Biochimica et Biophysica Acta, 436 (1976) 190–198

BBA 77308

RELATIONSHIP BETWEEN EFFLUX OF IONIC CALCIUM AND PHOSPHORUS DURING EXCITATION OF PANCREATIC ISLETS WITH GLUCOSE

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(Received December 1st, 1975)

SUMMARY

Simultaneous rates of [^{32}P]orthophosphate and $^{45}Ca^{2+}$ efflux from prelabeled rat pancreatic islets have been evaluated to assess whether these ions move in concert throughout all phases of "stimulus-secretion coupling". Perifusion with stimulatory concentrations of glucose elicited immediate but transitory increases in ^{32}P outflow accompanied by initial retardations and subsequent augmentations in net $^{45}Ca^{2+}$ outflows. These monophasic ^{32}P and biphasic $^{45}Ca^{2+}$ responses to secretory stimulation were abolished completely by membrane stabilization with tetracaine. However, certain manipulations enabled individual components to be modified separately. During stimulation with glucose, inhibition of insulin release by Ni^{2+} abolished the late increases in $^{45}Ca^{2+}$ outflow without affecting the initial retentions of $^{45}Ca^{2+}$ or the increased releases of ^{32}P. Under basal conditions, the ionophore A23187 "triggered" increased releases of $^{45}Ca^{2+}$ and insulin without prior retentions of $^{45}Ca^{2+}$ or enhancements of ^{32}P efflux. Thus, the immediate retardations of $^{45}Ca^{2+}$ outflow and heightened efflux of ^{32}P may reflect early events in stimulus-secretion coupling which can be dissociated from the augmented release of $^{45}Ca^{2+}$ accompanying activated emiocytosis.

INTRODUCTION

We have reported recently that stimulation of isolated ^{32}P-prelabeled rat pancreatic islets with D-glucose elicits an almost immediate, transient heightened efflux of [^{32}P]orthophosphate (the "phosphate flush") [1]. This packet-pulse release of ^{32}P can be triggered by other nutrient islet secretagogues such as D-mannose or L-leucine but not by sugars which do not affect insulin secretion, such as D-fructose, D-galactose, D-myoinositol or L-glucose [1, 2]. The "phosphate flush" does not require transcellular entry of oxidizable substrates since it also occurs during islet exposure to the insulin-secretory b(−) isomer of the nonmetabolizable leucine analogue, 2-aminonorbornane-2-carboxylic acid [3]. The phenomenon can be abolished by membrane stabilizers but it persists during islet stimulation even when

extrusion of insulin is inhibited by addition of ionic nickel to the extracellular medium or omission of calcium [1, 2]. The latter ability to dissociate the "phosphate flush" from emiocytosis has suggested that it may reflect an early event in stimulus recognion by islets. Our experience with glucose anomers has strengthened this proposition. Thus, the "phosphate flush" is triggered by the α rather than the β anomer of glucose whereas the transmembrane transport of glucose into islets and intracellular phosphorylation to hexose phosphate do not display similar anomeric specificities [4].

Since translocations of ionic calcium have long been implicated in "stimulus-secretion coupling" [5] in pancreatic islets [6–10], and possible counterbalancing anions have not been evaluated previously, the present studies were initiated. We have attempted to correlate the concurrent patterns of $^{45}Ca^{2+}$ and ^{32}P efflux during stimulation of prelabeled islets with glucose. Our studies have indicated that some relationships between calcium and phosphate transfers may obtain but that certain components may be manipulated separately under appropriate experimental conditions.

MATERIALS AND METHODS

Our techniques for isolating, prelabeling and perifusing rat pancreatic islets have been described elsewhere [1, 2, 4]. In brief, groups of 250–400 islets which had been isolated by collagenase digestion were labeled during preincubation for 90 min. at 37 °C in an atmosphere of 95 % O_2/5 % CO_2, in the presence of $^{45}Ca^{2+}$ (200–400 μCi/ml) or [^{32}P]orthophosphate (150 μCi/ml) (New England Nuclear, Boston, Mass.). The islets were suspended in 1 ml of modified Krebs-Ringer-bicarbonate buffer of the following composition (mM): Na^+ 144, K^+ 4.8, Ca^{2+} 0.85, Mg^{2+} 1.2, Cl^- 126.0, HCO_3^- 25.0, SO_4^{2-} 1.2, and HPO_4^{2-} 0.5. To this medium were added 0.5 mg/ml of bovine serum albumin (Fraction V, Armour Pharmaceutical Company, Chicago, Ill.), 0.1 mg/ml of streptomycin sulfate, 100 units/ml penicillin and 3.0 mg/ml of glucose. These conditions differ from those previously reported [1–4] in that ionic calcium was reduced from 2.4 mM to 0.8 mM and glucose concentration was increased from 1.0 mg/ml to 3.0 mg/ml. These changes were instituted because preliminary experiments indicated that they improved the accumulation of $^{45}Ca^{+2}$ by islets during the incubation for prelabeling.

At the end of prelabeling, the islets were submitted to repeated washes with a similar Krebs-Ringer-bicarbonate buffer which contained no isotopes and in which the glucose concentration was reduced to 0.5 mg/ml and the HPO_4^{2-} concentration was increased to 1 mM. 50–100 islets were then placed in small chambers [1] and perifused at 37 °C with Krebs-Ringer-bicarbonate solution of the same composition as used for the washings, which was gassed continuously with 95 % O_2/5 % CO_2. The perifusate was delivered via a peristaltic pump at a rate of 0.8–0.9 ml/min; the total volume of perifusion chamber and associated tubing was 2.8 ml, so that the dead time of the entire system at the usual flow rate was approximately 3 min. The perifusion medium was changed at the 25th and 45th min in accordance with experimental objectives (vide infra). Total effluents were collected at intervals of 5, 2 or 1 min (as shown in the figures) and analyzed for radioactivity and insulin content.

Aliquots from each collection were mixed with 10 ml of Aquasol (New England Nuclear, Boston, Mass.) and counted in a Nuclear Chicago Mark I liquid scintilla-

tion counter (Nuclear Chicago Corp., Des Plaines, Ill.). Values for efflux rates of $^{45}Ca^{2+}$ and ^{32}P (cpm/min) in individual channels were expressed as percent of the mean values observed in the five 1-min collections obtained between the 20th and 25th min. High voltage ionophoresis corroborated that more than 95 % of the radioactivity in the effluent at all time points consisted of [^{32}P]orthophosphate [2].

In most experiments, four channels (i.e. two containing islets prelabeled with $^{45}Ca^{2+}$ and two containing islets prelabeled with [^{32}P]orthophosphate were run in parallel. This permitted control and experimental observations to be secured with each isotope at the same time. Total immunoreactive insulin was determined by radioimmunoassay as previously described [1, 2, 4]. All reagents were analytical grade; tetracaine hydrochloride was secured from Schwarz/Mann Co. The ionophore A23187 was kindly provided by Eli Lilly and Co., Indianapolis, Ind. Means$\pm$S.E.M. were calculated from experiments performed on separate occasions with separate preparations of islets.

RESULTS AND DISCUSSION

Characteristic efflux patterns for ^{32}P and $^{45}Ca^{2+}$ are depicted in Fig. 1. As previously described [1, 2] relatively constant rates of ^{32}P efflux were obtained within 15–20 min of the start of perifusion. Increasing the glucose concentration in the perifusate from 0.5 to 3.0 mg/ml at the 25th min of perifusion induced a significant increase in the rate of ^{32}P efflux within 4 min. Peak values for efflux from islets prelabeled in this fashion were 630$\pm$53 % of the basal values and the total duration of heightened ^{32}P efflux was 10–12 min. By contrast, rates of $^{45}Ca^{2+}$ efflux were declining measurably throughout perifusion and this decrement persisted in curvilinear fashion when perifusion with non-stimulatory amounts of glucose (0.5 mg/ml) was continued for 65 min (see cross-hatched area in Fig. 1). When perifusate glucose was increased to 3.0 mg/ml glucose at the 25th min, a biphasic change in $^{45}Ca^{2+}$ efflux supervened. An initial retardation of efflux was manifested coinciding exactly with the onset of the heightened outpouring of ^{32}P. 2–3 min later (i.e. at min 31 or 32), $^{45}Ca^{2+}$ efflux increased markedly and then returned to basal rates 6–7 min thereafter (approximately coincident with the return of ^{32}P to basal). Peak rates of $^{45}Ca^{2+}$ and ^{32}P efflux occurred at the same time. Malaisse has described a similar transitory biphasic change in the release of $^{45}Ca^{2+}$ from prelabeled islets in response to secretory stimulation [10], and this clearly deviates at least initially (i.e. at min 29 and 30) from the monophasic pattern of heightened ^{32}P release (Fig. 1). To see whether one of the two phases of the $^{45}Ca^{2+}$ response to glucose is associated with the ^{32}P response, attempts were made to dissociate these events. We have previously shown that membrane stabilization with the local anesthetic tetracaine abolishes the effect of secretagogues upon insulin release and ^{32}P efflux [1, 2]. In confirmation of our earlier findings, inclusion of tetracaine (1 mM) in the perifusate abolished the heightened efflux of ^{32}P which should have occurred when glucose was increased from 0.5 to 3.0 mg/ml at the 25th min (Fig. 2). At the same time, tetracaine also abolished both phases of the $^{45}Ca^{2+}$ response so that $^{45}Ca^{2+}$ outflow did not deviate from control levels (the cross-hatched bars; Fig. 1).

Because ionic nickel, in contrast to tetracaine, can inhibit insulin release in response to secretory stimulation without abolishing enhanced ^{32}P efflux [1, 2], the

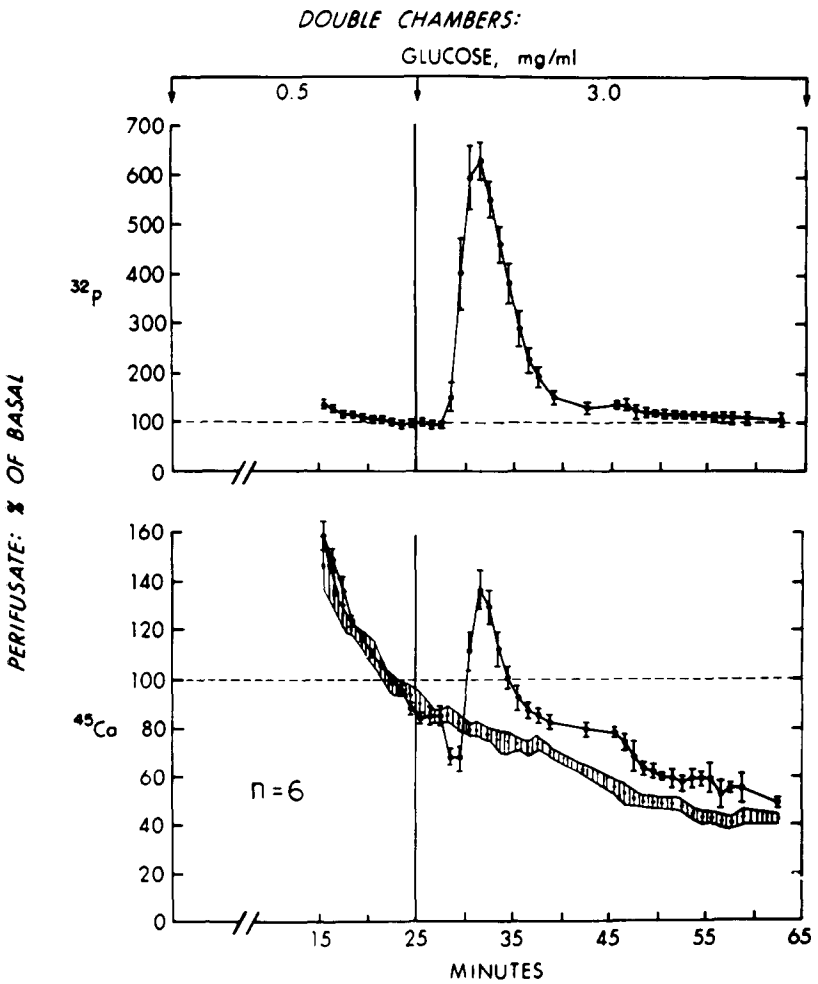

Fig. 1. Effect of glucose on efflux of ^{32}P and $^{45}Ca^{2+}$ from prelabeled pancreatic islets. Glucose concentration in the perifusate is shown in the upper part of the figure. Values at each time point (Mean ±S.E.M.) have been expressed as % of basal as described in text. On this and all subsequent illustrations, *n* denotes the number of separate experiments, performed on different days, in which ^{32}P and $^{45}Ca^{2+}$ prelabeled islets were exposed to stimulants. Cross-hatched bars represent mean ±S.E.M. from four individual control experiments in which glucose concentration was maintained at 0.5 mg/ml throughout the 65 min perifusion.

effects of 2 mM Ni^{2+} on the biphasic calcium response were investigated. As shown in Fig. 3, stimulation with 3.0 mg/ml glucose in the presence of 2 mM Ni^{2+} elicited heightened ^{32}P efflux and a concurrent retardation in the outflow of $^{45}Ca^{2+}$. However, in this situation, where there is no release of insulin [1, 2], subsequent heightened efflux of $^{45}Ca^{2+}$ did not occur. It was not seen until nickel was withdrawn (at the 45th min) and the stimulatory effects of 3.0 mg/ml glucose on insulin secretion became manifest [1, 2]. The augmented release of $^{45}Ca^{2+}$ (and insulin [1, 2]) at this time were not accompanied by a second increase in ^{32}P outflow (Fig. 3). Additional con-

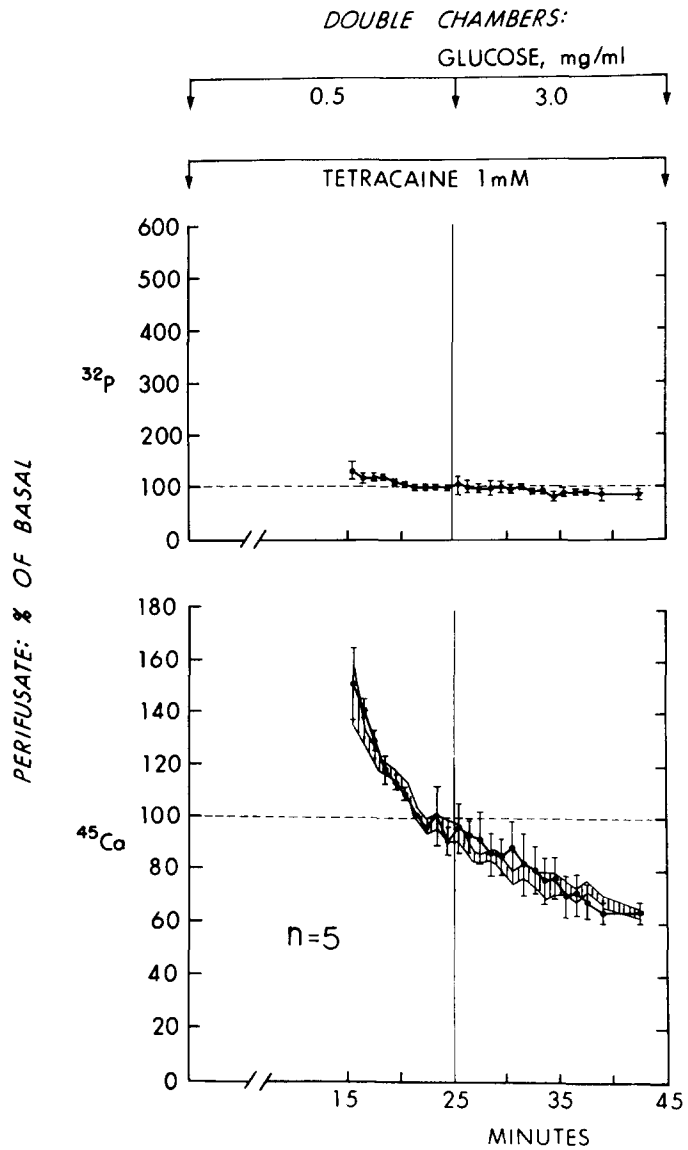

Fig. 2. Effect of tetracaine on efflux of ^{32}P and $^{45}Ca^{2+}$ from prelabeled pancreatic islets. Glucose and tetracaine concentrations in the perifusate are shown in the upper part of the figure. For purpose of comparison, the control values for $^{45}Ca^{2+}$ from Fig. 1 are also shown by the cross-hatched bars.

trol experiments (not shown) have demonstrated that Ni^{2+} per se does not affect basal ^{32}P and $^{45}Ca^{2+}$ efflux when added in the presence of non-stimulatory glucose concentrations. Thus, Ni^{2+} appears to dissociate events that might be triggered during the early interaction between glucose and its recognition system from later events more closely associated with the final emiocytotic release of insulin. Such an interpretation is consistent with the recent experiments of Dormer et al. [11] in which

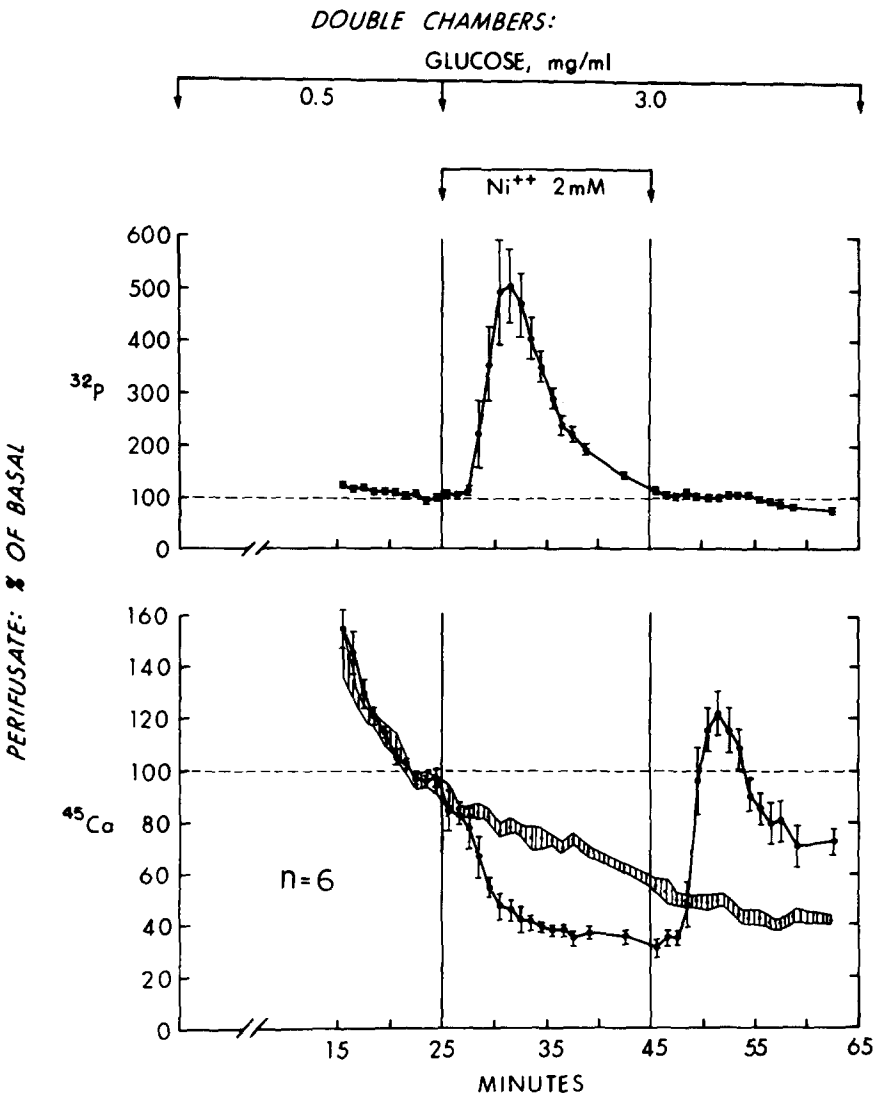

Fig. 3 Effect of Ni^{2+} on efflux of ^{32}P and $^{45}Ca^{2+}$ from prelabeled pancreatic islets. Data are presented as in Figs. 1 and 2.

Ni^{2+} rapidly and reversibly inhibited the exocytotic release of several hormones from various tissues without affecting the changes in cyclic AMP or energy metabolism that characterize secretory activation in these structures.

If the ^{32}P efflux and the early retention of $^{45}Ca^{2+}$ outflow reflect some initial interaction between stimulus and receptor site, whereas the subsequent heightened outflow of $^{45}Ca^{2+}$ connotes later events associated with the emiocytotic extrusion of insulin, then no perturbation of phosphorus nor immediate retardation of calcium should arise when the primary interaction between stimulus and receptor is bypassed and secretion is triggered at some later phase in the "stimulus-secretion" sequence.

The calcium ionophore A23187 was utilized to test this hypothesis since it has been shown to effect direct release of hormones or enzymes from a variety of secretory

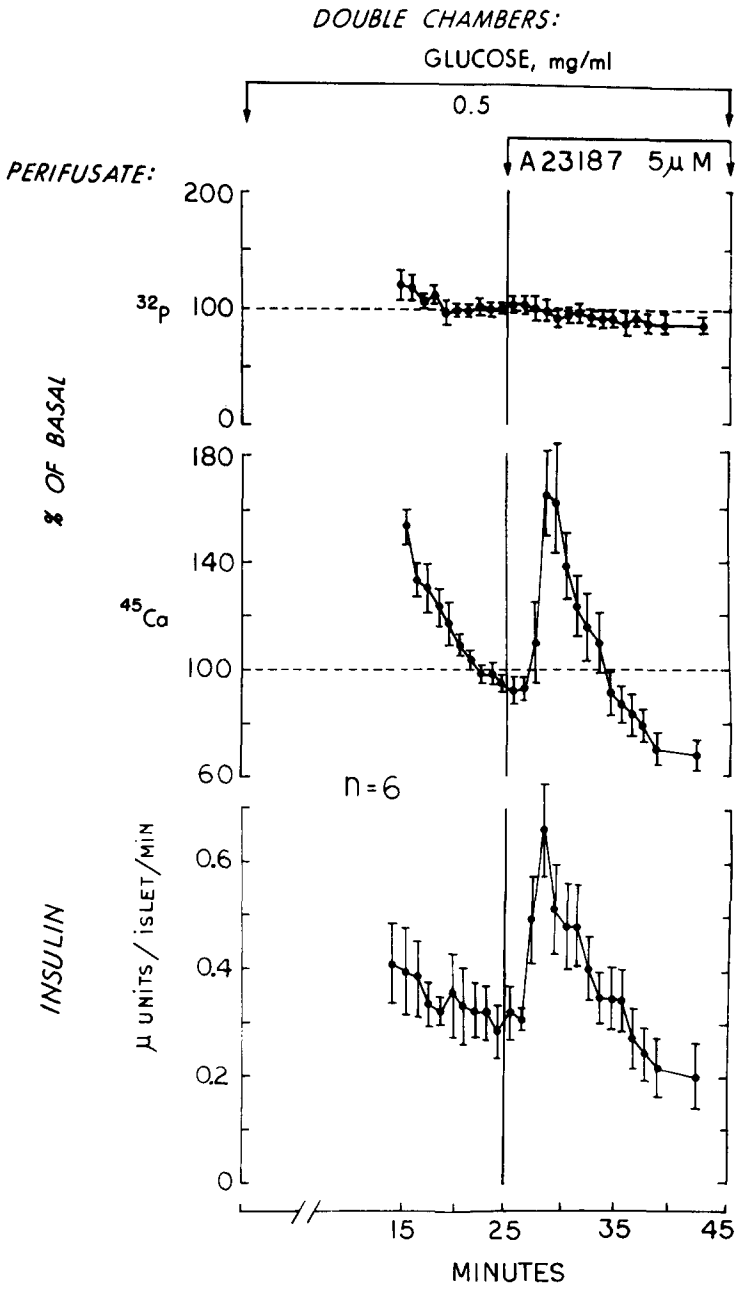

Fig. 4. Effect of the ionophore A23187 on efflux of ^{32}P, $^{45}Ca^{2+}$ and insulin from prelabeled pancreatic islets. Glucose and ionophore concentrations are shown in the upper part of the figure. Data are presented as in Figs. 1–3.

cells, including pancreatic islets [12–15], by increasing transcellular and/or intracellular calcium translocations. Experiments with A23187 are summarized in Fig. 4. Under basal conditions of perifusion with non-stimulatory concentrations of glucose (0.5 mg/ml), the addition of 5 μM A23187 to the perifusate did not increase ^{32}P outflow nor retard $^{45}Ca^{2+}$ efflux (Fig. 4). However, it effected an almost immediate transitory pulse of insulin release and a concurrent heightened out flow of $^{45}Ca^{2+}$ (Fig. 4).

In summary, we have described three experimental situations in which the increased rates of $^{45}Ca^{2+}$ and ^{32}P efflux that normally occur during islet excitation (Fig. 1), can be dissociated from each other: (a) when emiocytosis of insulin is inhibited by ionic nickel (Fig. 3); (b) when nickel is subsequently removed from the perifusion medium in the presence of stimulatory glucose concentrations (Fig. 3); and (c) when the ionophore A23187 is used to bypass the initial interactions between specific stimulant secretagogue and receptor (Fig. 4). However, we have not been able to dissociate the early increase in ^{32}P efflux from the initial simultaneous inhibition of $^{45}Ca^{2+}$ outflow which is triggered by glucose stimulation (Figs. 1–3). This suggests that immediate net transcellular release of phosphate and cellular retention of calcium may coexist as early events of the stimulus-secretion sequence and that these antedate the subsequent increase in calcium outflow associated with activated secretion. Although the precise linkage remains to be established, it is tempting to consider that those factors which underly the initial change in calcium flux during the initiation of excitation may be related phenomenologically to the same membrane-receptor interactions that "trigger" the transient increase in ^{32}P outflow.

ACKNOWLEDGEMENTS

These studies were supported by Research Grant AM-10699 and Training Grant AM-05071 from the National Institute of Arthritis and Metabolic Disease, National Institutes of Health, Bethesda, Maryland.

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