Somatostatin- and Epinephrine-Induced Modifications of $^{45}Ca^{++}$ Fluxes and Insulin Release in Rat Pancreatic Islets Maintained in Tissue Culture

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ABSTRACT The effects of somatostatin and epinephrine have been studied with regard to glucose-induced insulin release and $^{45}Ca^{++}$ uptake by rat pancreatic islets after 2 days in tissue culture and with regard to $^{45}Ca^{++}$ efflux from islets loaded with the radioisotope during the 2 days of culture. $^{45}Ca^{++}$ uptake, measured simultaneously with insulin release, was linear with time for 5 min. $^{45}Ca^{++}$ efflux and insulin release were also measured simultaneously from perifused islets.

Glucose (16.7 mM) markedly stimulated insulin release and $^{45}Ca^{++}$ uptake. Somatostatin inhibited the stimulation of insulin release by glucose in a concentration-related manner (1–1,000 ng/ml) but was without effect on the glucose-induced stimulation of $^{45}Ca^{++}$ uptake. Similarly, under perifusion conditions, both phases of insulin release were inhibited by somatostatin while no effect was observed on the pattern of $^{45}Ca^{++}$ efflux after glucose.

Epinephrine, in contrast to somatostatin, caused a concentration-dependent inhibition of the stimulation of both insulin release and $^{45}Ca^{++}$ uptake by glucose. Both phases of insulin release were inhibited by epinephrine and marked inhibition could be observed with no change in the characteristic glucose-evoked pattern of $^{45}Ca^{++}$ efflux (e.g., with 10 nM epinephrine). The inhibitory effect of epinephrine on $^{45}Ca^{++}$ uptake and insulin release appeared to be mediated via an α-adrenergic mechanism, since is was abolished in the presence of phentolamine.

Somatostatin inhibits insulin release without any detectable effect upon the handling of calcium by the islets. In contrast, inhibition of insulin release by epinephrine is accompanied by a partial inhibition of glucose-induced Ca^{++} uptake.

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INTRODUCTION

Somatostatin (1–6) and epinephrine (7–12) inhibit the stimulation of insulin release by a variety of agents, including sugars, amino acids, and activators of the adenylate cyclase-cyclic AMP system. In searching for the mode of inhibition it has been suggested that they interfere with calcium handling by the pancreatic B cells. Thus, Curry and Bennett showed that the inhibitory effect of somatostatin on glucose-stimulated insulin release from the perfused rat pancreas was attenuated when the calcium concentration in the medium was increased (13, 14). These results, and others (15, 16), suggested a connection between somatostatin and calcium, either by somatostatin inhibition of calcium uptake, or by calcium interference with somatostatin receptor-binding or action. Malaisse-Lagae and Malaisse showed epinephrine to inhibit glucose-stimulated net uptake of $^{45}Ca^{++}$ by isolated islets when measured after 90 min of incubation (17). Brisson and Malaisse later reported that epinephrine increased the outward flux of $^{45}Ca^{++}$ from preloaded islets and postulated that the inhibitory effect on insulin release might be via lowering of the cytosolic Ca^{++} concentration (18). These studies on calcium uptake and efflux were, however, performed in the presence of very high epinephrine concentrations (10–100 μM). As epinephrine inhibits insulin release at much lower concentrations, it seemed necessary to reexamine the effect of epinephrine on calcium fluxes in islets. In view of the few reports on the effect of somatostatin on calcium handling, it seemed of particular interest also to study its effect on calcium uptake and efflux.

In the study reported here, collagenase-isolated islets were used after a maintenance period of 2 days under tissue culture conditions. This procedure was used for two reasons. First, somatostatin is relatively

ineffective on freshly isolated islets (4, 19, 20), whereas the sensitivity is increased after a period of culture (21). Second, by loading the islets with $^{45}Ca^{++}$ during the 2 days of culture, isotopic equilibrium is assured throughout the B-cell calcium compartments, and interpretation of $^{45}Ca^{++}$ efflux facilitated thereby. For the studies on calcium uptake a method has been developed which allows the simultaneous measurement of $^{45}Ca^{++}$ uptake and insulin release by the same islets.

METHODS

Isolation and tissue culture maintenance of the islets. Pancreatic islets were isolated by the collagenase technique (22) from male Wistar rats weighing 220–270 g. After washing, 200–250 islets were suspended in 5 or 6 ml of medium 199 containing 10% heat-inactivated calf serum, 14 mM sodium bicarbonate, 8.3 mM glucose, 400 IU/ml sodium penicillin G, and 200 μg/ml streptomycin sulphate. The suspended islets were placed in 60-mm diameter plastic Petri dishes as used for bacteriological culture, and kept at 37°C in an incubator gassed with air and CO_2. The islets do not attach to the Petri dishes and remain as individual islets throughout the 45–47-h maintenance period. Islets used for $^{45}Ca^{++}$ efflux studies were labeled with $^{45}CaCl_2$ during the entire maintenance period. 100 μCi of $^{45}CaCl_2$/ml of culture medium was used for this purpose. $CaCl_2$ in the culture medium was 1.8 mM and the final specific radioactivity was approximately 54 μCi/μmol.

$^{45}Ca^{++}$ uptake measurement. After the maintenance period the islets were washed twice at room temperature with a modified Krebs-Ringer bicarbonate buffer (KRB-Hepes)[1] containing 5 mM $NaHCO_3$, 1 or 2.5 mM $CaCl_2$, 250 kallikrein inhibitory U/ml Trasylol, 0.5% dialyzed bovine serum albumin, 10 mM Hepes, and 2.8 mM glucose, pH 7.4. In the experiments with epinephrine the buffer also contained 1.1 mM ascorbic acid. The ascorbic acid had no influence alone on insulin release, $^{45}Ca^{++}$ uptake, or $^{45}Ca^{++}$ efflux under conditions of either low or high glucose. The islets were distributed into polyethylene tubes (0.4 ml volume) containing 200 μl of a mixture of dibutyl- and dinonylphthalate (10:3 vol/vol) layered on top of 20 μl of 6 M urea. The 50 μl of KRB-Hepes buffer containing the 10 islets was carefully placed against the walls of the tubes to leave an air-layer between the buffer and the oil mixture. The incubation was started by adding another 50 μl of warm (37°C) KRB-Hepes buffer containing glucose and other test substances to yield appropriate final concentrations, 0.8 μCi of $^{45}CaCl_2$ and 1.4 μCi [6,6′(n)^{3}H] sucrose (4 μM), as a marker of the extracellular space (23, 24). The tubes were incubated in a water bath at 37°C without shaking. The incubation was stopped and the islets were separated from the incubation buffer (usually after 5 min of incubation) by centrifugation for 15 s at 8,000 *g* in a Greiner microfuge (type 2F1). By this procedure (25–28) the islets were effectively separated from the buffer by passage through the phthalate mixture and into the urea layer. Insulin release by the islets was assayed on an aliquot of the supernatant buffer. The bottoms of the tubes were cut above the urea layer and placed in 5 ml Instagel for liquid scintillation spectrometry. In every experiment blanks containing cut microfuge tubes without islets, standards of the radioactive medium (20 μl), and samples containing $^{45}Ca^{++}$ only for the estimation of spillover of $^{45}Ca^{++}$ counts into the ^{3}H channel were added for spectrometry. Blanks without islets did not differ from the background counts. Ca^{++} uptake was calculated from the $^{45}Ca^{++}$ space in excess of the [^{3}H]sucrose space. The sucrose space became maximal within 1 min of incubation and remained constant over 30 min. At 5 min the extracellular space was 1.22±0.10 nl/islets (n = 15) (mean±SEM) in the presence of 2.8 mM glucose and 1.25±0.11 nl/islet (n = 14) in the presence of 16.7 mM glucose.

The insulin release determined on the same islets as the Ca^{++} uptake, was corrected to indicate the true release over the 5-min incubation period by subtraction of the values measured at zero time. Immunoreactive insulin (IRI) was measured by the method of Herbert et al. (29) with rat insulin as standard. Neither epinephrine nor somatostatin interfered with the immunoassay at the concentrations employed.

Perifusion system and measurement of $^{45}Ca^{++}$ efflux. For the measurement of $^{45}Ca^{++}$ efflux, islets that had been preloaded during 45–47 h with $^{45}CaCl_2$ were perifused using 40 islets per chamber. The perifusion system has been described in detail elsewhere (30, 31), but the following modifications were made: the volume of the chamber was decreased to 70 μl and two rotating oxygen distributors also serving as medium reservoirs were connected to each chamber. The dead space of the system was approximately 1.4 ml and the flow rate was 1.4 ml/min. The perifusate consisted of KRB buffer containing 2.5 mM $CaCl_2$, 0.5% dialyzed bovine serum albumin, and 2.8 mM glucose. The islets were placed directly in the perifusion chamber without washing. From zero time to 46 min the islets were perifused with KRB buffer containing 2.8 mM glucose. At 46 min the glucose concentration was increased to 16.7 mM and the stimulation period continued for another 45 min. Somatostatin or epinephrine were added during the stimulation period only. No sample collections were made during the 40 min of the equilibration period. Fractions were then collected every minute between 41 and 65 min and thereafter every 5th min. An aliquot of the sample was assayed for IRI. To 0.8 ml of the samples 8 ml of Instagel was added for assay of $^{45}Ca^{++}$ by liquid scintillation spectrometry. After background subtraction, the counts per minute were normalized by setting the mean counts per minute of the five samples collected between 41 and 45 min to 100% and expressing the subsequent values as a percentage of this mean. The mean basal efflux ranged between 100 and 200 cpm. Statistical analysis was by Student's t test.

The materials employed and their sources were as follows: collagenase, 150 U/mg (Worthington Biochemical Corp., Freehold, N. J.), medium 199 and Hepes solution (Grand Island Biological Co., Grand Island, N. Y.), sodium penicillin G (Pfizer Inc., New York), streptomycin sulphate (Novo Industri A.S., Copenhagen, Denmark), plastic Petri dishes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.), bovine serum albumin (Behring-Werke AG., Marburg, W. Germany), Trasylol (kindly provided by Dr. H. Rüf, Bayer Pharma A.G., Zurich, Switzerland), l-ascorbic acid and l-epinephrine (Sigma Chemical Co., St. Louis, Mo.), phentolamine (Regitine, Ciba Geigy A.G., Basal, Switzerland), somatostatin (cyclic) was provided by Dr. R. Guillemin, (The Salk Institute, La Jolla, Calif.), guinea-pig anti pork insulin serum was a generous gift from Dr. P. Wright (the University of Indiana, Indianapolis, Ind.), rat insulin was provided by Dr. J. Schlichtkrull (Novo Research Institute, Copenhagen, Denmark), $^{45}CaCl_2$ and [6,6′(n)^{3}H] sucrose (The Radiochemical Centre, Amersham, England), and Insta-

[1] *Abbreviations used in this paper*: IRI, immunoreactive insulin; KRB buffer, Krebs-Ringer bicarbonate buffer.

gel (Packard Instrument International S. A., Zurich, Switzerland).

RESULTS

Time course of $^{45}Ca^{++}$ uptake and insulin release. $^{45}Ca^{++}$ uptake and insulin release were measured in the presence of either 2.8 or 16.7 mM glucose over different time periods between 0 and 30 min. As is shown in Fig. 1 $^{45}Ca^{++}$ uptake by the islets was biphasic. At both glucose concentrations an initial rapid phase, which was linear with time for 5 min, was followed by a phase with a lower rate of $^{45}Ca^{++}$ uptake. The uptake was stimulated by high glucose relative to low glucose at 1 min ($P < 0.02$) and at all subsequent time points. Insulin was released at a low basal rate in the presence of 2.8 mM glucose. The release rate was markedly stimulated by 16.7 mM glucose resulting in significantly stimulated cumulative insulin release at 2 min ($P < 0.005$) and thereafter. The sigmoidal pattern of insulin release in the presence of high glucose in this incubation system is explained by the biphasic release of insulin seen in the dynamic system (compare Fig. 3).

Since $^{45}Ca^{++}$ uptake was linear with time over the initial 5 min of incubation, subsequent measurements of Ca^{++} uptake were performed over 5 min only.

Effect of somatostatin on $^{45}Ca^{++}$ uptake and insulin release. As already observed 16.7 mM glucose caused a large increase in IRI release relative to 2.8 mM glucose when measured over the 5-min incubation period (Fig. 2). Somatostatin was tested at concentrations between 1 and 1,000 ng/ml (0.54 nM to 0.54 μM) in the presence of 16.7 mM glucose. All somatostatin concentrations used significantly inhibited IRI release. The inhibition was 36, 45, 72, and 73% with 1, 10, 100, and 1,000 ng/ml somatostatin, respectively. The change of glucose from 2.8 to 16.7 mM was associated with a doubling in $^{45}Ca^{++}$ uptake. Strikingly, somatostatin was without effect on $^{45}Ca^{++}$ uptake, even at the highest concentration of 1,000 ng/ml.

Effect of somatostatin on $^{45}Ca^{++}$ efflux and insulin release. Two concentrations of somatostatin 100 and 1,000 ng/ml were employed. The results of experiments with 1,000 ng/ml are shown in Fig. 3. Under control conditions, 16.7 mM glucose stimulated insulin release in the characteristic biphasic manner. Both phases of insulin release were inhibited by somatostatin. In this perifusion system with a dead space time of 1 min, the five 1-min collections between 47 and 51 min corre-

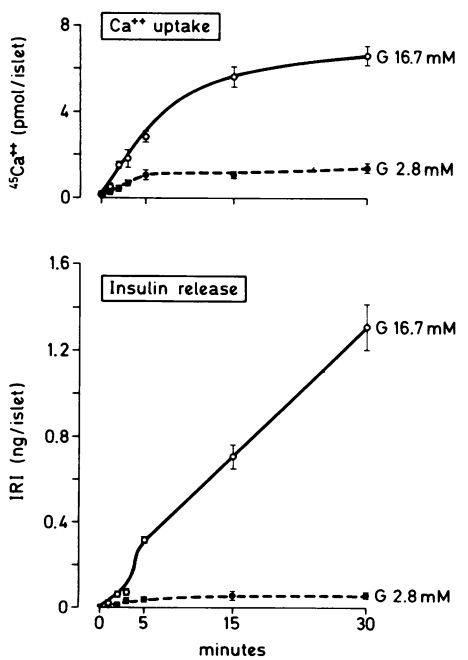

FIGURE 1 Time course of $^{45}Ca^{++}$ uptake and insulin release by islets incubated in the presence of 1 mM Ca^{++} and either 2.8 or 16.7 mM glucose. The mean values±SEM of four–five observations are shown. G, glucose.

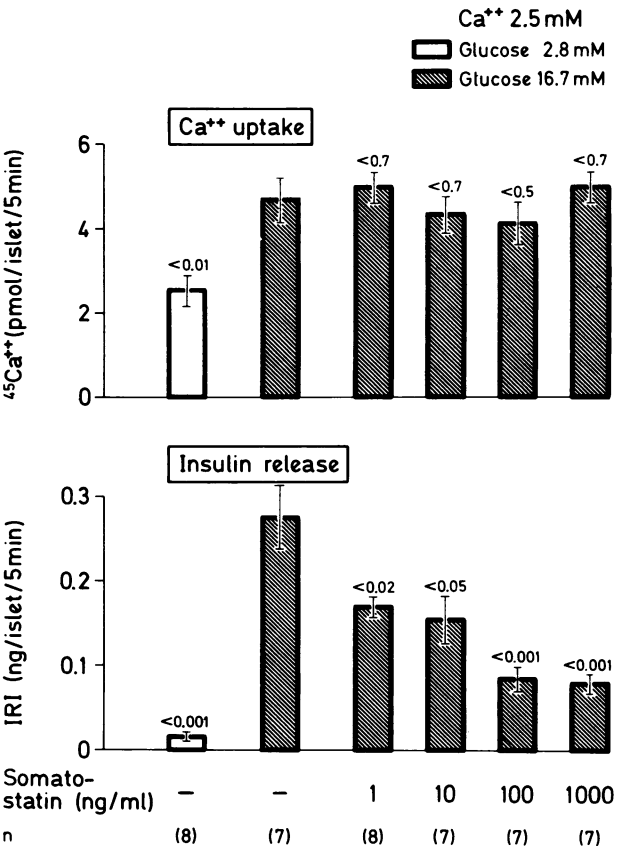

FIGURE 2 Effect of different somatostatin concentrations on $^{45}Ca^{++}$ uptake and insulin release by incubated islets in the presence of 16.7 mM glucose. The numbers above the bars indicate P values relative to 16.7 mM glucose alone. Vertical lines represent mean±SEM. n, number of observations.

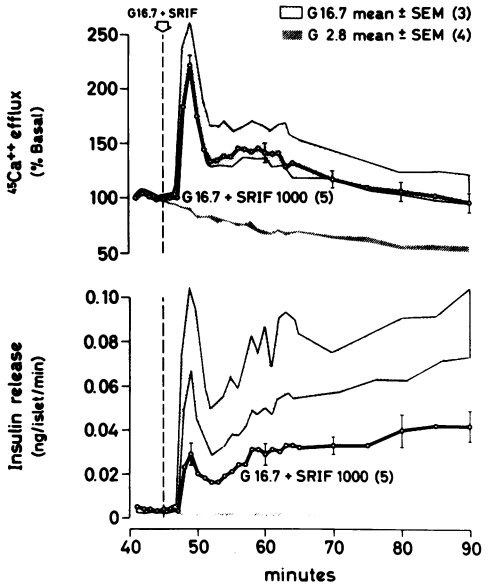

FIGURE 3 Effect of somatostatin (1,000 ng/ml) on glucose-stimulated insulin release (lower panel) and $^{45}Ca^{++}$ efflux (upper panel) from perifused islets. After 45 min the perifusate containing 2.8 mM glucose was replaced by medium containing 16.7 mM glucose and somatostatin. The results of paired control experiments in the absence of somatostatin are indicated by the dotted areas. Results of perifusions in the presence of 2.8 mM glucose throughout are depicted as hatched areas. Vertical lines represent mean±SEM. The number of observations is in parentheses. G, glucose; SRIF, somatostatin.

spond to the 5-min period used for the $^{45}Ca^{++}$ uptake studies and are used to assess the first phase of insulin release. The subsequent period from 52 to 90 min was considered to represent the second phase. The results of the planimetric analysis are as follows. The first phase was inhibited 72% and the second phase 55% by 1,000 ng/ml somatostatin. As is shown in Fig. 3 the stimulation with 16.7 mM glucose caused an increase of $^{45}Ca^{++}$ efflux which started within 1 min of the glucose reaching the islets and peaked 2 min later. $^{45}Ca^{++}$ efflux then decreased to about 50% of the peak value and remained elevated throughout the glucose stimulation. No inhibition of $^{45}Ca^{++}$ efflux by somatostatin was observed (Fig. 3). Similar results were obtained when 100 ng/ml of somatostatin was used. First phase insulin release was inhibited by 37% and second phase by 43% in the absence of any change in the characteristic $^{45}Ca^{++}$ efflux pattern.

Effects of epinephrine on $^{45}Ca^{++}$ uptake and insulin release. The effects of 16.7 mM glucose and four concentrations of epinephrine on $^{45}Ca^{++}$ uptake and insulin release were studied. In Fig. 4 are shown the results when 0.1 and 10 nM, 1 μM, and 0.1 mM epinephrine were employed. 16.7 mM glucose stimulated insulin release fourfold relative to 2.8 mM glucose. The glucose-stimulated insulin release was inhibited by epinephrine in a dose-related manner by 39, 52, 112, and 113% with 0.1 and 10 nM, 1 μM, and 0.1 mM epinephrine, respectively.

It is also shown in Fig. 4 that 16.7 mM glucose caused the expected increase of $^{45}Ca^{++}$ uptake. Epinephrine at 0.1 nM decreased $^{45}Ca^{++}$ uptake but the inhibition was not statistically significant. 10 nM, 1 μM, and 0.1 mM epinephrine all significantly inhibited Ca^{++} uptake.

The α-adrenergic blocking agent, phentolamine, was used to examine the nature of the inhibitory effect of epinephrine on $^{45}Ca^{++}$ uptake. As shown in Table I, 10 μM phentolamine abolished the inhibitory effect of 10 nM epinephrine on $^{45}Ca^{++}$ uptake and insulin release in the presence of 16.7 mM glucose.

Effect of epinephrine on $^{45}Ca^{++}$ efflux and insulin release. In these experiments epinephrine was tested at 0.1 nM, 10 nM, 1 μM, and 1 mM in the presence of 16.7 mM glucose. The results are shown in Fig. 5. 0.1 nM epinephrine had no effect on glucose-

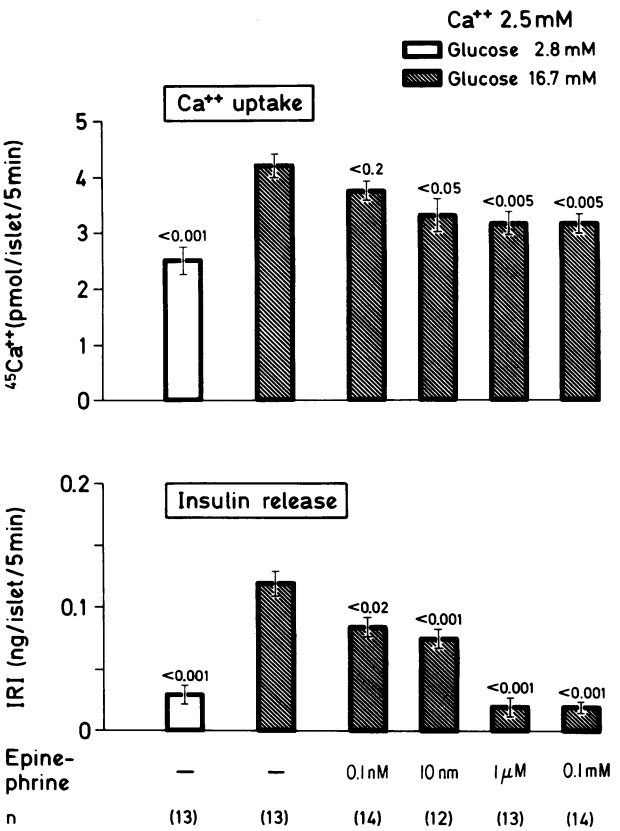

FIGURE 4 Effect of different epinephrine concentrations on insulin release and $^{45}Ca^{++}$ uptake by incubated islets in the presence of 16.7 mM glucose. The numbers above the bars indicate *P* values relative to 16.7 mM glucose alone. Vertical lines represent mean±SEM. *n*, number of observations.

TABLE I

Effect of Phentolamine on Epinephrine-Induced Inhibition of Glucose-Stimulated $^{45}Ca^{++}$ Uptake and Insulin Release from Incubated Islets

	$^{45}Ca^{++}$ uptake	P*	Insulin release	P*	n
	pmol/islet/5 min		*ng/islet/5 min*		
Glucose, 2.8 mM	0.69±0.17	<0.001	0.09±0.02	<0.001	5
Glucose, 16.7 mM	2.41±0.27	—	0.21±0.02	—	8
Glucose, 16.7 mM + epinephrine, 10 nM	1.60±0.13	<0.02	0.14±0.01	<0.005	9
Glucose, 16.7 mM + phentolamine, 10 μM	2.40±0.35	0.98	0.20±0.02	<0.50	9
Glucose, 16.7 mM + epinephrine, 10 nM + phentolamine, 10 μM	2.36±0.36	0.95	0.23±0.02	<0.30	7

The medium contained 1 mM Ca^{++}. Values expressed as mean±SEM.
* Relative to 16.7 mM glucose.

stimulated insulin release, nor did it affect the pattern of $^{45}Ca^{++}$ efflux. With 10 nM epinephrine, however, the first phase of insulin release was inhibited by 49% and the second phase by 62%. Despite these marked inhibitory effects, epinephrine at this concentration had no effect upon the pattern of $^{45}Ca^{++}$ efflux. From the results in Fig. 6, it can be seen that 1 μM epinephrine essentially abolished glucose-stimulated insulin release. The first phase release was inhibited by 95% and the second phase by 100%. This contrasted markedly with the slight nonsignificant effect of 1 μM epinephrine on glucose-stimulated $^{45}Ca^{++}$ efflux, which was decreased by 38% during the first phase and by 16% during the second phase.

Finally, two experiments were carried out in the presence of the very high epinephrine concentration of

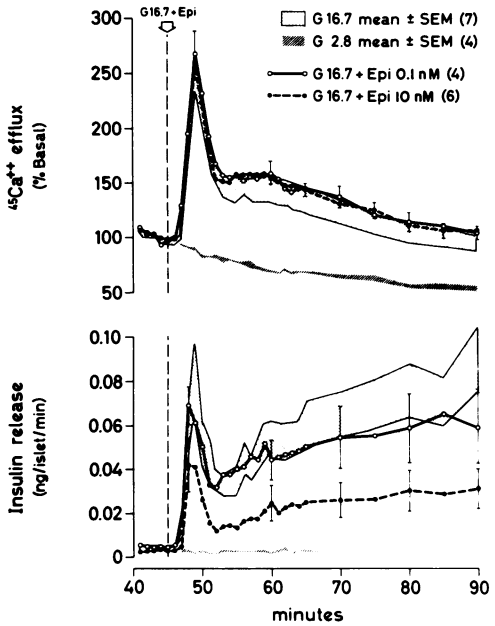

FIGURE 5 Effect of low concentrations of epinephrine (0.1 and 10 nM) on glucose-stimulated insulin release (lower panel) and $^{45}Ca^{++}$ efflux (upper panel) from perifused islets. After 45 min the perifusate containing 2.8 mM glucose was replaced by medium containing 16.7 mM glucose and the epinephrine concentration under test. The results of paired control experiments in the absence of epinephrine are indicated by the dotted areas. Results of perifusions in the presence of 2.8 mM glucose throughout are depicted as hatched areas. Vertical lines represent mean±SEM. The number of observations is in parentheses. G, glucose; Epi, epinephrine.

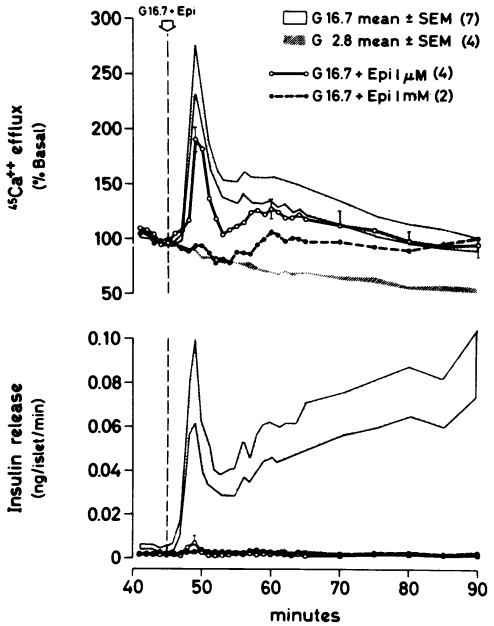

FIGURE 6 Effect of high concentrations of epinephrine (1 μM and 1 mM) on glucose-stimulated insulin release (lower panel) and $^{45}Ca^{++}$ efflux (upper panel) from perifused islets. After 45 min the perifusate containing 2.8 mM glucose was replaced by medium containing 16.7 mM glucose and the epinephrine concentration under test. The results of paired control experiments in the absence of epinephrine are indicated by the dotted areas. Results of perifusions in the presence of 2.8 mM glucose throughout are depicted as hatched areas. Vertical lines represent mean ±SEM. The number of observations is in parentheses. G, glucose; Epi, epinephrine.

1 mM. In this case both phases of insulin release were abolished, as expected. 1 mM epinephrine inhibited $^{45}Ca^{++}$ efflux by 83% during the first 5 min after the addition of high glucose and by 53% subsequently (Fig. 6).

DISCUSSION

Insulin release. The biphasic insulin release induced by 16.7 mM glucose is similar to that observed in the perfused pancreas with a rapid first phase, a clearly distinguishable nadir, and a slowly increasing second phase reaching about the same level of secretion rate as the first phase. The perifusion of isolated islets maintained under tissue culture conditions for 2 days therefore appears well suited for studies on insulin release.

$^{45}Ca^{++}$ uptake and efflux. The method of measuring $^{45}Ca^{++}$ uptake used in this study has been used previously for other tissues (25–27)and islets (28, 32) but has been modified to allow the simultaneous measurement of insulin release. As $^{45}Ca^{++}$ uptake was linear with time for 5 min, all experiments were performed over this period. Measurement of $^{45}Ca^{++}$ uptake over longer periods was not studied because without linearity the $^{45}Ca^{++}$ in the islet can no longer be interpreted solely in terms of uptake. It is clear that glucose caused a prompt and marked increase in the rate of $^{45}Ca^{++}$ uptake and insulin release. The values of uptake reported here are somewhat less than those reported for the larger islet from the ob-ob mouse (33, 34).

In previous studies on $^{45}Ca^{++}$ efflux from rat islets, the islets were loaded with $^{45}Ca^{++}$ for either 60 (35, 18) or 90 min (36). The pattern of $^{45}Ca^{++}$ efflux in response to glucose in these three studies was an initial inhibition of $^{45}Ca^{++}$ followed by a stimulation of efflux. In our studies with islets loaded with $^{45}Ca^{++}$ during 2 days in culture conditions and perifused in a micro (70-μl) perifusion chamber, the effect of glucose to inhibit $^{45}Ca^{++}$ efflux is not seen. Only the stimulation of $^{45}Ca^{++}$ efflux is observed. In other experiments to be reported elsewhere[2] the inhibitory effect of glucose on $^{45}Ca^{++}$ efflux was detected in the 2-day loaded islets by the device of lowering the external Ca^{++} concentration. Thus, the inhibitory effect of glucose on $^{45}Ca^{++}$ efflux is operative but is probably obscured by the rapidity of the effect of glucose to also stimulate $^{45}Ca^{++}$ efflux. In fact, in the results reported here, the stimulation of $^{45}Ca^{++}$ efflux peaks within 3 min of exposure to glucose, i.e., at a time when the inhibitory effect reported by others (35, 36) has not reached the nadir of the initial decrease of $^{45}Ca^{++}$ efflux.

Effect of somatostatin. Somatostatin inhibited glucose-stimulated insulin release in a dose-related fashion. The first phase insulin release measured over 5 min in the static system showed a similar sensitivity to somatostatin, as has been reported for the perfused pancreas (1, 2, 19) for monolayer cultures of the endocrine pancreas (3) and for isolated islets after 2 days in culture (21). When high concentrations of somatostatin were used (100 and 1,000 ng/ml) both phases of insulin release measured in the dynamic system were inhibited to a similar extent. Despite potent inhibition of insulin release, no effect of somatostatin on glucose-stimulated $^{45}Ca^{++}$ uptake or $^{45}Ca^{++}$ efflux could be detected. The present results appear to conflict with the recent reports by Oliver (37) and by Bhathena et al. (15) who observed an inhibition of glucose-stimulated retention of $^{45}Ca^{++}$ by 1,000 and 2,000 ng/ml somatostatin, respectively. The experimental conditions differed, however, in that the $^{45}Ca^{++}$ retained by the islets after 60 or 90 min incubation was measured rather than the rate of Ca^{++} uptake. Thus, the observed inhibition might be indirect or secondary to the action of somatostatin to decrease insulin release perhaps by influencing metabolism. For instance, somatostatin has recently been reported to inhibit glucose metabolism by isolated islets (6) and Sener et al. have shown that $^{45}Ca^{++}$ net uptake is proportional to glycolysis (38). The present study does not offer an explanation for the reported effects of calcium to attenuate the inhibitory action of somatostatin (13–16).

Effect of epinephrine. In the present study 0.1 nM epinephrine, a concentration approximating plasma levels in resting man (39), was the threshold concentration for inhibition of glucose-stimulated insulin release. The inhibition by 0.1 nM epinephrine was associated with a small, but nonsignificant, decrease in Ca^{++} uptake. Higher concentrations of epinephrine inhibited both release and Ca^{++} uptake. Like the effect of epinephrine to inhibit insulin release (40–44), the inhibition of $^{45}Ca^{++}$ uptake appears to be mediated by α-adrenergic mechanisms. It is of interest to note that when the insulin release in response to high glucose was abolished in the presence of 1 μM and 0.1 mM epinephrine, Ca^{++} uptake was only inhibited by 60%. Since Ca^{++} antagonists, like verapamil, could inhibit glucose-stimulated Ca^{++} uptake completely,[3] the possibility exists that epinephrine affects only one compartment of calcium uptake. Previous studies have dealt with the effect of high concentrations of epinephrine to inhibit $^{45}Ca^{++}$ retention by islets stimulated with glucose during prolonged incubations (15, 17, 37) rather than initial $^{45}Ca^{++}$ uptake.

In contrast to its effects on Ca^{++} uptake, epinephrine (with the exception of the extraordinarily high con-

[2] Kikuchi, M., C. B. Wollheim, G. S. Cuendet, A. E. Renold, and G. W. G. Sharp. Studies on the dual effects of glucose on $^{45}Ca^{++}$ efflux from isolated rat islets. Manuscript submitted for publication.

[3] Wollheim et al. Unpublished observations.

centration of 1 mM) did not alter significantly $^{45}Ca^{++}$ efflux evoked by glucose. Brisson and Malaisse (18), who applied 10 μM to 0.1 mM epinephrine during stimulation with glucose, observed an immediate decrease in $^{45}Ca^{++}$ efflux when extracellular Ca^{++} was present at normal concentrations, and an increase in $^{45}Ca^{++}$ efflux in the absence of Ca^{++}.

General considerations. The hypothesis that glucose initiated insulin release by increasing the concentration of ionized calcium in a critical compartment of the β-cell, is favored by the following data: (*a*) glucose stimulates uptake and increases net content of $^{45}Ca^{++}$ as assessed by four different methods (17, 28, 33, 34); (*b*) glucose increases the amount of calcium in the β-cells, as localized histochemically by the pyroantimonate precipitation technique (45); (*c*) calcium is able to induce insulin release either alone in high concentrations (46, 47) or in the presence of ionophore A23187 (48–50); (*d*) substances which interfere with Ca^{++} uptake, like Mg^{++} (51), Co^{++} (28), La^{+++} (52), and verapamil (53), inhibit glucose-induced insulin release. Since somatostatin, as well as epinephrine, inhibits insulin release stimulated by a variety of substances, it appears natural to focus on a site in the release mechanism common to many secretagogues when looking for the mode of action of the two inhibitors. The data presented here exclude an action of somatostatin on the Ca^{++} uptake mechanism of the islets. Somatostatin clearly caused a potent inhibition of insulin release without affecting either the influx or efflux of $^{45}Ca^{++}$. Epinephrine, on the other hand, did inhibit glucose-stimulated $^{45}Ca^{++}$ uptake and this could indicate a possible mode of action of this hormone (17) as could the inhibition of adenylate cyclase (54–56) and the lowering of islet cyclic AMP levels (57, 58). The latter seems unlikely since epinephrine inhibits insulin release even in the presence of dibutyryl cyclic AMP (9). Efendic et al. (59), measuring [^{3}H]cyclic AMP formation in freshly isolated islets, could not observe an effect of high somatostatin concentrations on islet cyclic AMP content, but in one condition cyclic AMP release into the medium was inhibited.

It has been suggested that at least part of the increase in $^{45}Ca^{++}$ efflux caused by high glucose concentrations is associated with insulin release and might be released together with the secretory granules (35). In the present study, a striking dissociation between insulin release and $^{45}Ca^{++}$ efflux was observed in that both somatostatin and epinephrine inhibited insulin release without affecting $^{45}Ca^{++}$ efflux. Thus, epinephrine at 1 μM abolished glucose-stimulated insulin release, while $^{45}Ca^{++}$ efflux was not significantly altered. It is clear, therefore, that the contribution of $^{45}Ca^{++}$ in the insulin containing granules to $^{45}Ca^{++}$ efflux after glucose stimulation is insignificantly small.

In conclusion, epinephrine could exert at least some of its inhibitory effect upon insulin release by inhibition of calcium uptake, whereas somatostatin is inhibitory to insulin release by a mechanism which does not affect glucose-induced changes in calcium fluxes.

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