Glucose-stimulated ^{45}Ca Uptake in Isolated Rat Islets

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SUMMARY

Net ^{45}Ca uptake (in excess of the extracellular ^{3}H-sucrose space) and insulin release were measured under low (2 mM) and high (20 mM) glucose conditions in collagenase-isolated rat islets. ^{45}Ca uptake curves were mathematically fitted and subjected to compartmental analysis.

Within the first 60 seconds after addition of trace ^{45}Ca, islets showed a similar rapid uptake of ^{45}Ca regardless of the glucose concentration or length of time of prior exposure to glucose. Net ^{45}Ca uptake continued to increase for 30-60 minutes, and the islets in high glucose showed approximately twofold greater maximum uptake than islets in low glucose. Islets *pre*incubated in low glucose and then incubated in high glucose showed a 5-15-minute delay in net ^{45}Ca uptake as compared with islets that had been preincubated in high glucose.

Insulin release was detectable by 10 minutes of incubation with high glucose.

Mathematical modeling of the low- and high-glucose net ^{45}Ca uptake curves suggests that there are at least two calcium "compartments" within the β-cell. One compartment is small, rapidly filled, and insensitive to glucose, while the other, larger compartment, is slowly filled and fills to a much greater extent in the presence of high glucose. A major proportion of the glucose-stimulated uptake is at the level of influx. DIABETES *27*:365-69, April, 1978.

Calcium must be present for the normal stimulation of insulin release by glucose, but the exact interactions of the calcium ions and glucose are unclear. Elucidation of the movements of calcium during glucose-stimulated insulin release may aid in the understanding of stimulus-secretion coupling in the β-cell.

Several authors have shown that glucose increases the rate of net uptake and maximum level of ^{45}Ca in isolated pancreatic islets.[1-4] This is supported by morphologic studies showing that glucose induces extensive Ca deposits within the "halos" of stimulated β-cells.[5-7] At least part of the net uptake of calcium may be due to an inhibition of ^{45}Ca efflux.[8]

The present studies are an attempt to measure and to mathematically model the kinetics of β-cell ^{45}Ca uptake during glucose-stimulated insulin release.

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MATERIALS AND METHODS

Isolation of islets. Adult, fed, male Long-Evans rats, weighing 350-375 gm., were used in all experiments. Islets were isolated by the collagenase method of Lacy and Kostianovsky,[9] as previously described.[10] The basal medium used for rinsing, preincubation, and incubation of the islets was a modified Krebs-Ringer bicarbonate buffer (KRB) with the following composition in mM: bicarbonate 29, phosphate 1.5, sodium 140, chloride 111, potassium 5.8, magnesium 1.2, calcium 2.35, HEPES buffer 25, human serum albumin 0.3 per cent, and glucose 2 or 20 mM, pH 7.4.

Measurement of ^{45}Ca uptake and insulin release. All uptake experiments utilized a nonwash, double-isotope technique, slightly modified from that described by Henquin and Lambert.[11]

The incubations were carried out in polyethylene tubes (0.4-ml. volume, Beckman Instruments) into which had been layered (from the bottom up) 25 μl. 6 M urea, 150 μl. silicone oil (Versilube F-50, General Electric), and 40 μl. KRB. The experiment was car-

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ried out in a 37°, nonshaking water bath with continual gassing with 95 per cent O_2 + 5 per cent CO_2. A 60-minute "preincubation" period was begun when batches of 10 islets in 10 μl. KRB were added to the top, 40-μl. (KRB) layer, of each tube. The 0.5-60-minute "incubation" period then began when 100 μl. KRB, containing ^{45}Ca (0.5-1 μCi.) and ^{3}H-sucrose (4 μCi.), was added to the top (KRB and islet) layer of each tube. ($^{45}CaCl_2$ and [6,6'(n)-^{3}H] sucrose were obtained from ICN Pharmaceuticals, Irvine, CA., and Amersham/Searle, Arlington Heights, IL., respectively.) High glucose (20 mM) was added as indicated during either the preincubation and incubation periods or during the incubation period alone. The incubation in all experiments was ended by a 20-second centrifugation of the tubes (10,000×g, Microfuge, Beckman Instruments). This rapidly forced the islets through the oil layer into the urea layer below, while leaving most extracellular fluid behind. An aliquot of the supernatant (KRB) was diluted and frozen for later insulin assay with rat insulin standards.[12] The levels measured represented the cumulative insulin release during both preincubation and incubation periods. The bottom of the test tube, containing the islets in the urea layer and a small portion of the oil layer, was cut off and counted for ^{45}Ca and ^{3}H. Calcium uptake was measured as ^{45}Ca uptake in excess of the extracellular ^{3}H-sucrose space[4] from each experiment.

The islets' extracellular (^{3}H-sucrose) spaces reached maximal levels by one to two minutes. Then, from 3-60 minutes, more than 90 per cent of the sucrose spaces were in the 0.5-2.5 nl./islet range. However, excessive sucrose spaces (up to 12 nl./islet) and high net ^{45}Ca spaces in excess of the sucrose space (up to sixfold the mean 60-minute level) were seen in 20 per cent and 50 per cent of the islets incubated for 90 and 120 minutes, respectively. Assuming that the abnormal ^{3}H-sucrose and ^{45}Ca uptake represented deterioration and increased permeability of the islets' cell plasma membranes, only incubations up tc 60 minutes were used for mathematical analyses.

Mathematical curve analysis and statistical analysis. The net ^{45}Ca uptake curves were fit by a least-squares fitting program that weighs all points equally and a PDP digital computer. The two-tailed Student *t*-test was used for all other statistical evaluations.

RESULTS

The effects of length of incubation with ^{45}Ca and glucose concentration (2 and 20 mM) on net ^{45}Ca uptake, ^{3}H-sucrose space, and insulin release are shown in figure 1. The maximum sucrose space, about 2 nl./islet, was reached within one to two minutes after beginning incubation with the ^{3}H-sucrose. Glucose had no effect on the sucrose space.

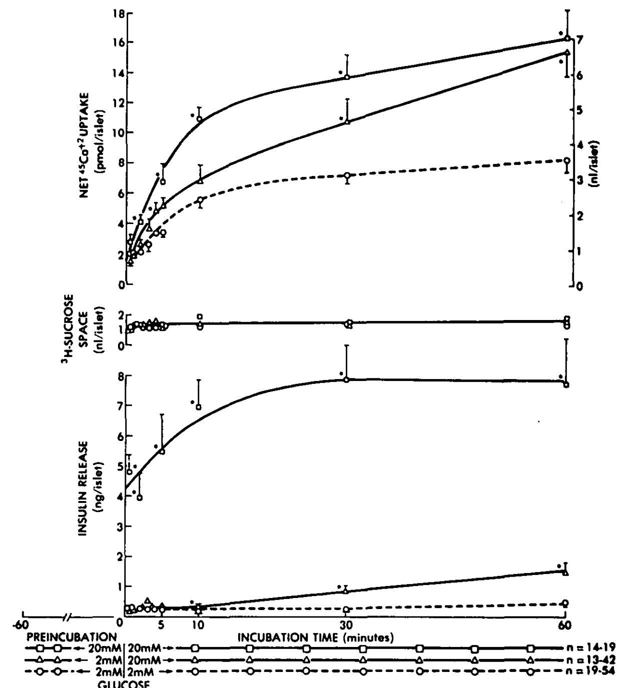

FIG. 1. Effect of glucose concentration and length of incubation on net ^{45}Ca uptake, ^{3}H-sucrose space, and insulin release from isolated rat islets. After 60-minute preincubation without isotopes (time −60 to 0), islets were incubated 0.5-60 minutes with $^{45}CaCl_2$ and ^{3}H-sucrose. Three procedures were studied: preincubation with 2 mM glucose followed by incubation with 2 mM; preincubation with 2 mM, then incubation with 20 mM; and preincubation with 20 mM, then incubation with 20 mM. "Net $^{45}Ca^{+2}$ uptake" refers to the net uptake of $^{45}Ca^{+2}$ in excess of the extracellular sucrose space, assuming the same specific activity as in the incubation medium. Each point represents the mean ± S.E.M. The stars indicate values from islets incubated in 20 mM glucose that are significantly different from the corresponding 2 mM-glucose-incubated islets (two-tailed Student-*t*, $p < 0.05$).

Islets maintained in 2 mM (low) glucose showed an initial (one-to-two-minute) rapid accumulation of added ^{45}Ca; uptake then approached a plateau at about 30 minutes. Islets that had been preincubated (time −60 to 0) in low glucose and then incubated from time 0 on in 20 mM (high) glucose, showed a similar initial accumulation of ^{45}Ca. However, net uptake became significantly different from the low-glucose islets at four to five minutes and continued to rise until 60 minutes. Islets that were both preincubated and incubated in high glucose showed the same

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initial accumulation of ^{45}Ca. Net uptake first became significantly different from the low-glucose islets at minute 2; net levels rose more quickly but then reached the same maximum level at 60 minutes as the islets preincubated in low glucose and then incubated in high glucose.

Insulin release remained low in those islets that had been both preincubated and incubated in low glucose. Islets that had been preincubated in low glucose and then incubated from time 0 on in high glucose showed significant glucose-stimulated insulin release at minute 10. Insulin levels were already elevated after 60-minute preincubation in high glucose and increased further during incubation with high glucose until about 30 minutes, at which time they appeared to reach a plateau.

The effect of length of incubation with high glucose on ^{45}Ca *influx* was measured in the following way: Islets were preincubated 60 minutes in low glucose, followed by incubations of 1-60 minutes in either low or high glucose. ^{45}Ca and ^{3}H-sucrose were added, and incubation was continued for one more minute (figure 2). Assuming that equilibration or efflux of ^{45}Ca within one minute is small, this technique would predominantly measure the effect of 1-60-minute incubation in high glucose on Ca *influx*. As seen in figure 2, no effect of prior incubation with high glucose was seen on the *one-minute* ^{45}Ca influx. These data suggest the presence of a small, glucose-insensitive calcium compartment within the β-cell.

Mathematical modeling was used to better understand the processes operative in net calcium uptake. The experimental data from figure 1 (2 mM → 2 mM glucose and 20 mM → 20 mM glucose) were fit simultaneously by least-squares by using a model that

TABLE 1

Glucose concentration (mM)	Equations describing net ^{45}Ca uptake curves
	Assuming glucose increases influx only:
2	pmol Ca^{+2}/islet = $1.81 + 6.08\,(1\text{-}e^{-0.081t})$
20	pmol Ca^{+2}/islet = $1.81 + 14.48\,(1\text{-}e^{-0.081t})$
	Assuming glucose decreases efflux only:
2	pmol Ca^{+2}/islet = $1.25 + 6.31\,(1\text{-}e^{-0.172t})$
20	pmol Ca^{+2}/islet = $1.25 + 15.30\,(1\text{-}e^{-0.071t})$

Where t = time in minutes.

1.81 and 1.25 = content of the small compartment when glucose is assumed to affect influx or efflux, respectively.

6.08 and 6.31 = content of the large compartment at "steady state" in the presence of only 2 mM glucose.

14.48 and 15.30 = content of the large compartment at "steady state" in the presence of 20 mM glucose.

assumes that there are two calcium "compartments" within the β-cell. One is a small (~ 1-2 pmol/islet), rapidly filling (t ½ < 15 seconds) compartment that is insensitive to glucose, and the other is a large (~ 14-15 pmol/islet), slowly filling (t ½ ~ 10 minutes) compartment that fills to a greater extent (2.4-fold) in the presence of high glucose. Table 1 and figure 3 show the results when glucose was assumed to cause a net ^{45}Ca uptake: (1) by increasing *influx* into the large, glucose-sensitive compartment, or (2) by decreasing *efflux* from that compartment. The best fit was obtained in case 1, in which an effect of glucose on influx was assumed.

DISCUSSION

This investigation was designed to study the kinetics of Ca uptake as it relates to glucose-stimulated

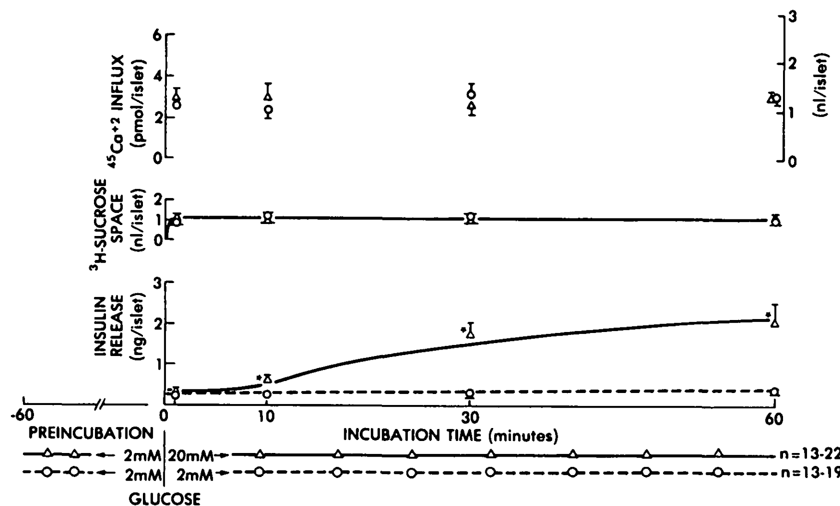

FIGURE 2

Effect of glucose concentration and length of incubation on "influx" of $^{45}Ca^{+2}$, ^{3}H-sucrose space, and insulin release from isolated rat islets. After 60-minute preincubation with only 2 mM glucose, islets were incubated for 1-60 minutes with 2 mM or 20 mM glucose and then for only one minute more with the medium supplemented with $^{45}CaCl_2$ and ^{3}H-sucrose. "$^{45}Ca^{+2}$ influx" refers to net uptake of $^{45}Ca^{+2}$ in excess of the extracellular sucrose space after only 60-second exposure to the $^{45}CaCl_2$ and ^{3}H-sucrose. Each point represents the mean ± S.E.M. The stars indicate significant differences (two-tailed Student-t, $p < 0.05$).

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insulin release. From analysis of the net ^{45}Ca uptake curves under low (2 mM) and high (20 mM) glucose conditions, it appears that ^{45}Ca accumulates within at least two different compartments within the β-cell. A small, rapidly filling compartment is unresponsive to glucose, while net uptake into the large compartment is more than doubled by the presence of high glucose. Our results and mathematical inferences strongly confirm those of Naber et al.,[4] who used isolated rat islets but a different experimental technique.

The batch-incubation technique used here measures accumulated insulin release and is not suitable, or sensitive enough, to measure a first spike of insulin release. In batch incubations such as these, it is also not possible to determine the exact time of onset of calcium influx into the glucose-sensitive, large compartment, since it is masked during the first minute or two by uptake into the small but rapidly filling glucose-insensitive compartment.

By extending the incubations as long as possible, we hoped to determine the total number of calcium compartments within the β-cell and the maximum calcium content of the islets at isotopic equilibrium. However, unexpectedly elevated net ^{45}Ca uptake levels and sucrose spaces[13] were seen in islets incubated for more than 60 minutes. This suggested a progressive, time-related islet deterioration or plasma membrane leak. Therefore, mathematical least-squares analysis was used to fit data only up to the 60-minute incubation, as shown.

For simplicity, it was assumed that isotopic equilibrium occurred at 60 minutes. However, this is only an approximation, since other ^{45}Ca-uptake studies in this laboratory suggest that islets are only 50-70 per cent labeled at 60 minutes and do not reach isotopic equilibrium until two to three hours' incubation (W. T. Imagawa and G. M. Grodsky, unpublished data). Because ^{45}Ca uptake probably was increasing slowly at 60 minutes in figure 1, it is likely that there is actually a "third" calcium compartment (or even more) within the β-cell. Thus, the formulae given in RESULTS somewhat incorrectly estimate the maximum size of the large compartment and ignore other possible compartments.

In the mathematical analyses the net ^{45}Ca uptake curves from figure 1 could not be described by a one-compartment model (single exponential) but two-compartment models were successful. Since the time constant for net uptake into the small compartment was too fast to measure accurately, the net ^{45}Ca uptake curves were equally well described by a model in

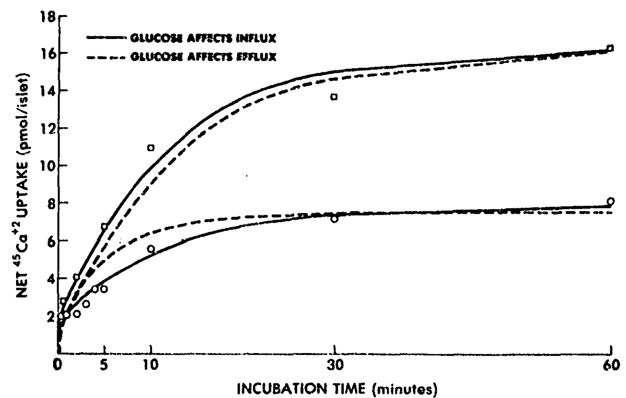

FIG. 3. Mathematical curve fitting of net $^{45}Ca^{+2}$ uptake curves, based on the assumptions that there are two parallel calcium compartments within the β-cell and that glucose increases the net uptake of ^{45}Ca into the large compartment solely by increasing influx or decreasing efflux. Experimental data (o and □) taken from figure 1. Formulae listed in table 1.

which the two calcium compartments are in series (^{45}Ca goes through the small compartment to enter the large), as by a model in which the calcium compartments are parallel (table 1 and figure 3). Therefore, the simpler, parallel configuration was assumed.

The existence of the small, rapidly filling, glucose-insensitive compartment was suggested by two findings: (1) all three net ^{45}Ca-uptake curves in figure 1 rose very steeply within the first 60 seconds, and no difference was seen between low and high glucose and, (2) previous exposure to high glucose for 1-60 minutes had no effect on the one-minute ^{45}Ca-uptake rate (figure 2). The small, glucose-insensitive compartment may represent some portion of the plasma membrane, whereas the large compartment may be some intracellular site.[14]

Since the small compartment was insensitive to glucose, glucose-stimulated uptake of ^{45}Ca may reflect an increased influx into the large compartment (2.4-fold), decreased efflux from that compartment, or a combination of both. Assuming that glucose affects only calcium influx gave a better fit than assuming that glucose affects only efflux (figure 3), and a combination of influx and efflux did not improve the degree of fit. Malaisse et al.[8] clearly showed that glucose does decrease ^{45}Ca efflux but did not quantify the effect. In our own studies,[15] high glucose decreased ^{45}Ca-efflux rates approximately 20 per cent. Therefore, the present data and those of Malaisse are consistent with a major effect of glucose on increasing ^{45}Ca influx into the large, glucose-sensitive compartment, but the data do not exclude the probability that glu-

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cose may exert some of its action by means of decreasing efflux.

Islets preincubated in low glucose showed a slower ^{45}Ca uptake during incubation in high glucose (2 → 20 mM glucose) than islets preincubated in high glucose (20 → 20 mM glucose). A reasonable mathematical fit could be obtained by assuming a 5-15-minute delay in the ability of high glucose to increase ^{45}Ca influx and might represent the time required for production or activation of a calcium pump or carrier.

In conclusion, these studies show that glucose causes a significant net uptake of ^{45}Ca by isolated rat islets. There are at least two, and possibly more, calcium "compartments" within the β-cell. A small compartment is rapidly filled with added ^{45}Ca and is unaffected by glucose. Net uptake into a large compartment is approximately doubled by glucose (20 mM)—either by increasing influx or by a combination of increased influx and decreased efflux. From the present data, it appears that high glucose increases net ^{45}Ca uptake into the larger compartment within two to four minutes of exposure. In addition, prior exposure to high glucose increases the rate at which this uptake reaches equilibrium.

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