Dual effects of glucose on the cytosolic Ca^{2+} activity of mouse pancreatic β-cells

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The cytosolic Ca^{2+} activity of mouse pancreatic β-cells was studied with the intracellular fluorescent indicator quin2. When the extracellular Ca^{2+} concentration was 1.20 mM, the basal cytosolic Ca^{2+} activity was 162 ± 9 nM. Stimulation with 20 mM glucose increased this Ca^{2+} activity by 40%. In the presence of only 0.20 mM Ca^{2+} or after the addition of the voltage-dependent Ca^{2+}-channel blocker D-600, glucose had an opposite and more prompt effect in reducing cytosolic Ca^{2+} by about 15%. It is concluded that an early result of glucose exposure is a lowering of the cytosolic Ca^{2+} activity and that this effect tends to be masked by a subsequent increase of the Ca^{2+} activity due to influx of Ca^{2+} through the voltage-dependent Ca^{2+} channels.

Cytosolic Ca^{2+} *Quin2* *Insulin secretion* *Glucose* *Voltage-dependent Ca^{2+}-channel*

1. INTRODUCTION

Glucose-stimulated insulin release is supposed to be initiated by an increased Ca^{2+} activity in the β-cell cytosol. This belief is based on indirect evidence, since cytosolic Ca^{2+} has never been determined in normal pancreatic β-cells. The requirements for large amounts of material have restricted measurements with the intracellular Ca^{2+} indicator quin2 to the clonal insulin-producing RINm5F cells [1,2]. A major disadvantage with these cells is that they do not respond to glucose, the main physiological stimulus for insulin secretion. Nevertheless, it was possible to make two principally important observations. Firstly, stimulation of insulin secretion by K^+ depolarization was associated with an increased cytosolic Ca^{2+} activity [1,2]. Secondly, it was shown that there are mechanisms by which glucose can lower cytosolic Ca^{2+} [1]. We have now measured the Ca^{2+} activity also in pancreatic β-cells by taking advantage of the obese-hyperglycemic mouse. In this syndrome there is a considerable increase of the total β-cell mass [3], and in animals with the present genetic background the β-cells respond normally to glucose and other modulators of insulin secretion [4]. By showing that glucose exerts a dual action on the cytosolic Ca^{2+} activity of the β-cells it was possible to reinforce previous arguments [1,5–8] that glucose-stimulated insulin release reflects a balance between increased entry of Ca^{2+} and the sequestration of the cation in intracellular stores.

2. MATERIALS AND METHODS

Adult non-inbred obese-hyperglycaemic mice of both sexes were taken from a local colony [3] and starved overnight. A collagenase technique was used to isolate 500–1000 pancreatic islets rich in β-cells. An islet cell suspension was prepared and washed essentially as in [9]. The cells were resuspended in 25 ml RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, 200 μg/ml gentamycin and 100 μg/ml streptomycin (Flow). This suspension was incubated for 24 h at 37°C in a 75-cm^2 Nunclon flask (Nunc) in an atmosphere of 5% CO_2 in air. To prevent cell attachment, the flasks were shaken gently. The cells were loaded with quin2 by

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adding 50 μM quin2 tetraacetoxymethyl ester (Calbiochem-Behring) as a 50 mM solution in dimethylsulphoxide (DMSO). After 60–75 min in the culture incubator the cells were spun down at 50 × g for 2 min. The cells were resuspended in 10 ml Hepes buffer (pH 7.4, 37°C), physiologically balanced in cations with Cl^- as the sole anion [10] followed by another centrifugation. Finally the cells were suspended in 1.25 ml Hepes buffer containing 1.20 mM Ca^{2+}. The cell suspension was incubated with constant stirring at 37°C in a 1-cm cuvette placed in a spectrofluorometer with excitation and emission wavelengths set at 339 and 492 nm, respectively. To avoid dilution of the suspension, test substances were added as concentrated solutions in water or DMSO (D-600). When the background fluorescence from cells lacking quin2 was studied in separate experiments, the introduction of test substances had no measurable effect. The intracellular Ca^{2+} activities were calculated [11] after the calibration procedure in [12] assuming a K_d for the Ca–quin2 complex of 115 nM [11]. Account was taken for fading of the quin2 fluorescence and release of the indicator from cells during the experiments [1]. Statistical significances were calculated from paired test and control data using Student's t-test.

3. RESULTS

Fig.1 shows fluorescence recordings from experiments where the effects of glucose, K^+ and D-600 were studied. The addition of the sugar increased the quin2 fluorescence from cells incubated in the presence of 1.20 mM Ca^{2+} and the fluorescence was further enhanced by K^+ depolarization (fig.1A). These effects were both reversed by D-600. Indeed, in the presence of this

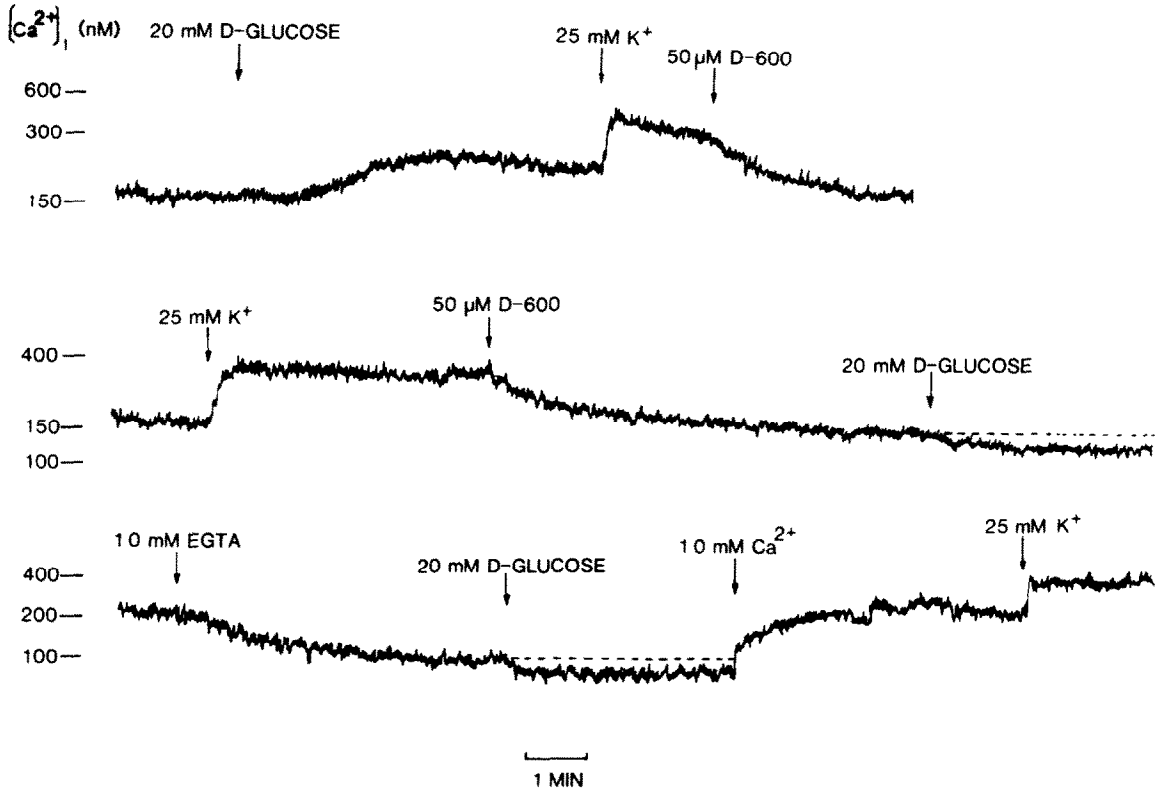

Fig.1. Fluorescence traces (339/492 nm) from mouse pancreatic β-cells loaded with quin2. At the beginning of the 3 experiments shown, the cuvette contained β-cells suspended in 1.25 ml medium with 1.20 mM Ca^{2+} (2 mg protein/ml). Approximate cytosolic Ca^{2+} activities (nM) are indicated as well as additions of glucose, K^+, D-600, EGTA and Ca^{2+}. (---) Linear extrapolations of the fluorescence signals.

voltage-dependent Ca^{2+}-channel blocker glucose suppressed the quin2 fluorescence (fig.1B). A glucose reduction was observed also after lowering the extracellular concentration of Ca^{2+} to 0.20 mM by addition of EGTA (fig.1C).

Calculations of the glucose effect on the cytosolic Ca^{2+} activity are shown in fig.2 which is based on experiments like those presented in fig.1. When the extracellular Ca^{2+} concentration was 1.20 mM, the basal cytosolic Ca^{2+} activity averaged 162 ± 9 nM (mean value ± SE, $n = 16$). After a delay of 1 min the addition of 20 mM glucose resulted in an increasing cytosolic Ca^{2+} activity

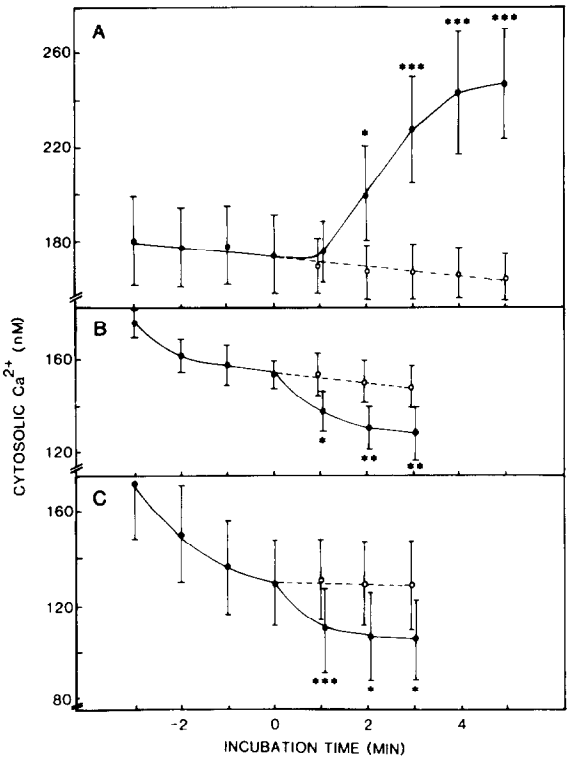

Fig.2. Effects of glucose on the cytosolic Ca^{2+} activity of mouse pancreatic β-cells. At 0 min 20 mM glucose was added to medium containing 1.20 mM Ca^{2+} (A), 1.20 mM Ca^{2+} and 50 μM D-600 (B) or 0.20 mM Ca^{2+} (C). The Ca^{2+} activities (nM) were calculated from original fluorescence traces like those in fig.1. (○) Ca^{2+} activities expected without modification of the medium. The validity of calculating these values from linear extrapolations of the fluorescence signal in each experiment was ascertained separately. Results are given as means ± SE of 11 (A), 5 (B) and 4 (C) experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

reaching a 40% higher level within 5 min (fig.2A). In the presence of D-600, glucose had a more prompt effect and there was a 15% reduction of cytosolic Ca^{2+} (fig.2B). When the extracellular Ca^{2+} concentration was lowered to 0.20 mM the basal cytosolic Ca^{2+} activity decreased by 44 ± 6% ($p < 0.001$). Also in this case glucose promptly reduced cytosolic Ca^{2+} by about 15% (fig.2C).

4. DISCUSSION

The present study is the first demonstration by direct measurements that exposure of β-cells to glucose results in an enhanced cytosolic Ca^{2+} activity. Apparently the increase was due to influx of Ca^{2+} through voltage-dependent Ca^{2+} channels, since the channel-blocking agent D-600 or a reduction of the extracellular Ca^{2+} concentration prevented this effect of glucose. It was consequently possible to confirm previous conclusions based on indirect evidence from several laboratories [5,13–15]. Nevertheless, it was important to demonstrate that the average cytosolic Ca^{2+} activity was enhanced, since it has also been suggested that glucose-stimulated insulin release is associated with a pronounced reduction of the cytosolic Ca^{2+} activity [16,17].

The initial Ca^{2+} handling after exposure to glucose has been the subject of even more controversy. The first phase of insulin release has been proposed to result from mobilization of intracellular Ca^{2+} [15]. It has also been suggested that glucose raises cytosolic Ca^{2+} by inhibiting the active extrusion of the ion by Na^+/Ca^{2+} countertransport [14,15,18]. Even if such a mechanism would appear to explain how the sugar rapidly inhibits ^{45}Ca efflux from β-cells, this inhibition may also reflect an initial lowering of cytosolic Ca^{2+} by stimulated sequestration of the cation in intracellular stores [5,6]. The latter alternative has recently received considerable support. A major argument against Na^+/Ca^{2+} countertransport as a site of action for the sugar is that glucose inhibits ^{45}Ca efflux also after complete removal of extracellular Na^+ [19]. In support for the idea that glucose stimulates the intracellular sequestration of Ca^{2+} the sugar has been found to promote a depolarization-independent net uptake of the cation by RINm5F cells exposed to μM concentrations of extracellular Ca^{2+} [7]. Indeed, it has been

possible to demonstrate with the quin2 technique that glucose can lower cytosolic Ca^{2+} in RINm5F cells [1]. Up to now the most forceful argument for a glucose-induced sequestration of Ca^{2+} in normal β-cells, is that under certain conditions glucose can inhibit insulin release [8]. Moreover, this sugar has been reported to decrease the activity of a Ca^{2+}-dependent protein kinase in pancreatic islets incubated in the absence of extracellular Ca^{2+} [20].

The glucose-induced reduction of cytosolic Ca^{2+} became evident when the entry of Ca^{2+} into the β-cells was decreased. This is not surprising since it is known that such an experimental situation uncovers the inhibitory effect of the sugar on ^{45}Ca efflux [21]. Also, the dynamics of the changes in the cytosolic Ca^{2+} activity parallel those of ^{45}Ca efflux, the glucose-induced reduction being prompt in comparison with the increase of the Ca^{2+} activity. In some of the experiments performed in the presence of 1.20 mM Ca^{2+} the rise of cytosolic Ca^{2+} was preceded by a decreased activity of the ion during the first min after exposure to the sugar. The notion that entry of Ca^{2+} masks the effect of the intracellular Ca^{2+} buffering pin-points an obstacle in the measurements of cytosolic Ca^{2+}. It should be kept in mind that the fluorescence signal represents an average and that the ion activity probably varies considerably within the cytosol [22]. Moreover, the average fluorescence signal may underestimate the mean Ca^{2+} activity, since there is no linear relationship between the two parameters within the concentration range of interest. Other problems are related to the relatively high concentrations of intracellular quin2 which have to be used. Rapid Ca^{2+} transients, like those evoked by K^+, may be distorted by binding of the ion to the indicator [1,23]. Also, the calcium-binding capacity of the cytosol may be significantly perturbed [23]. Even if the quantitative data should be interpreted with caution, the basal steady-state Ca^{2+} activity of the β-cells was similar to that obtained with the quin2 technique in other cells [1,2,11,12,23]. The basal Ca^{2+} activity is fairly close to the threshold for Ca^{2+}-stimulated insulin release from β-cells made permeable to the cation [24].

The present study has demonstrated that an early result of glucose exposure is a lowering of the cytosolic Ca^{2+} activity; an effect which tends to be masked by a subsequent increase of the Ca^{2+} activity due to influx of Ca^{2+} through the voltage-dependent channels. Since the K^+ conductance of the β-cell membrane appears essentially to be under the control of cytosolic Ca^{2+} [16,17], the reduction of the calcium activity may be an initiating factor in the depolarizing effect of glucose. In contradiction to the stimulatory component in the action of glucose, the inhibitory one can be expected to become less pronounced with time due to a limited capacity for calcium sequestration. The concept of the dual glucose action on insulin release may consequently explain as yet unexplained phenomena such as the appearance of a slowly increasing second phase and the fact that the secretory response is improved after priming with the sugar [5,8].

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