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Effects of Glucose on the Calcium Content of Intact β-cells and Cellular Organelles

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INTRODUCTION

The calcium ion has a fundamental role in insulin secretion. The presence of extracellular calcium is not only a prerequisite for the secretory response to glucose and other stimulators of insulin secretion (8, 11, 12), but it is also possible to initiate the secretory process in a glucose-free medium by merely raising the Ca^{2+} concentration (3, 9). It is therefore generally assumed that insulin secretagogues stimulate the net uptake of calcium into the pancreatic β-cell. Indirect support for this idea is obtained from studies with ^{45}Ca. Glucose stimulates the net uptake of ^{45}Ca into intact β-cells (4, 14, 18, 21) and the in situ uptake into mitochondria and secretory granules (6, 7, 17). Glucose also inhibits ^{45}Ca efflux from preloaded β-cells (5, 16). These effects of glucose on the β-cells can be reproduced in islets believed to be in isotopic equilibrium (20). However, so far there has been no convincing demonstration of glucose effects on the calcium content of the β-cells by direct methods. In the present study this was attempted by recording the effects of glucose on the total calcium content of intact β-cells and isolated organelles as measured by graphite-furnace atomic absorption spectroscopy.

METHODS

Experiments were performed with collagenase-isolated pancreatic islets from ob/ob-mice starved overnight or for 3 days. Each experimental group consisted of 70-80 pancreatic islets which were incubated for 120 min in a modified Krebs-Ringer medium (8) containing 1.28 mM $^{40}Ca^{2+}$ with or without ^{45}Ca supplementation or 2.56 mM $^{40}Ca^{2+}$ (intracellular Ca in Fig. 1). Glucose (1 or 20 mM) was also added to the medium. In one series of experiments (intracellular Ca in Fig. 1) this incubation was followed by a lanthanum wash procedure to remove external calcium (10). Subcellular fractions of the islets were prepared by differential centrifugation under conditions minimizing calcium redistribution (17). Graphite-furnace atomic absorption spectroscopy was employed for measurements of ^{40}Ca (2) in islet homogenates and subcellular islet fractions. When using tracer techniques, the radioactivity was expressed in terms of mmole ^{45}Ca

assuming the same specific activity as used in the media for loading the islets. The contents of ^{40}Ca or ^{45}Ca were always related to the protein of the sample. The results shown in the figures are presented as mean values ± SEM of 5 to 9 experiments.

RESULTS AND DISCUSSION

The stimulatory effect of glucose on ^{45}Ca uptake into pancreatic β-cells is well documented (4, 14, 18, 21). This phenomenon can partly be explained by an enhanced calcium-calcium exchange (1, 15). A glucose effect on the net content of β-cell calcium should therefore be expected to be of less magnitude than that obtained with the tracer technique. Fig 1 shows measurements of both radioactive and total calcium in homogenates from islets exposed to a low or a high glucose concentration. Glucose increased the ^{45}Ca uptake (*P<0.001), but no statistically significant effect was attained when measuring total calcium in similarly treated islets. There was no effect of glucose on total calcium even after prolonged starvation, a condition associated with an augmented ^{45}Ca uptake in response to glucose (13). It is obvious when comparing the measurements of ^{45}Ca and ^{40}Ca that the high background of ^{40}Ca makes the detection of a small glucose effect difficult. Measures were therefore taken to reduce this background. In another series of experiments intracellular calcium was measured after removal of external calcium by lanthanum washing. However, also this procedure proved insufficient for the demonstration of a glucose effect.

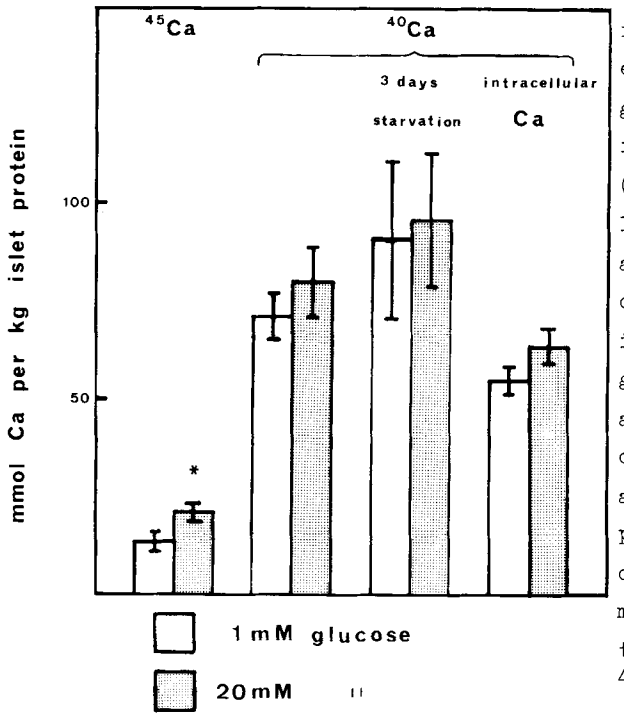

Fig. 1. Effects of glucose on the calcium content of β-cell rich pancreatic islets.

The glucose stimulation of ^{45}Ca uptake into intact β-cells can in part be explained by an enhanced incorporation into mitochondria and secretory granules (6, 7, 17). Attention should therefore be paid to how the organelles contribute to the islet content of calcium. Detection of a glucose effect on the subcell-

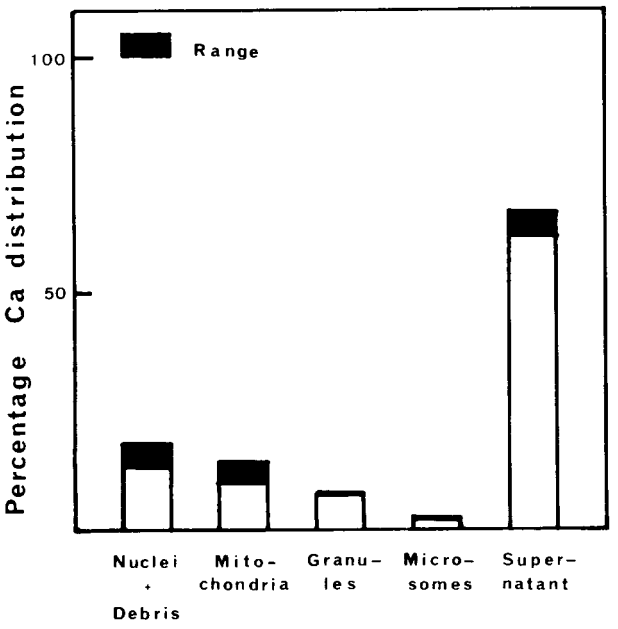

Fig. 2. Percentage distribution of β-cell calcium among the subcellular fractions.

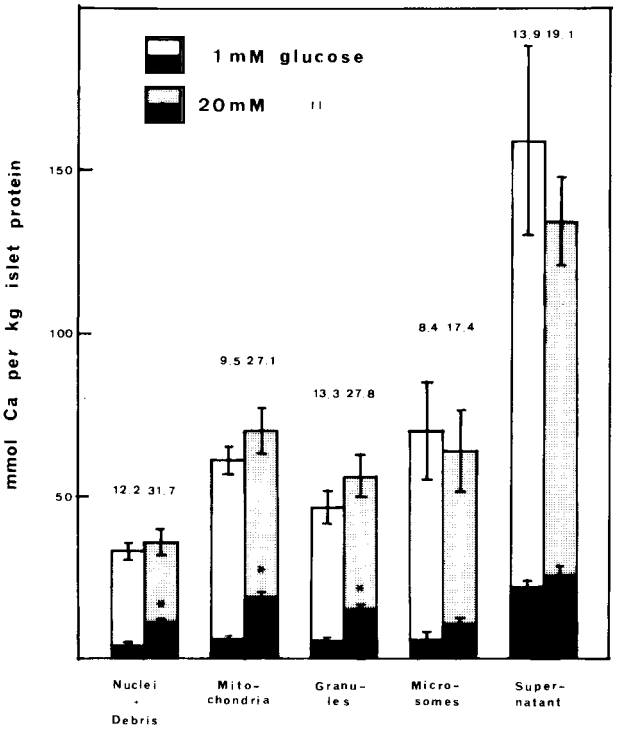

Fig. 3. Effects of glucose on the content of radioactive (black bars) and total calcium (entire bars) of β-cell fractions isolated from mice starved overnight. The figures above each bar denote the percentage of total calcium accounted for by ^{45}Ca.

ular fractions might also be facilitated by a reduction of the background through elimination of "physiologically inert" calcium. Fig. 2 shows that only a small proportion of the total β-cell calcium is recovered from the sedimentable fractions. It is difficult to assess the origin of the calcium in the supernatant. Some of it can be expected to be released from organelles disrupted during homogenization. Calcium bound superficially to cellular organelles would also be recovered from the supernatant by EDTA chelation. Furthermore, the possibility should not be excluded that the cytoplasm may contain substantial amounts of calcium despite a low concentration of ionized Ca^{2+}.

Fig. 3 presents measurements of both total and ^{45}Ca calcium in the β-cell fractions. Glucose stimulated ^{45}Ca uptake into nuclei + cell debris, mitochondria and secretory granules (*$P<0.001$) but had no significant effect on either microsomes, supernatant or on total calcium in any of the

fractions. There was a marked increase in the proportion of calcium accounted for by ^{45}Ca in the glucose stimulated β-cells, and this effect was particularly prominent in the first 3 fractions.

In the mouse, the effectiveness of glucose to promote incorporation of ^{45}Ca into the β-cell increases linearly with time of starvation for at least 3 days (13). However, even after such a long period of starvation it was not possible to demonstrate statistically significant effects of glucose when measuring total calcium in the subcellular fractions (Fig. 4).

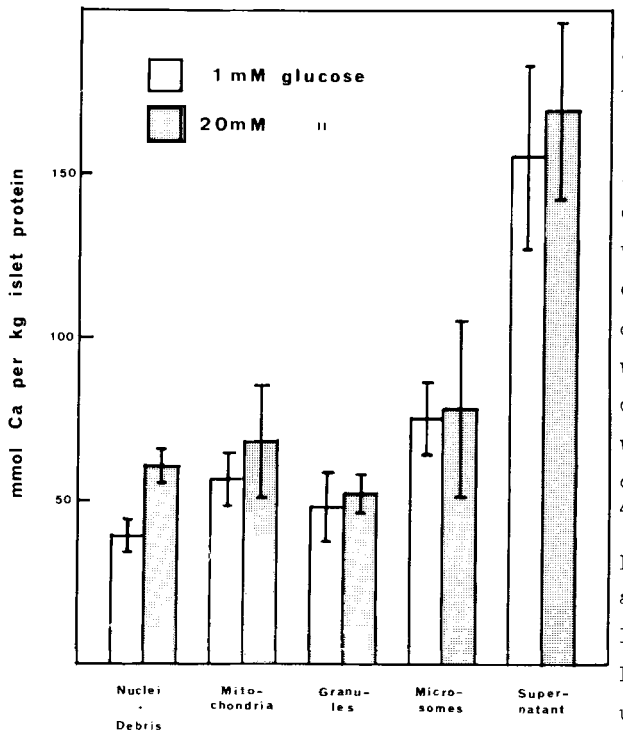

Fig. 4. Effects of glucose on the content of total calcium of β-cell fractions isolated from mice starved for 3 days.

With the present experimental approach it was possible to obtain data about the subcellular distribution of calcium in the β-cells. It is therefore interesting to compare the organelle content of calcium with present and earlier data on ^{45}Ca (6). The percentage distributions of ^{40}Ca and ^{45}Ca were virtually identical provided that the loading with ^{45}Ca was performed at a low glucose concentration. The fact that ^{45}Ca accounted for a similar proportion of total calcium in all fractions from low glucose loaded islets is evident from Fig. 3. In contrast to the uniform labelling of different calcium pools at the low glucose concentration (Fig. 3), there was a preferential stimulation of ^{45}Ca incorporation into mitochondria and secretory granules in the presence of 20 mM glucose. The latter observation is consistent with an effect of glucose also on the intracellular handling of Ca^{2+}.

In chromaffin cells, the percentage distribution of calcium is different (19). The ratio secretory granules/supernatant is about 4 as compared to 0.12 in the β-cells. Although the differences are less striking when expressing the data per unit protein the β-cell supernatant still contains much more calcium. It is difficult to assess to what extent these dissimilarities reflect real differences in calcium distribution. Factors which may distort the results

include differences in protein distribution, organelle fragility and experimental procedures.

In the present study it has not been possible to demonstrate statistically significant effects of glucose on the total islet content of calcium or on the calcium content of the subcellular fractions. This is not surprising since the magnitude of the glucose effect on ^{45}Ca uptake is small in relation to the total islet content of calcium. Furthermore, the ^{45}Ca uptake represents at least in part a calcium/calcium exchange. In each case when glucose stimulated ^{45}Ca incorporation there was also a higher mean content of total calcium. It is therefore likely that glucose not only stimulates the uptake of ^{45}Ca but also increases the net uptake of calcium. Buffering of this calcium by mitochondria and secretory granules might represent a mechanism for regulation and termination of the secretory response.

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