Glucose-Stimulated ^{45}Calcium Efflux from Isolated Rat Pancreatic Islets

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ABSTRACT Kinetics of ^{45}Ca efflux and insulin release were studied in collagenase-isolated rat islets during 2-h perifusions with calcium-depleted (0.05 mM) bicarbonate-phosphate buffer containing 2.2 mM glucose. Addition of glucose (16.7 mM) suppressed ^{45}Ca efflux by 30%. Removal of glucose caused an "off response" of insulin release. The perifusion of a normal concentration of Ca (2.3 mM) greatly stimulated ^{45}Ca efflux, indicating Ca ↔ ^{45}Ca exchange. When Ca and glucose were superimposed, the effects on ^{45}Ca efflux and insulin release depended upon the order of presentation of the stimuli: when Ca was added to an ongoing 16.7-mM glucose perifusion, biphasic patterns of ^{45}Ca and insulin release were seen; when glucose was superimposed on a Ca perifusion, an inhibition of the Ca-stimulated ^{45}Ca efflux occurred, and a reduced but clearly biphasic insulin response was seen. The subsequent insulin off response after withdrawal of the glucose was also reduced.

Mathematical "peeling" of ^{45}Ca efflux curves from unstimulated islets suggests that there are at least two, and probably three, different intracellular Ca compartments (not including the extracellular sucrose space). At the beginning of perifusion, these three compartments (I, II, III) contain 25, 56, and 19% of the intracellular ^{45}Ca, and their rates of efflux are 6.7, 1.2, and 0.1 %/min, respectively. Glucose appears to suppress efflux from the largest compartment (II); Ca appears to exchange with ^{45}Ca from a more inert compartment (III). The relationship between insulin and ^{45}Ca release is not stoichiometric.

INTRODUCTION

The role of calcium (Ca) ion movements during glucose-stimulated insulin release from the pancreatic β-cell is unclear. Several studies (1–4) have shown that Ca is necessary for insulin secretion. In studies of ^{45}Ca efflux, Malaisse et al. (5), and later Bukowiecki and Freinkel (6), showed that an increased glucose load (with approximately 1 mM Ca), caused a short depression followed immediately by an impressive spike of ^{45}Ca efflux. This spike occurred about the time that the first phase of insulin release is usually seen (7). At a very low external Ca level with EGTA in the medium, glucose only depressed ^{45}Ca efflux (5). Glucose, in the presence of external Ca, causes an increased net uptake of ^{45}Ca (8–10). It is the free cytosolic Ca, or Ca in a small compartment, which is believed to be directly related to biphasic insulin release and the electrical phenomena which accompany it (11). Both decreased efflux and increased influx across the plasma membrane could lead to increasing this Ca. It, and insulin secretion, may also be increased by solubilization of plasma membrane-bound Ca or release from an intracellular organelle. Histochemical studies indicate that Ca deposits are found within the "halo" of the beta granule, near the plasma membrane, and in the mitochondrial matrix and endoplasmic reticulum of the β-cell (12–14). In the presence of a high level of glucose, the granules' stores and those on the inner side of the plasma membrane appear to increase while the mitochondrial and endoplasmic reticulum ones tend to dissipate. Thus, glucose-stimulated movements of Ca are complex and probably the summation of many different processes.

In the present studies with isolated perifused islets, the effect of glucose and Ca on the kinetics of ^{45}Ca efflux and insulin release were studied. Efflux during the absence of stimulation was also studied to attempt mathematical estimation of the number, size, and efflux rates of the intracellular Ca pools within the islets.

METHODS

Islets were isolated by the collagenase method of Lacy and Kostianovsky (15) from 350- to 375-g, fed, Long-Evans rats

A preliminary report of some of these data has appeared previously in 1977. *Clin. Res.* **25**: 103A. (Abstr.)

Received for publication 9 December 1977 and in revised form 20 April 1978.

and incubated at 37°C for 60 min in the presence of ^{45}Ca (100–150 μCi) in 0.5 ml of medium. The incubation medium was a bicarbonate-phosphate buffer (pH 7.4) from which all Ca except radioactive Ca was omitted; the total Ca concentration was approximately 0.3 mM. The buffer was otherwise composed of 29 mM bicarbonate, 1.5 mM phosphate, 140 mM sodium, 111 mM chloride, 58 mM potassium, 1.2 mM magnesium, 0.3% human serum albumin, and 16.7 mM glucose. Preliminary uptake experiments showed that islets reached 50–70% isotopic equilibrium after 60 min incubation with ^{45}Ca. Saturation occurred at 2.5–3 h. After the incubation, 150–200 islets were washed once and perifused for 120 min at a flow rate of 1.0 ml/min, during which ^{45}Ca efflux and insulin release were measured. The medium used for washing and perifusion of the islets was identical to the incubation medium, except that the isotope was omitted and the glucose concentration was lowered to 2.2 mM. The Ca concentration, as determined by atomic absorption spectrophotometry, was 0.05 mM ("calcium-depleted medium"). Most of this Ca was probably not free, but rather bound to the albumin in the medium. The perifusion technique used was that described by Lacy et al. (16). In some experiments after perifusion for 60 min, the glucose and Ca concentrations in the perifusate were brought to 16.7 and 2.3 mM, respectively; these reagents were dissolved in 0.9% saline solution and added by a Harvard infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.) at a rate of 0.05 ml/min. These stimuli were added in saline solution because it was not possible to dissolve the 20-fold concentration of Ca in a phosphate-containing buffer. The stimulation period was 45 min. In other experiments, glucose and Ca were infused concomitantly during the middle one-third of the stimulation period. From each collected sample, 0.4 ml was removed for liquid scintillation counting in Aquasol (New England Nuclear, Boston, Mass.). The remainder of the sample was saved for insulin determination by solid-phase, single-antibody radioimmunoassay (17). At the end of the perifusion, the filter that contained the islets and 0.4 ml perifusate was counted in Aquasol. All counts and insulin values were expressed per 200 islets. To establish the time required for extracellular washout of isotope, the extracellular space marker [6,6′(n)-^{3}H]sucrose (2 mCi) (ICN Pharmaceuticals, Inc., Cleveland, Ohio) was added to the incubation medium after 50 min (n = three experiments), and [^{3}H]sucrose washout was monitored. The [^{3}H]sucrose counts decreased to near background levels by 20 min (see Fig. 1). Therefore, in all the subsequent ^{45}Ca efflux perifusions, collections were begun after 20 min.

Analysis of the perifusion control curves was as follows. Each of six experiments, in which the effluent was collected from 1 to 120 min, was normalized to 100% at 20 min. The resulting values were then multiplied by the average counts per minute of all the curves at 20 min, giving a set of individual curves, each normalized to the average 20-min value. The mean of these normalized curves was then analyzed by simple peeling, with the BMD-X nonlinear, least-squares-fitting routine of the University of California (San Francisco) IBM 360-50 computer (IBM Corp., White Plains, N. Y.). First, a slow compartment (II) was fitted with the later points in the curve. After subtraction of this compartment, the fast compartment (I) was fitted. A third, much slower compartment (III) was calculated by subtracting the calculated number of counts remaining in compartments I and II from the average number of counts measured in the islets at the end of the experiments. The release rate from this third compartment was not resolvable within the practical perifusion period and therefore was assumed to be approximately zero.

In the top portion of Figs. 1 through 5, ^{45}Ca efflux (counts per minute/200 islets) is presented as a semilogarithmic plot. Displayed in this manner, efflux at a constant rate from a single compartment would appear as a straight line. To reduce the statistical variation among experiments, these data are also presented as the fractional efflux rate[1] (percent of islet content effluxed per minute, where islet content at time t is the sum of the counts remaining in the islets at the end of the 120-min perfusion plus the counts not yet effluxed during the period from t to 120 min). Estimated counts remaining in the islets at each time period are also shown. Data shown as mean±SEM.

Differences in specific activity of the incubation medium (100–150 μCi/0.5 ml) and(or) actual differences in uptake by different batches of islets led to variations in the absolute counts effluxed during individual experiments. This difference is most notable in the top of Fig. 5, where the counts per minute effluxed at any one time for the two experimental curves differs by about twofold. However, the fact that the efflux curves are parallel on a log scale indicates that their rates of efflux are identical. This is seen when the efflux was recalculated as fractional efflux (percent counts per minute; middle of Fig. 5). Therefore, differences in labeling do not invalidate the comparison of the rates of efflux and acute changes in the rate of efflux in response to a stimulus.

RESULTS

Fig. 1 shows the efflux of ^{45}Ca and [^{3}H]sucrose from perifused islets incubated for 60 and 10 min, respectively, with the isotopes. Counts per minute are reported on a logarithmic scale to show the kinetics of ^{45}Ca and [^{3}H]sucrose washout. The extracellular marker, [^{3}H]sucrose, was eliminated within 25 min. Calculated fractional efflux rate of ^{45}Ca was not constant throughout the 120-min experiment, but declined from 8 to 0.5 %/min. Throughout most of the efflux period, therefore, the total islet content of ^{45}Ca fell very slowly; release during any 1 min was only a fraction of the total ^{45}Ca in the islets. As expected, almost no insulin release occurred in the calcium-depleted medium.

Control for the subsequent experiments (Fig. 2) showed no effect of saline solution infusion on ^{45}Ca efflux or insulin release.

As seen in Fig. 3, and again in Fig. 4, by increasing the glucose concentration after 60 min from 2.2 to 16.7 mM in calcium-depleted medium we suppressed the efflux of ^{45}Ca by about 30% within 5 min. This effect was maintained throughout the 45-min period of glucose perifusion. When the glucose perifusion was stopped, efflux returned to normal (Fig. 3) and a marked overshoot of insulin release occurred (Fig. 4). The addition of 2.3 mM Ca during the middle one-third of the glucose perifusion caused biphasic insulin release and biphasic Ca efflux and potentiated the insulin overshoot when the glucose was removed.

Fig. 5 shows that, during the solo perifusion of 2.3 mM Ca, the rate of ^{45}Ca efflux increased for approximately 25 min and then remained constant at about twice the control level. When Ca perifusion was

[1] Essentially the same as Borle's Efflux Rate Coefficient (20).

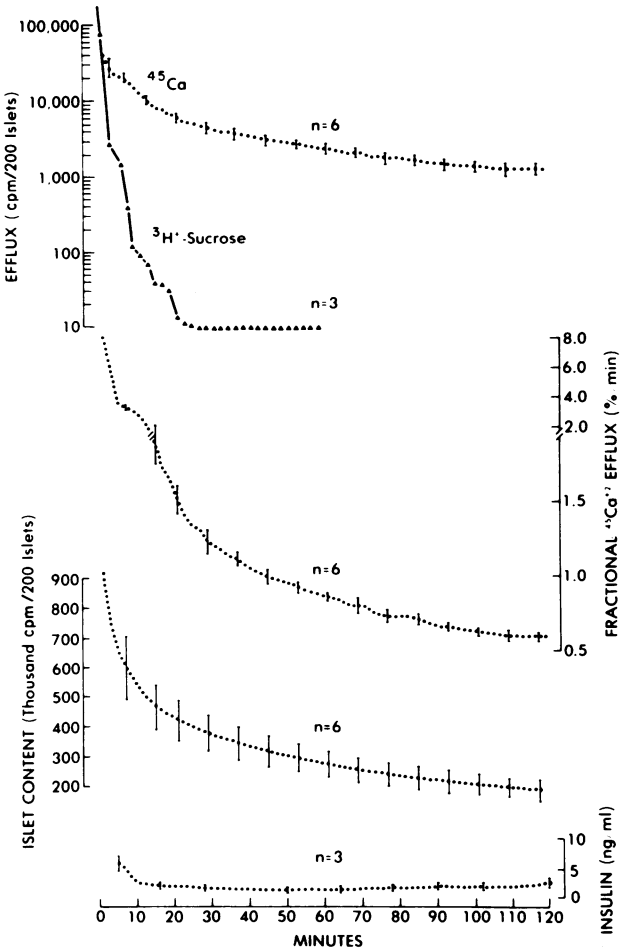

FIGURE 1 Efflux of ^{45}Ca from prelabeled rat islets. [^{3}H]Sucrose was used as a marker for the extracellular space. Basal perifusate contained 2.2 mM glucose and 0.05 mM Ca^{+2}. Fractional ^{45}Ca efflux was calculated at 2-min intervals as explained in Methods. Data shown as mean±SEM.

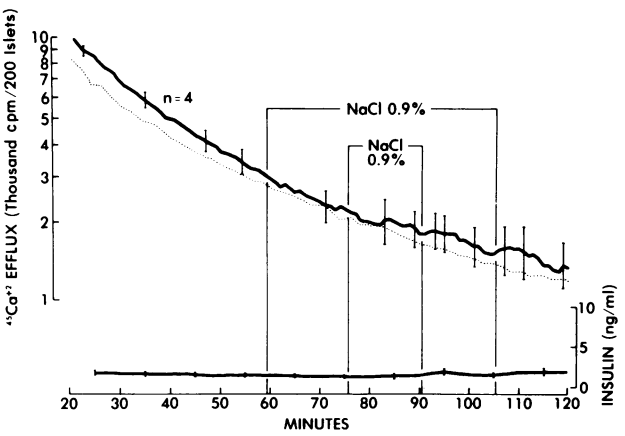

FIGURE 2 Lack of effect of saline solution infusion on efflux of ^{45}Ca from prelabeled rat islets. Basal perifusate contained 2.2 mM glucose and 0.05 mM Ca^{+2}. Data shown as mean ±SEM. Control experiments (n = 8) shown by dotted line.

stopped, ^{45}Ca efflux promptly fell toward the control level without overshoot. By raising the glucose concentration from 2.2 to 16.7 mM in the middle of the Ca perifusion we suppressed the ongoing ^{45}Ca efflux and caused biphasic insulin release. Changes in ^{45}Ca efflux and insulin release were opposite to each other at this time. The amount of insulin released was somewhat less[2] than in those experiments in which the order of addition of Ca and glucose was reversed (Fig. 4). Removal of glucose in experiments of either design was usually associated with an overshoot of insulin release and ^{45}Ca efflux (Figs. 4 and 5).

In both types of experiments, the observed efflux reflected only slight changes in the curves showing ^{45}Ca remaining in the islets. This indicated that islet ^{45}Ca content was an insensitive measure of the effect of glucose on Ca efflux.

Analysis of the kinetics of ^{45}Ca efflux during the control (unstimulated) experiments suggested that the efflux was from at least two (I and II), and probably a third (III), separate Ca compartments within the islets. These compartments and their rates of efflux are shown in Figs. 6 and 7. At the beginning of efflux, in medium containing 0.05 mM Ca and 2.2 mM glucose, the approximate distribution of ^{45}Ca in the compartments was: 25%(I), 56%(II), and 19%(III). After 60 min perifusion, compartment I was nearly empty (emptying rate 6.7 %/min); II was one-half empty (emptying rate 1.2 %/min); and compartment III was nearly unchanged (emptying rate 0.1 %/min). By increasing the glucose concentration to 16.7 mM we reduced efflux (Fig. 3)

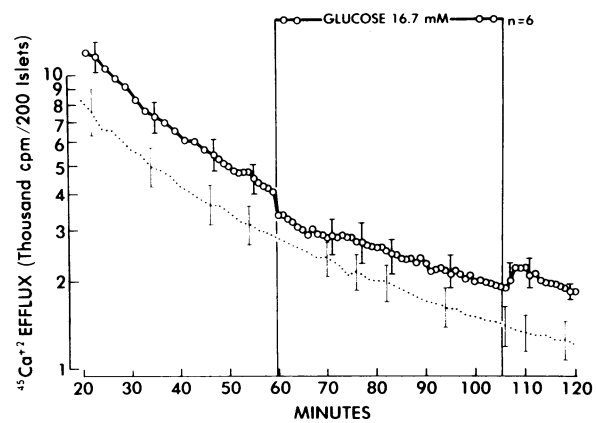

FIGURE 3 Effect of glucose (16.7 mM) on efflux of ^{45}Ca from prelabeled rat islets. Basal perifusate contained 2.2 mM glucose and 0.05 mM Ca. Data shown as mean±SEM. Control experiments (n = 8) shown by dotted line. (These data taken from Fig. 4 and presented here on an expanded scale.)

[2] Although mean release was reduced by 60%, this difference was not significant because of the large variation: 145±60 vs. 59±15 ng/15 min (SEM).

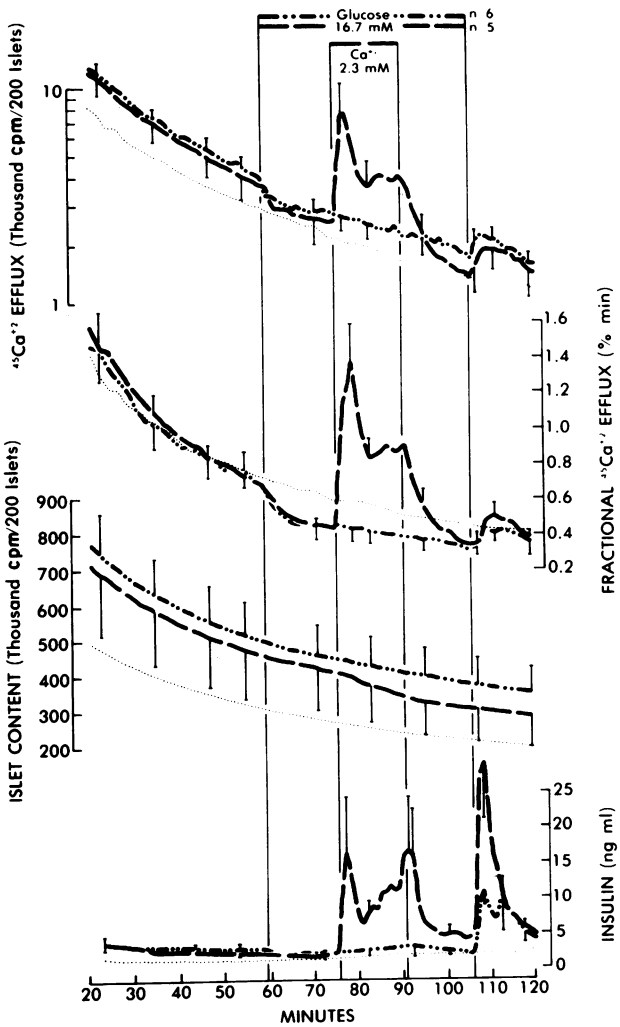

FIGURE 4 Effect of glucose (16.7 mM) without and with the addition of unlabeled Ca (2.3 mM) on efflux of ^{45}Ca from prelabeled rat islets. Basal perifusate contained 2.2 mM glucose and 0.05 mM Ca. Data shown as mean±SEM. Control experiments (n = 8) shown by dotted lines.

in a pattern that could be approximated by decreasing the release rate from compartment II by about 30%. By increasing the Ca concentration to 2.3 mM we increased total efflux about twofold (Fig. 5).

DISCUSSION

The function of Ca ion movement during glucose-stimulated insulin release is unclear. The present studies attempted to clarify the relationship between Ca + glucose-stimulated insulin release and concomitant changes in ^{45}Ca efflux.

Computer analysis of the mean ^{45}Ca efflux curve from unstimulated islets (Fig. 1) revealed the existence of at least two intracellular compartments (I and II) exclusive of the extracellular (sucrose) space. A third intracellular Ca compartment (III) was assumed to exist in order to better fit the experimental efflux curve and the large number of counts remaining in the islets after 120 min. ^{45}Ca in the extracellular space, which bound very loosely to the outsides of the islets and which was released from a possibly very labile compartment (18), was presumably washed away during the first 20–25 min of perifusion (19) ("[^{3}H]sucrose space"). This first 20-min period of ^{45}Ca efflux was not shown in subsequent Figs. 2–5. Compartment sizes were determined by extrapolation of the efflux from each of the three compartments back to zero time (beginning of perifusion) while assuming a constant rate of emptying throughout the experimental period. It was further assumed that the three compartments were parallel (i.e., that one compartment did not release into another compartment before release to the exterior of the cell). Also, efflux from each compartment was assumed inde-

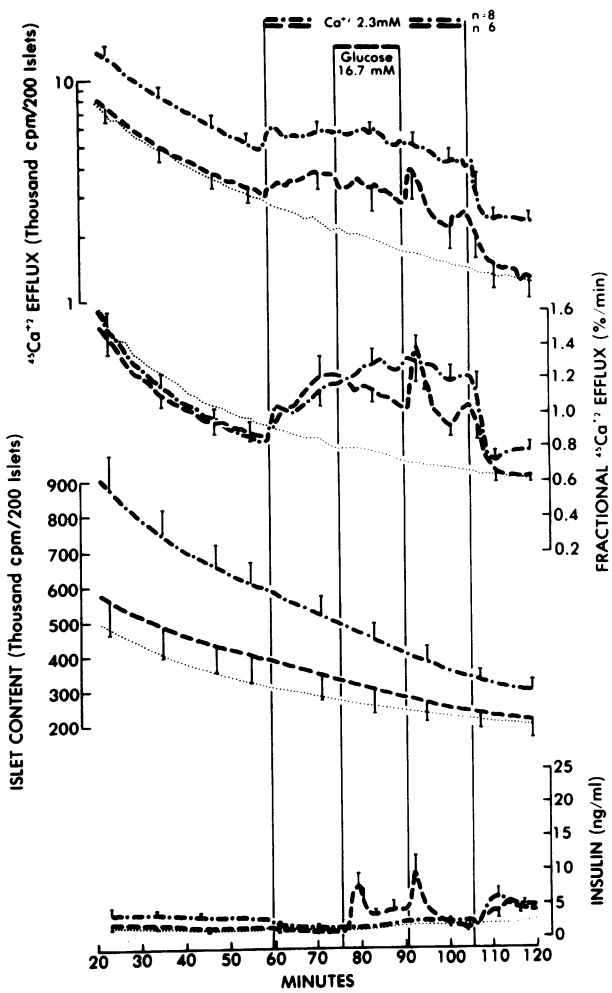

FIGURE 5 Effect of unlabeled Ca (2.3 mM) without and with the addition of glucose (16.7 mM) on efflux of ^{45}Ca from prelabeled rat islets. Basal perifusate contained 2.2 mM glucose and 0.05 mM Ca^{+2}. Data shown as mean±SEM. Control experiments (n = 8) shown by dotted lines.

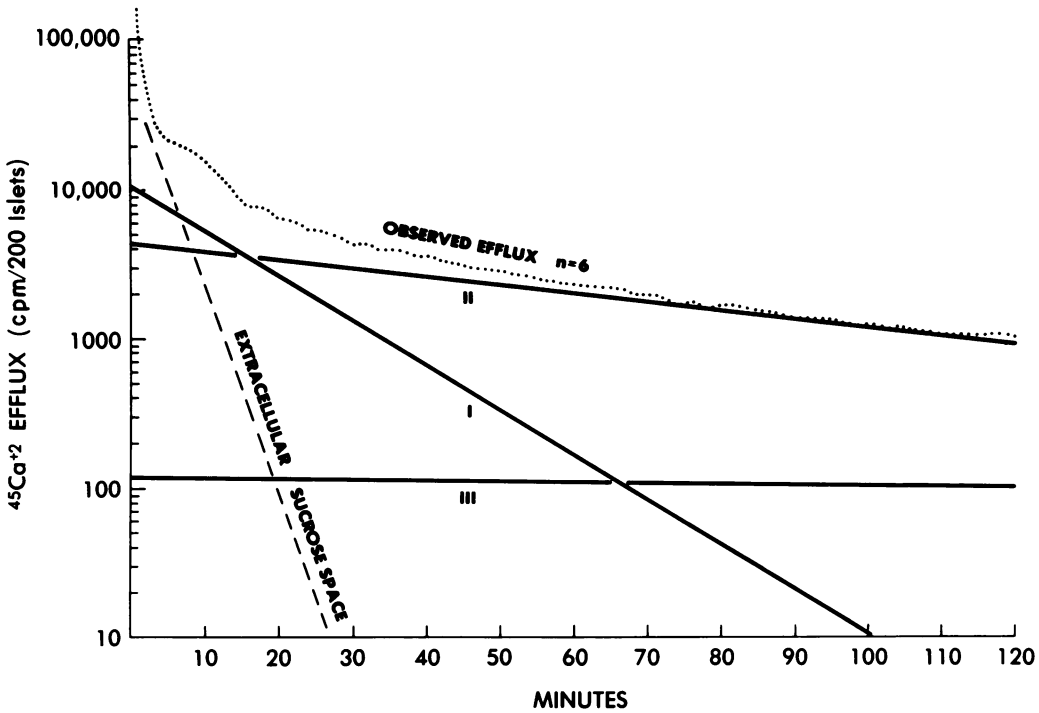

FIGURE 6 Hypothetical division of observed ^{45}Ca efflux (dotted line) into efflux from three parallel, intra-islet, calcium compartments, not including the extracellular sucrose space (broken line). Basal perifusate contained 2.2 mM glucose and 0.05 mM Ca. Observed efflux was the mean of six experiments, each normalized with reference to the 20-min point. (Relative size of compartments from which efflux occurs shown in Fig. 7.)

pendent of Ca movements in the others. However, the data are not incompatible with the possibility that the compartments are in series (e.g., ^{45}Ca release from a "slow" compartment into a rapidly emptying compartment and then to the exterior) (20).

From the studies of isolated kidney cells (21) Borle found three phases of ^{45}Ca-efflux which he tentatively ascribed to extracellular, cytoplasmic, and mitochondrial ^{45}Ca compartments. He further suggested that the mitochondrial pool may be inhomogeneous, consisting of bound and free components. By comparison with these (21) and other (22) studies it is possible that the islets' most rapidly emptying intracellular compartment (I) may correspond to the cytosol. Compartment II, which is larger and empties more slowly, may correspond to the Ca-storing mitochondria (23), insulin granules (12–14), plasma membrane (13), or other intracellular Ca-storing sites (24). The nature of compartment III is more difficult to define. In the absence of the stimulators, glucose and Ca, compartment III is relatively inert and may correspond to the more inert (bound Ca?) portions of the mitochondria, granules, plasma membrane, etc. However, there is no assurance that Ca compartments in pancreatic islet cells resemble those in kidney and liver.

Malaisse et al. (5) have previously shown inhibition of ^{45}Ca efflux from islets by glucose (16.7 mM) in medium without added Ca and containing EGTA. Fig. 3 shows that this suppressive effect is fairly constant throughout a 45-min glucose infusion. Based on the theoretical efflux from each compartment (Fig. 6), it can be seen that compartments I and III contribute only about 10% of the total ^{45}Ca efflux during this period (60–105 min) in unstimulated islets. Therefore, at the time of glucose infusion, efflux was primarily from compartment II, and the suppressive effect of glucose was probably directed toward this compartment. However, the present data cannot distinguish between an inhibi-

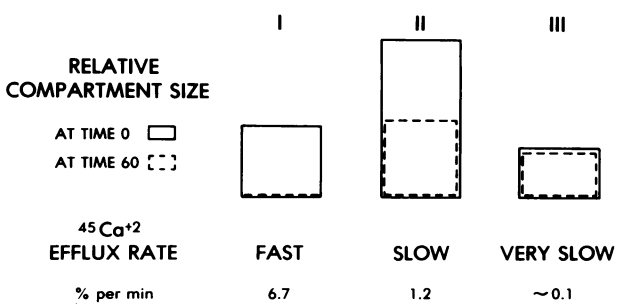

FIGURE 7 Theoretical calcium compartments within the β-cell. Relative size of the ^{45}Ca-filled compartments is calculated for the beginning of perifusion, time 0 (solid line), and at 60 min (broken line). (Efflux from these compartments shown in Fig. 6.)

tion of efflux at the plasma membrane and an inhibition at some intracellular organelle or site. Inhibition of the normal movement of Ca out through the plasma membrane might cause a temporary accumulation of Ca within the cytosol. However, because there was not a significant overshoot of ^{45}Ca efflux after we stopped the glucose infusion (Fig. 4), the excess ^{45}Ca from compartment II may have been redeposited into a more inaccessible site.

In contrast to the effect of glucose, perifusion of 2.3 mM Ca in glucose-depleted medium caused a marked ^{45}Ca efflux which was sustained at about 200% of the control level (Fig. 5). At the end of the Ca infusion (105 min), if the excess ^{45}Ca had come from compartment II, and if that compartment had gone unreplenished, it would have been about one-half as full, and efflux would have fallen to about 50% of the control level. However, because efflux returned to near the control level, the external Ca appears to have mobilized or exchanged with ^{45}Ca from a more inert compartment (III?). Studies with isolated heart mitochondria have shown an approximate 1:1 exchange of unlabeled Ca for labeled ^{45}Ca (25). From studies of isolated adipocyte plasma membranes (22), it seems unlikely that there would have been enough labeled ^{45}Ca bound to the plasma membrane to have sustained the high level of ^{45}Ca efflux for the entire 45-min period. The Ca-induced ^{45}Ca efflux probably was not a manifestation of islet damage, because Ca-stimulated ^{45}Ca efflux was immediate and biphasic when Ca was superimposed on glucose infusion, and because the biphasic insulin release promptly ceased after termination of the Ca perifusion. Raising the Ca concentration from 2.3 to 4.6 mM in similar studies produced a small but definite increase in ^{45}Ca efflux (data not shown). Thus, these results suggest that islets, like other tissues (25, 26), possess a Ca $\leftrightarrow$ ^{45}Ca exchange system which may occur in the mitochondria (25), or other β-cell locations which accumulate Ca (granules, plasma membrane, endoplasmic reticulum, Golgi apparatus) (12–14).

A further complication to the precise interpretation of these compartmental results is that the isolated rat islet only consists of about 60–70% β-cells—the remainder being α, δ, pancreatic polypeptide, other?, and endothelial cells (27–29). Efflux from the non-β-cells of the islet may complicate the compartmental analysis. The development of a pure β-cell technique would help solve this question.

The order in which glucose and Ca were presented to the islets influenced insulin secretion. When glucose was perifused first, a larger biphasic insulin release occurred. Thus, a potentiating effect of glucose can occur in a Ca-depleted medium. As seen in the perfused pancreas (30), prior exposure to glucose can potentiate the insulin response, possibly by increasing proinsulin, metabolic intermediates, ATP, cyclic AMP, or microtubule protein (31–34).

In experiments in which glucose was superimposed 15 min after addition of Ca (Fig. 5), glucose suppressed ^{45}Ca efflux even though it stimulated insulin release. These experiments differ in design but are consistent with recent reports (35, 36) in suggesting that calcium efflux does not occur stoichiometrically with insulin release, as was originally suggested (5).

It is interesting to note that, in all instances in which glucose was stopped during perifusion, an overshoot or "off response" of insulin release and a possible ^{45}Ca off response occurred. The relationship between ^{45}Ca and insulin was not stoichiometric, and the cause of this off response is not clear. However, studies by Blackard et al. (37) suggest that the abrupt decrease in osmolarity on removal of glucose may have caused at least the insulin off response.

Although the various changes in efflux were easily discernible when the minute-by-minute efflux of ^{45}Ca was measured, the rates of efflux were small. Therefore, as illustrated in Figs. 1,4, and 5, the attempt to detect changes in efflux by measuring the small depletion of islet content may not always be sufficient for monitoring acute changes in efflux (9).

The use of ^{45}Ca to analyze storage and efflux of Ca could depend upon the conditions of prior ^{45}Ca loading. We used stimulatory levels of glucose and low levels of unlabeled Ca because, as noted by others (8), this caused enhanced loading of the isotope and more sensitive measurement of efflux. Loading was for 60 min, corresponding to 50–70% of isotopic equilibrium. In comparative studies (data not shown), in which loading occurred at normal Ca levels over a 20-h period in culture to ensure isotopic equilibrium within the islets, efflux curves were in qualitative agreement with the results shown in Figs. 1, 3, 4, and 5.

In conclusion, the present study suggests that ^{45}Ca efflux was derived from at least three compartments within the unstimulated islets. Glucose decreased efflux from the largest compartment in the presence or absence of previously added Ca. Calcium caused a marked ^{45}Ca efflux from a relatively stable compartment, indicating the presence of a Ca pump or a rapidly exchangeable Ca-binding site. ^{45}Ca efflux and insulin release were dependent upon the order of addition of glucose and Ca consistent with the possibility that glucose can affect intracellular Ca distribution and potentiate subsequent insulin release even in low extracellular Ca. During stimulation with glucose, Ca efflux does not occur stoichiometrically with insulin release.

ACKNOWLEDGMENTS

We are indebted to Mary Ann Jones, Michele Manning, and Florence Schmid for technical assistance.

This investigation was supported in part by grant AM-01410 of the National Institute of Arthritis, Metabolism, and Digestive Diseases; the Kroc Foundation of Santa Ynez, Calif.; and the Juvenile Diabetes Foundation of New York.

REFERENCES

1. Grodsky, G. M., and L. L. Bennett. 1966. Cation requirements for insulin secretion in the isolated perfused pancreas. *Diabetes.* **15:** 910–913.
2. Curry, D. L., L. L. Bennett, and G. M. Grodsky. 1968. Dynamics of insulin secretion by the perfused rat pancreas. *Endocrinology.* **83:** 572–584.
3. Davis, G., G. Somers, and W. J. Malaisse. 1975. Stimulation of insulin release by calcium. *Biochem. Biophys. Res. Commun.* **67:** 525–529.
4. Davis, G., and N. R. Lazarus. 1976. An in vitro system for studying insulin release caused by secretory granules-plasma membrane interaction. Definition of the system. *J. Physiol. (Lond.).* **256:** 709–729.
5. Malaisse, W. J., G. R. Brisson, and L. E. Baird. 1973. Stimulus-secretion coupling of glucose-induced insulin release. X. Effect of glucose on ^{45}Ca efflux from perifused islets. *Am. J. Physiol.* **224:** 389–394.
6. Bukowiecki, L., and N. Freinkel. 1976. Relationship between efflux of ionic calcium and phosphorus during excitation of pancreatic islets with glucose. *Biochim. Biophys. Acta.* **437:** 190–198.
7. Lenzen, S. 1978. The immediate insulin-secretory response of the rat pancreas to glucose compared with tolbutamide and other secretagogues. *Diabetes.* **27:** 27–34.
8. Malaisse-Lagae, F., and W. J. Malaisse, 1971. Stimulus-secretion coupling of glucose-induced insulin release. III. Uptake of ^{45}calcium by isolated islets of Langerhans. *Endocrinology.* **88:** 72–80.
9. Naber, S. P., M. L. McDaniel, and P. E. Lacy. 1977. The effect of glucose on the acute uptake and efflux of calcium^{45} in isolated rat islets. *Endocrinology.* **101:** 686–693.
10. Frankel, B. J., J. A. Kromhout, W. T. Imagawa, H. D. Landahl, and G. M. Grodsky. 1978. Glucose-stimulated ^{45}Ca uptake in isolated rat islets. *Diabetes.* **27:** 365–369.
11. Matthews, E. K., and P. M. Dean. 1970. Electrical activity in islet cells. *In* The Structure and Metabolism of the Pancreatic Islets. Wenner-Gren Symposium No. 16. S. Falkmer, B. Hellman, and I-B. Täljedal, editors. Pergamon Press Ltd., Oxford. 305–313.
12. Herman, L., T. Sato, and C. N. Hales. 1973. The electron microscopic localization of cations to pancreatic islets of Langerhans and their possible role in insulin secretion. *J. Ultrastruct. Res.* **42:** 298–311.
13. Schäfer, H-J., and G. Klöppel. 1974. The significance of calcium in insulin secretion. 1974. Ultrastructural studies on identification and localization of calcium in activated and inactivated B cells of mice. *Virchows Arch. A Pathol. Anat. Histol.* **362:** 231–245.
14. Ravazzola, M., F. Malaisse-Lagae, N. Amherdt, A. Perrelet, W. Malaisse, and L. Orci. 1976. Patterns of calcium localization in pancreatic endocrine cells. *J. Cell Sci.* **27:** 107–117.
15. Lacy, P. E., and M. Kostianovsky. 1967. Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes.* **16:**35–39.
16. Lacy, P. E., M. M. Walker, and C. J. Fink. 1972. Perifusion of isolated rat islets in vitro. Participation of the microtubular system in the biphasic release of insulin. *Diabetes.* **21:** 987–998.
17. Lundquist, I., R. Fanska, and G. M. Grodsky. 1976. Interaction of calcium and glucose on glucagon secretion. *Endocrinology.* **99:** 1304–1312.
18. Hellman, B., J. Sehlin, and I-B. Täljedal. 1976. Calcium and secretion: Distinction between two pools of glucose-sensitive calcium in pancreatic islets. *Science (Wash. D. C.).* **194:** 1421–1423.
19. Hellman, B., J. Sehlin, and I-B. Täljedal. 1976. Effects of glucose on $^{45}Ca^{2+}$ uptake by pancreatic islets as studied with the lanthanum method. *J. Physiol. (Lond.).* **254:** 639–656.
20. Borle, A. B. 1975. Methods for assessing hormone effects on calcium fluxes *in vitro*. *Methods Enzymol.* **39:** 513–573.
21. Borle, A. B. 1972. Kinetic analysis of calcium movements in cell culture. V. Intracellular calcium distribution in kidney cells. *J. Membr. Biol.* **10:** 45–66.
22. McDonald, J. M., D. E. Bruns, and L. Jarett. 1976. Characterization of calcium binding to adipocyte plasma membranes. *J. Biol. Chem.* **251:** 5345–5351.
23. Borle, A. B. 1973. Calcium metabolism at the cellular level. *Fed. Proc.* **32:** 1944–50.
24. Moore, L., T. Chen, H. R. Knapp, Jr., and E. J. Landon. 1975. Energy-dependent calcium sequestration activity in rat liver microsomes. *J. Biol. Chem.* **250:** 4562–4568.
25. Crompton, M., M. Künzi, and E. Carafoli. 1977. The calcium-induced and sodium-induced effluxes of calcium from heart mitochondria. Evidence for a sodium-calcium carrier. *Eur. J. Biochem.* **79:** 549–558.
26. Blaustein, M. P., and J. M. Russell. 1975. Sodium-calcium exchange and calcium-calcium exchange in internally dialyzed squid giant axons. *J. Membr. Biol.* **22:** 285–312.
27. Hoftiezer, V., and A-M. Carpenter. 1973. Comparison of streptozotocin and alloxan-induced diabetes in the rat, including volumetric quantitation of the pancreatic islets. *Diabetologia.* **9:** 178–184.
28. McEvoy, R. C., and O. D. Hegre. 1977. Morphometric quantitation of the pancreatic insulin-, glucagon-, and somatostatin-positive cell populations in normal and alloxan-diabetic rats. *Diabetes.* **26:** 1140–1146.
29. Larsson, L-I., F. Sundler, and R. Håkanson. 1976. Pancreatic polypeptide—A postulated new hormone: Identification of its cellular storage site by light and electron microscopic immunocytochemistry. *Diabetologia.* **12:** 211–226.
30. Grodsky, G. M., D. Curry, H. Landahl, and L. Bennett. 1969. Further studies on the dynamic aspects of insulin release in vitro with evidence for a two-compartmental storage system. *Acta Diabetol. Lat.* **6**(Suppl. 1): 554–579.
31. Pipeleers, D. G., M. Marichal, and W. J. Malaisse. 1973. The stimulus-secretion coupling of glucose-induced insulin release. XIV. Glucose regulation of insular biosynthetic activity. *Endocrinology.* **93:** 1001–1011.
32. Lambert, A. E. 1976. The regulation of insulin secretion. *Rev. Physiol. Biochem. Pharmacol.* **75:** 97–159.
33. Charles, M. A., J. Lawecki, R. Pictet, and G. M. Grodsky. 1975. Insulin secretion. Interrelationships of glucose, cyclic adenosine 3′:5′-monophosphate, and calcium. *J. Biol. Chem.* **250:** 6134–6140.
34. Pipeleers, D. G., M. A. Pipeleers-Marichal, and D. M. Kipnis. 1976. Microtubule assembly and the intracellular transport of secretory granules in pancreatic islets. *Science (Wash. D. C.).* **191:** 88–90.
35. Wollheim, C. B., M. Kikuchi, A. E. Renold, and G. W. G. Sharp. 1977. Somatostatin- and epinephrine-induced modifications of $^{45}Ca^{++}$ fluxes and insulin release in rat pancreatic islets maintained in tissue culture. *J. Clin. Invest.* **60:** 1165–1173.
36. Gylfe, E., and B. Hellman. 1978. Calcium and pancreatic β-cell function. II. Mobilization of glucose-sensitive ^{45}Ca from perifused islets rich in β-cells. *Biochim. Biophys. Acta.* **538:** 249–257.
37. Blackard, W. G., M. Kikuchi, A. Rabinovitch, and A. E. Renold. 1975. An effect of hypoosmolarity on insulin release in vitro. *Am. J. Physiol.* **228:** 706–713.