Ca^{2+}_i obtained with tolbutamide in a single β-cell loaded with fura-2 after culture overnight.

about half the peak value. The signal-to-noise characteristics of the recording of the glucose effect is apparent from the inset of Fig. 2. Calculations of Ca^{2+}_i in single β-cells from 14 animals indicated an initial reduction of 12 ± 1 nM ($P < 0.001$) when the subsequent peak increase was 121 ± 12 nM ($P < 0.001$). 3-0-Methylglucose used as an osmotic control did not affect Ca^{2+}_i in 3 experiments (not shown).

Addition of 100 μM tolbutamide to a perifusion medium containing 3 mM glucose resulted in a rapid increase of Ca^{2+}_i without signs of preceding reduction (Fig. 3). The effect was similar to the stimulatory phase obtained with glucose in subsequently declining to about half the peak value. Measurements of the tolbutamide effect in 9 animals indicated a peak value corresponding to a stimulation of 133 ± 12 nM ($P < 0.001$). Occasionally β-cells responded to tolbutamide when not reacting to glucose.

DISCUSSION

The cytoplasmic Ca^{2+} concentrations of the resting β-cells were lower than those obtained when measuring the fura-2 fluorescence in suspensions of cells prepared from the ob/ob-mouse islets (3,6). Corresponding differences have been reported also for other types of cells when comparing analyses of single cells with suspensions (14-16). The resting Ca^{2+}_i in the β-cells was actually similar to that observed with the same technique in single glucagon-producing α_2-cells (12). It should be kept in mind that the absolute values for Ca^{2+}_i reported depend on calibration procedures

(17-19) based on the dubious assumption that the Ca^{2+}_i measured is evenly distributed and confined to the cytoplasm.

The results establish that glucose has a dual action on the cytoplasmic Ca^{2+} activity of single pancreatic β-cells. Although such observations may only seem confirmatory (3,6), it is actually the first unequivocal demonstration that both the glucose-induced lowering of Ca^{2+}_i and the subsequent increase take place in the same cell. It became particularly important to clarify this matter when it was found that glucose lowers Ca^{2+}_i in the pancreatic α_2-cells (3,12). The β-cells are exceptional in also being depolarized by the sugar, which results in the subsequent increase of Ca^{2+}_i after opening of the voltage-dependent Ca^{2+} channels. The glucose-induced lowering of Ca^{2+}_i was relatively small compared to the following increase. Accordingly, the detection of the initial reduction requires a high signal-to-noise ratio in the measurements. It is therefore easy to understand why several studies of non-tumour islet cells (17,20,21) have been able to demonstrate only the stimulatory phase of the glucose action.

Single β-cells can be expected to differ in some aspects from those present in their natural environment within the islets. Studies of purified fractions of β-cells have indicated that a reduced insulin secretory response to glucose (22,23) is not accounted for by impaired metabolism of the sugar (24) but is at least in part due to lowering of cyclic AMP after elimination of the glucagon source (23). Cyclic AMP promotion of the secretory process may to some extent reflect a potentiation of the glucose-stimulated entry of Ca^{2+}, but it is essentially the result of increased sensitivity of the secretory machinery to the Ca^{2+} signal (2-4). In support for the conservation of the fundamental glucose effects on the Ca^{2+} handling in isolated β-cells, the sugar has been found to stimulate the net uptake of ^{45}Ca in purified suspensions (25). Moreover, electrophysiological patch clamp studies have indicated that exposure to glucose induces electrical activity with opening of voltage-dependent Ca^{2+} channels also in single β-cells (26).

It was of interest to compare the glucose action on Ca^{2+}_i with that obtained when depolarizing the β-cells with tolbutamide. This sulfonylurea has similar characteristics to glucose in reducing the K^+ permeability of β-cells but not of α_2-cells (3). In analogy to what has been reported for insulin-releasing tumour cells (27), the exposure to tolbutamide resulted in a prompt increase of Ca^{2+}_i in the single β-cell. The sulfonylurea-induced increase of Ca^{2+}_i was more rapid than the corresponding effect of glucose and not preceded by an initial reduction. The observed differences in the time required to raise Ca^{2+}_i correspond to the initiation of insulin release by glucose and sulfonylureas (28).

The glucose-induced increase of Ca^{2+}_i was always preceded by an initial lowering. However, it was also apparent from the present studies that the glucose effect varied considerably and that some cells failed to respond to glucose although being sensitive to tolbutamide. This observation raises the question of whether the glucose recog-

nition mechanism is particularly vulnerable to the procedures of isolation and culture of the β-cells. It is also possible that there may exist functionally different subpopulations of β-cells. Indeed, previous studies of single β-cells have revealed a similar heterogenity for other effects of glucose, suggesting that the pancreatic islets normally contain some β-cells, which are unresponsive to the sugar (29).

With the observation that exposure to glucose results in a temporary reduction of Ca^{2+}_i it is possible to explain why the sugar has a similar effect on the efflux of radioactivity from islets preloaded with ^{45}Ca (1-3). In both cases the glucose--induced lowering will be sustained when preventing the increase of Ca^{2+} influx ob-ability of glucose to stimulate the removal of Ca^{2+} from the cytoplasm requires previous depletion of ATP and calcium (21,30). However, the present study provides ample support that the phenomenon has physiological significance in demonstrating that there is a lowering of Ca^{2+}_i also when raising the glucose concentration from 3 mM in the presence of a normal concentration of extracellular Ca^{2+}.

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