Cytosolic Free-Calcium Concentrations in Normal Pancreatic Islet Cells

Effect of Secretagogues and Somatostatin

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SUMMARY

We have assessed the effect of somatostatin on glucose-, potassium-, forskolin-, and dibutyryl cAMP–induced changes in cytosolic free [Ca^{2+}] in normal rat pancreatic islet cells with the new Ca^{2+} indicator fura 2. The cytosolic free [Ca^{2+}] in islet cells incubated with nonstimulatory concentrations of glucose (30 mg/dl) ranged from 54 to 64 nM. In the presence of extracellular Ca^{2+} (1 mM), glucose (300 mg/dl) rapidly increased the cytosolic free [Ca^{2+}] to a level of 90–110 nM. In the absence of extracellular Ca^{2+}, glucose failed to increase the cytosolic free [Ca^{2+}], which remained at a level of 55–60 nM. Somatostatin inhibited glucose-induced increases in cytosolic free [Ca^{2+}] in a dose-dependent manner (maximal inhibition was 34%). Half-maximal inhibition was observed at 10^{-9} M somatostatin, which correlated well with somatostatin binding to islet cells (K_d = 2.6×10^{-10} M). Potassium (50 mM) rapidly increased the cytosolic free [Ca^{2+}] to 110–120 nM, and its effect was not influenced by the presence of somatostatin. Forskolin (20 μM) and dibutyryl cAMP (1 mM) rapidly increased cytosolic free Ca^{2+} both in the presence and absence of extracellular Ca^{2+}. More than 80% of the overall increase in cytosolic free-Ca^{2+} levels could be accounted for by the mobilization of intracellular Ca^{2+} stores. Somatostatin effectively blocked the forskolin effect (32% inhibition) but not the dibutyryl cAMP–induced effect. Somatostatin appears to inhibit secretagogue-induced increases in cytosolic free [Ca^{2+}] by interfering with cAMP production and probably with Ca^{2+} transport across the cell membrane. *Diabetes* 36:571–77, 1987

An increase in cytoplasmic free [Ca^{2+}] is commonly regarded as a key event in promoting insulin secretion (1–8). Rorsman et al. (9) and Deleers et al. (10) were the first to measure cytosolic free [Ca^{2+}] in normal islet cells. With the fluorescent Ca^{2+} indicator quin 2, these authors observed glucose-induced increases in cytosolic free Ca^{2+} in mouse and albino rat islet cells.

Although there is little doubt that glucose influences Ca^{2+} fluxes, it is unclear whether glucose is capable of mobilizing Ca^{2+} from intracellular stores. Similarly, the effect of cAMP on cytosolic free [Ca^{2+}] remains enigmatic. Direct measurements of the cytosolic Ca^{2+} in islet cells from *ob/ob* rats (11) and in the RINm5f cell line (12) failed to demonstrate a significant influence of cAMP. In contrast, Ca^{2+}-transport studies strongly suggested that cAMP might modulate cytosolic free [Ca^{2+}].

The precise mechanism of somatostatin action in inhibiting exocytotic hormone release is not fully understood. Somatostatin has been shown to interfere with cAMP production (13–15), phosphorylation of proteins by cAMP-dependent protein kinase (13,16), and increases in cytosolic free [Ca^{2+}] (17–19). The latter has been demonstrated in GH_4, GH_3, and AtT-20 pituitary cell lines. The effect of somatostatin on cytosolic free [Ca^{2+}] in pancreatic islet cells has not been evaluated.

We used the fluorescent probe fura 2 to determine the effect of somatostatin on glucose-, potassium-, forskolin-, and dibutyryl cAMP–induced changes in islet cell cytosolic free [Ca^{2+}]. The recent introduction of a new group of Ca^{2+} probes (20) has greatly improved our ability to evaluate changes in cytosolic free [Ca^{2+}]. Fura 2 has advantages over the previously used quin 2 method and allows determination of cytosolic free [Ca^{2+}] in relatively few cells (20).

MATERIALS AND METHODS

Materials. Fura 2 and fura 2-AM were obtained from Molecular Probes (Junction City, OR). Culture medium (TCM-199),

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Received for publication 25 September 1986 and accepted in revised form 12 December 1986.

fetal calf serum (FCS), Dulbecco's phosphate-buffered saline (PBS), and antibiotics were purchased through Gibco (Grand Island, NY). Collagenase (CLS-IV) was obtained from Worthington Biochemical (Freehold, NJ). All other biochemicals were purchased from Sigma (St. Louis, MO). Male Sprague-Dawley rats weighing 250–350 g were obtained from Simonson (Gilroy, CA). The animals were allowed food and water ad libitum. Anesthesia was induced with injections of pentobarbital sodium (45 mg/kg body wt i.p.).

Islet cell isolation technique. Pancreatic islets (1000–3000 islets) were isolated with a standard Ficoll isolation technique as previously described (21). After isolation, the islets were placed into 3 ml of TCM-199 tissue culture medium containing 10% FCS, 30 or 300 mg/dl glucose, 100 U/ml of penicillin, and 100 μg/ml of streptomycin for 18 h at 37°C under 5% CO_2 in air. On the day of the experiment, islets were centrifuged for 5 min at 500 × *g* at 4°C and resuspended in 3 ml Dulbecco's PBS buffer containing 50 mg/dl glucose and 1 mg/ml trypsin. Islets were aspirated 5 times through a 14-gauge needle at room temperature and incubated at 37°C for 3 min with gentle agitation. Islets were then disrupted into individual cells with 5 gentle aspirations through a 20-gauge needle at 4°C. Cells were centrifuged at 500 × *g* for 3 min at 4°C, resuspended, and aspirated 5 times through a 20-gauge needle in 2 ml of Krebs-HEPES buffer containing 118.4 mM NaCl, 4.69 mM KCl, 1.2 mM $MgCl_2$, 1.18 mM KH_2PO_4, 1.25 mM $NaHCO_3$, 20 mM HEPES, 5 mg/ml bovine serum albumin (BSA), and 30 mg/dl glucose. A cell count was then obtained in the presence of 0.04% trypan blue. The cell yield ranged between 250 and 400 cells per islet with cell viability >85%.

Calibration of fluorescence spectra. The fluorescence spectra for the indicator fura 2 were determined in the absence and presence of Ca^{2+} as described by Grynkiewicz et al. (20). Both excitation and emission spectra were determined at saturation (1 mM Ca^{2+}) and at zero (1 mM EGTA) [Ca^{2+}]. The spectrum of 1.5 μM fura 2 was recorded in 2.4 ml of Krebs-HEPES buffer, pH 7.4, while thermostated at 37°C and in the intracellular ionic milieu (same as above, except 20 mM NaCl and 133.8 mM KCl). Sample spectra curves are shown in Fig. 1. There was no difference between the two buffers used. The excitation maximum of the fura 2–Ca^{2+} complex was found at a wavelength of 347 nm, compared to 370 nm for the free probe. A clear crossover point in the spectra of free- and bound-Ca^{2+} forms of fura 2 was at 360 nm. Emission maxima for the fura 2–Ca^{2+} complex and free probe were 479 and 477 nm, respectively.

Utilizing the spectra data for fura 2 at zero and saturating Ca^{2+} at an excitation wavelength of 347 nm (λ_1) and 387 nm (λ_2), we obtained the following: R_{min} = 0.707, R_{max} = 9.550, and Sf_2/Sb_2 = 5.983 with K_d = 224 nM, where R is ratio of fluorescence at λ_1/λ_2, and Sf_2 and Sb_2 are the fluorescence intensities of fura 2 in the free and bound forms at λ_2. Emission was determined at 478 nm.

Fura 2 loading. The loading procedure was performed according to the method described by Grynkiewicz et al. (20). Briefly, 5 μl of 1.249 mM fura 2-AM dissolved in dimethyl sulfoxide (DMSO) was added to 2 ml of cell suspension (in the presence of 1 mM $CaCl_2$) to yield a fura 2 concentration of 3.11 μM. Tubes were covered with aluminum foil to protect them from light and were incubated for 40 min at 37°C. Under

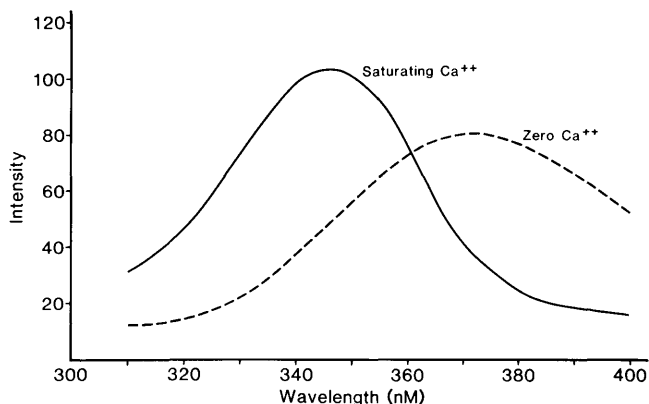

FIG. 1. Excitation spectra of 1.5 μM fura 2 in 2.4 ml of Krebs-HEPES buffer, pH 7.45, containing 1 mM EGTA before (zero Ca^{2+}) and after (saturating Ca^{2+}) addition of 2 mM $CaCl_2$. Emission was at 478 nm.

these conditions, fura 2-AM permeates the cells, is hydrolyzed to fura 2, and binds cytosolic free Ca^{2+}. To remove the unincorporated probe, the cells were centrifuged for 3 min at 500 × *g* at 4°C, washed in Krebs-HEPES buffer, and resuspended in this buffer at a concentration of 4–12 × 10^4 islet cells/ml. For the [Ca^{2+}] measurements, 2.4 ml of cell suspension (1–3 × 10^5 cells) was transferred to a 3-ml thermostated cuvette (37°C), where the cells were continuously stirred during the entire measurement.

Measurement of intracellular Ca^{2+}. The fluorescence of control and fura 2–loaded islet cells was measured at excitation wavelengths of 347 and 387 nm and at an emission wavelength of 478 nm with a Turner model 430 spectrofluorometer (fitted with a magnetic stirrer and a thermostated cuvette holder). Additions of concentrated test substances were made directly to the cuvettes to yield the desired concentrations. The changes in fluorescence measurements were recorded, and the 347:387 ratio (R) was determined. The free intracellular [Ca^{2+}] before and after the addition of the agents under study was calculated from the equation

$$[Ca^{2+}] = K_d \times \frac{R - R_{min}}{R_{max} - R} \times \frac{Sf_2}{Sb_2}$$

Introducing our calibration constants, we obtained

$$[Ca^{2+}] = 224\text{ nM} \times \frac{R - 0.707}{9.550 - R} \times 5.983$$

Weekly examination of the fluorescence spectra demonstrated the stability of each ratio measured.

Because of concerns that islet cell viscosity may alter the values of cytosolic free [Ca^{2+}] obtained with this method (22,23), we performed additional experiments in which maximum and minimum fluorescence (F_{max} and F_{min}) were estimated as previously reported for quin 2 (24). In these experiments, the cells were lysed with 0.04% Triton X-100, and the F_{max} was measured in the presence of 1 mM $CaCl_2$. The F_{min} was then measured in the presence of 2 mM EGTA and 50 mM Tris base (pH >8.3). The fluorescence of the extracellular fura 2 was estimated by adding $MnCl_2$ (50 μM), which quenches extracellular fura 2. $MnCl_2$ was then chelated by

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the addition of 100 μM pentetic acid. The estimate of extracellular fura 2 was made before stimulation of the cells with secretagogues. The results obtained with this method (method II) were compared with the results obtained by the ratio method (method I).

Somatostatin binding. ^{125}I-labeled somatostatin (SRIF; 1-Tyr-SRIF) with the specific activity of 2.231 Ci/μM was purchased from New England Nuclear.

The binding buffer contained 118.4 mM NaCl, 4.7 mM KCl, 1.2 mM $MgCl_2$, 1.18 mM KH_2PO_4, 1.33 mM $NaHCO_3$, 20 mM HEPES, 16.6 mM glucose, 0.5% BSA, and 800 KIU/ml trasylol, pH 7.6.

The binding reaction (~20,000 cells/tube, 40 pM ^{125}I-SRIF, total vol 250 μl) was carried out at 30°C for 30 min. Unbound ligand was removed by centrifugation in a Beckman microfuge (1 min at 10,000 rpm). The pellet was washed once with ice-cold Krebs-HEPES buffer, and its radioactivity was determined. The binding of the ligand in the presence of 10^{-7} M unlabeled somatostatin was considered nonspecific binding.

RESULTS

Initially, we determined the absorbance spectra and fluorescent properties of fura 2 in the presence and absence of Ca^{2+} with a Turner 430 spectrofluorometer. Figure 1 demonstrates excitation spectra of 1.5 μM fura 2 in buffer containing either 1 mM EGTA (zero Ca^{2+}) or 1 mM $CaCl_2$. Similar spectra were examined for five different concentrations of fura 2 (data not shown). In all experiments, the maximum fluorescence of fura 2 saturated with Ca^{2+} was found at a wavelength of 347 nm, whereas fluorescence of free fura 2 was detected at a wavelength of 370 nm. The crossover points were consistently found at a wavelength of 360 nm. The fluorescence of either the buffers used in these studies or tissues (without fura 2) was 10–12.5% that observed with the cells loaded with the probe. Cellular or buffer autofluorescence did not change in response to either glucose or other secretagogues.

In the initial experiment, we studied the effect of glucose on cytosolic free [Ca^{2+}] in the presence and absence of extracellular Ca^{2+}. In the presence of extracellular Ca^{2+} (1 mM), glucose increased cytosolic free [Ca^{2+}] from 55 ± 4 to 113 ± 8 nM ($P < .01$). However, in the absence of extracellular Ca^{2+} (Ca^{2+}-free Krebs-HEPES buffer with 1 mM EGTA), glucose failed to increase the cytosolic free [Ca^{2+}], which remained at a level of 55–60 nM (Fig. 2).

The effect of EGTA on basal cytosolic free [Ca^{2+}] was minimal. EGTA (1 mM) reduced the cytosolic free [Ca^{2+}] by 7 ± 1.1 nM (range 0–10 nM). To rule out the possibility that the EGTA-induced decrease in cytosolic free [Ca^{2+}] masks the glucose-induced rise, we performed the following experiments. The cells were incubated with glucose 30 mg/dl and no Ca^{2+} for 3 min, and cytosolic free [Ca^{2+}] was measured (47 nM). The glucose concentration was then raised to 300 mg/dl simultaneously with the addition of EGTA (1 mM). Cytosolic free [Ca^{2+}] was measured at 15, 30, 60, 120, 180, 240, and 300 s. Again, glucose failed to increase intracellular free [Ca^{2+}], which remained at the basal level. Moreover, when the islets were incubated without extracellular Ca^{2+} but in the absence of EGTA, glucose was ineffective in raising cytosolic free [Ca^{2+}]. These observations indicate that the presence of EGTA does not mask the glucose effect.

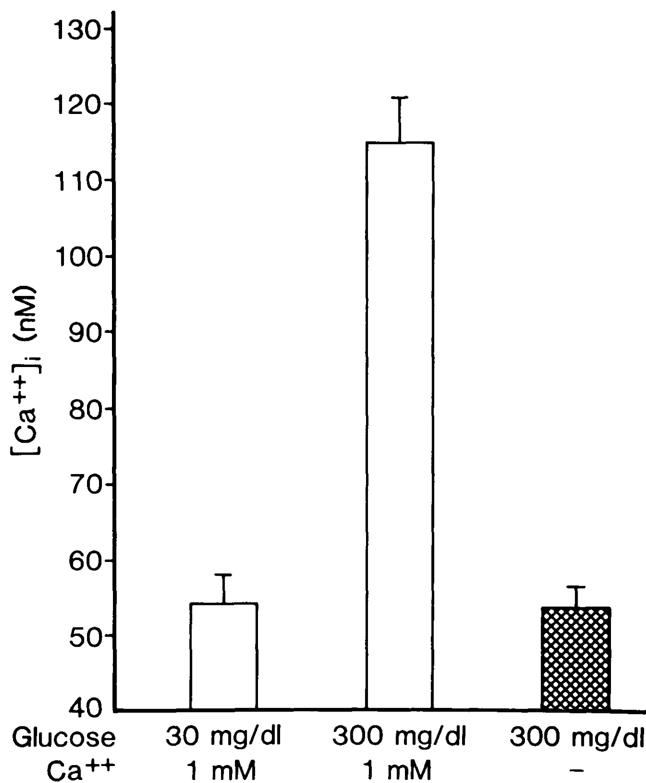

FIG. 2. Cytosolic free [Ca^{2+}] in islet cells incubated with high (300 mg/dl) glucose concentrations in absence or presence of 1 mM Ca^{2+}. Results represent means ± SE of 14 separate experiments.

Partial degranulation of the islet cells produced by incubating the islets in 16.7 mM glucose for various intervals (up to 24 h) before cell isolation did not alter either the basal or glucose-stimulated intracellular [Ca^{2+}]. Furthermore, there was no leakage of the fura 2–Ca^{2+} complex from the cells into the incubation media, as determined by Mn^{2+} displacement of Ca^{2+}. $MnCl_2$ does not enter the cell but may displace Ca^{2+} from its complex with fura 2. Therefore, if a drop in fluorescence is seen after addition of $MnCl_2$, it would indicate that the fura 2–Ca^{2+} complex is leaking into the media. At various stages of the experimental incubations, $MnCl_2$ (1 mM) was added to the media. There was no change in the fluorescence ratio, suggesting that the fura 2–Ca^{2+} complex does not leak out of cells. Similarly, the buffer (in which the cells were incubated during experiments) demonstrated identical fluorescence before and after the incubations and measurements.

In the subsequent experiments, we assessed the effect of somatostatin on glucose-induced increases in cytosolic free [Ca^{2+}]. The cells were preincubated with various concentrations of SRIF for 10 min at 37°C before being exposed to 300 mg/dl of glucose. SRIF dose-dependently inhibited the glucose-induced increase in intracellular [Ca^{2+}] (Fig. 3). Maximal inhibition (34%) of the glucose-induced increase in intracellular [Ca^{2+}] was achieved with 10^{-6} M SRIF (Fig. 4), and half-maximal inhibition was observed at 10^{-9} M (Fig. 5).

This concentration of SRIF yielded >50% inhibition of ^{125}I-SRIF binding to isolated pancreatic islets or dispersed pan-

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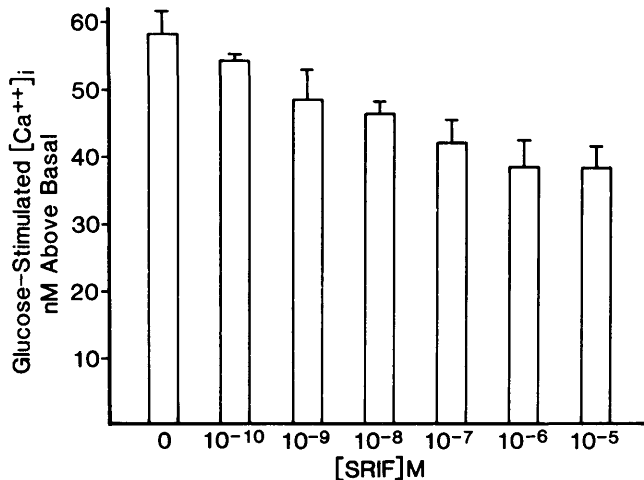

FIG. 3. Effect of somatostatin on glucose-induced increase in cytosolic free [Ca^{2+}] in isolated rat islet cells. Cells were preincubated with SRIF for 10 min at 37°C and then challenged with glucose (300 mg/dl). Measurements were obtained before and 5 min after addition of glucose. Results represent means ± SE of 5 experiments. *P* values versus control values are <.02 at 10^{-9} M and <.01 at all higher concentrations of somatostatin.

creatic cells. K_d for ^{125}I-SRIF binding was 2.6 × 10^{-10} M, with ~23,000 binding sites per cell.

To evaluate the mechanism of SRIF inhibition of the glucose-induced Ca^{2+} increase, we assessed somatostatin action on Ca^{2+} entry via voltage-dependent channels. Depolarization of the islet cells with 50 mM K^+ resulted in a rapid increase in intracellular [Ca^{2+}]. This effect of K^+ was not suppressed by SRIF (Fig. 6).

Because an increase in the cellular cAMP content is associated with insulin release, we examined the effects of forskolin, an activator of adenylate cyclase, and dibutyryl cAMP on the cytosolic free [Ca^{2+}]. Both agents rapidly increased [Ca^{2+}] in the presence and absence of extracellular Ca^{2+} (Fig. 7). Their effect was ~20% greater in the presence of extracellular Ca^{2+}. These data suggest that cAMP is capable of raising intracellular [Ca^{2+}].

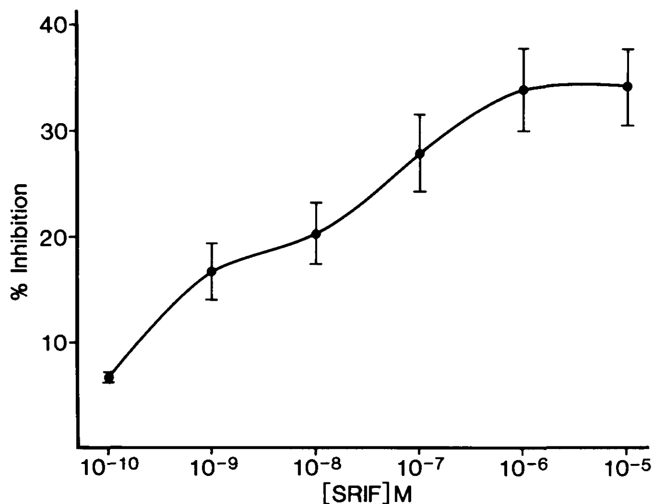

FIG. 4. Effect of somatostatin on glucose-induced increase in cytosolic free [Ca^{2+}] expressed as percent inhibition. Results represent means ± SE of 5 experiments.

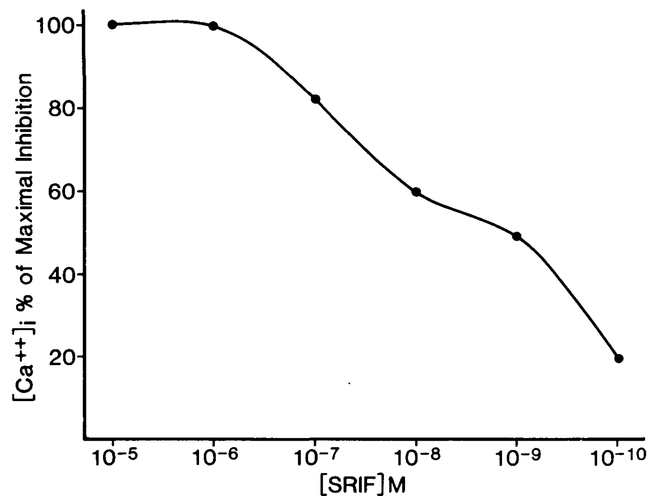

FIG. 5. Effect of somatostatin on glucose-induced increase in cytosolic free [Ca^{2+}] expressed as percent of maximal effect of somatostatin. Results represent mean values of 5 experiments.

SRIF inhibited the forskolin-induced but not the dibutyryl cAMP–induced rise in intracellular [Ca^{2+}], indicating that SRIF exerts its action by blocking forskolin-induced cAMP formation. The SRIF effect was identical in the presence and absence of extracellular Ca^{2+}.

Recent observations that tissue viscosity may change the parameters R_{min} and R_{max} calculated from the ratio method (22,23) prompted us to perform additional experiments in which F_{max} and F_{min} were estimated as previously reported for the quin 2 method. The results obtained with both methods are summarized in Table 1. As can be seen, the results obtained with the ratio method (method 1) are lower than those obtained with cell solubilization (method 2). The factor between the two methods is 1.95 ± 0.11 (n = 24).

DISCUSSION

This investigation was undertaken to examine the effect of various secretagogues on the regulation of cytosolic free

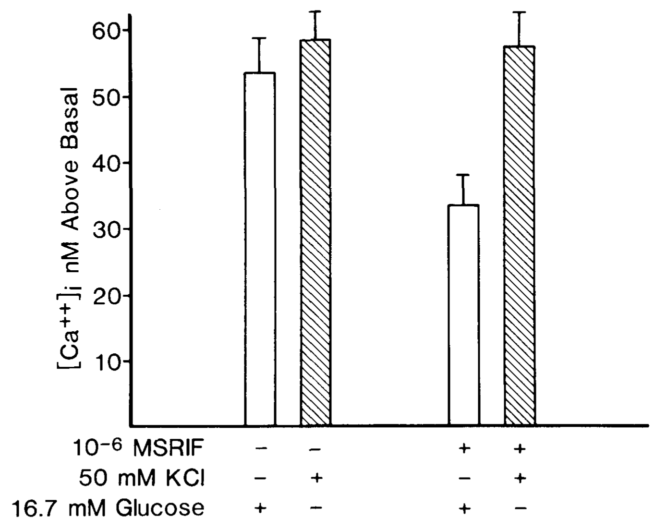

FIG. 6. Effect of somatostatin on K^+-induced increase in cytosolic free [Ca^{2+}] in pancreatic islet cells. Cells were preincubated with SRIF for 10 min at 37°C and challenged with glucose (*open bars*) or KCl (*hatched bars*). Results represent means ± SE of 5 experiments.

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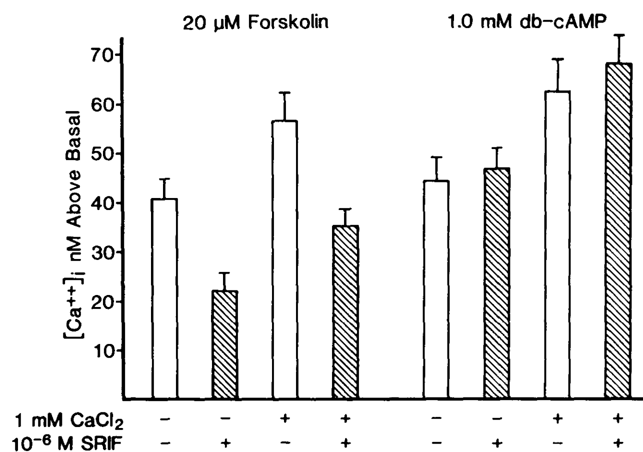

FIG. 7. Effect of somatostatin on forskolin- and dibutyryl cAMP–induced increase in cytosolic free [Ca^{2+}]. Cells were preincubated with (*hatched bars*) or without (*open bars*) somatostatin for 10 min at 37°C and then challenged with forskolin or dibutyryl cAMP (db-cAMP). Experiments were conducted with or without extracellular Ca^{2+} as indicated. Results represent means ± SE of 4 experiments.

[Ca^{2+}] in isolated islets. Particular attention was paid to the interrelationship between various secretagogues and somatostatin in producing alterations in cytosolic [Ca^{2+}].

Initially the data seemed to suggest that secretagogues act at different sites in promoting increases in cytosolic free [Ca^{2+}], some agents exerting their principal effects on promoting Ca^{2+} influx from the extracellular compartment and other agents exerting a predominant role in the mobilization of intracellular Ca^{2+} stores. Thus, glucose may exert its major action on promoting Ca^{2+} influx, whereas agents that act by cAMP-dependent pathways seem to evoke intracellular mobilization of Ca^{2+}.

The results of our experiments strongly suggest that in the absence of extracellular Ca^{2+}, glucose cannot increase intracellular free [Ca^{2+}]. These observations may indicate that the effect of glucose on cytosolic free [Ca^{2+}] is due to influx of extracellular Ca^{2+} into the cell rather than mobilization of intracellular Ca^{2+}. Previous experimental data point to the effect of glucose on promoting a net influx of extracellular Ca^{2+} into the β-cells (5,25–30). Although evidence exists that glucose can mobilize Ca^{2+} from the intracellular stores (5,7), it has not been confirmed by direct measurements in the absence of extracellular Ca^{2+}. In fact, our data suggest that glucose is unable to mobilize intracellular Ca^{2+} in the absence of extracellular Ca^{2+}. This failure of glucose to increase intracellular free [Ca^{2+}] in our experiments may be a result of rapid efflux of intracellular Ca^{2+} into the Ca^{2+}-deprived media. This rapid efflux may overcome and/or mask a glucose-induced increase in cytosolic free Ca^{2+}. Our experiments in which high glucose concentrations were added to the islet cells simultaneously with EGTA were undertaken to rule out this possibility. Indeed, the simultaneous addition of glucose and EGTA into the medium containing 1 mM Ca^{2+} resulted in no increase in cytosolic free [Ca^{2+}]. These observations suggest that rapid efflux does not account for the failure of glucose to raise intracellular Ca^{2+} in the absence of extracellular Ca^{2+}. A possible, albeit unlikely, explanation of these data is that the addition of EGTA simultaneously with glucose may still produce rapid and immediate efflux of Ca^{2+}, which would mask a putative increment in intracellular [Ca^{2+}] induced by glucose.

The most likely explanation of our findings is that extracellular Ca^{2+} is needed for the initial events of glucose action. It has been suggested that an early response to glucose stimulation is the breakdown of phosphatidylinositol bisphosphate and generation of inositol phosphates, including inositol triphosphate (IP_3) (31–34). Furthermore, an increased concentration of IP_3 has been proposed to be a crucial step in secretagogue-induced insulin release (35). However, recent data from several laboratories indicate that in islets, the generation of IP_3 may require extracellular Ca^{2+} (31,36). Moreover, it has been shown that an increase in IP_3 did not correlate with cellular Ca^{2+} efflux (37). Our results in normal rat islet cells are consistent with the notion that extracellular Ca^{2+} must be present for glucose to increase intracellular free [Ca^{2+}].

Our data and previously published reports of Rorsman et al. (9) and Deleers et al. (10) are in disagreement with the recent study of Boyd et al. (38) in cultured HIT cells, in which glucose did not increase cytosolic free [Ca^{2+}] even in the presence of extracellular Ca^{2+}, although it evoked insulin release. The authors suggested that an increase in intracellular [Ca^{2+}] may not be the primary signal that initiates glucose-stimulated insulin release in this β-cell line.

Assuming that somatostatin acts mainly by suppressing cAMP production (13–15), note that somatostatin inhibits glucose-induced but not K^+-induced increases in cytosolic free-Ca^{2+} levels. It would seem that glucose-induced Ca^{2+} increases are at least partly mediated by cAMP-dependent pathways, whereas the changes in cytosolic free Ca^{2+} induced by K^+ (50 μM) do not involve cAMP.

Forskolin and dibutyryl cAMP both increase cytosolic free [Ca^{2+}]. The status of our understanding of the interactions of cAMP-dependent pathways with Ca^{2+} fluxes and cytosolic free [Ca^{2+}] is not complete (39,40). Numerous studies have suggested that cAMP may produce an enhancement in Ca^{2+} influx and efflux and increase cytosolic free [Ca^{2+}] (41–45). However, two attempts to measure cytosolic free [Ca^{2+}] in pancreatic islets (one in *ob/ob* mice and another in clonal RINm5F cells) stimulated with forskolin, dibutyryl cAMP, or IBMX failed to demonstrate significant changes (11,12). Normal rat islet cells as used in our study may be more sensitive to cAMP stimulation. The increase in intracellular [Ca^{2+}] evoked by forskolin and dibutyryl cAMP in the absence of

TABLE 1
Comparison of two methods of calculating intracellular [Ca^{2+}] in pancreatic islet cells*

	Method 1† (nM)	Method 2‡ (nM)
30 mg/dl glucose	61.3 ± 8.4	105.1 ± 15.5
300 mg/dl glucose +	135.6 ± 9.9	262.9 ± 17.6
10^{-7} M SRIF	84.8 ± 17.1	128.9 ± 13.3
20 μM forskolin +	142.1 ± 11.1	246.5 ± 8.2
10^{-7} M SRIF	124.7 ± 12.8	176.9 ± 3.8
1 mM dibutyryl cAMP	142.5 ± 6.4	278.4 ± 21.7
50 mM KCl	117.6 ± 20.8	313.7 ± 36.9

*Factor between the 2 methods is 1.95 ± 0.11 (n = 24).
†Method based on ratios determined from fluorescence of nonviscous calibration buffers.
‡Method based on maximal and minimal fluorescence obtained after cell solubilization at end of experiment.

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extracellular Ca^{2+} was ~80% of that achieved in the presence of extracellular Ca^{2+}. These data suggest that the major site of cAMP action in increasing intracellular [Ca^{2+}] appears to be at the level of mobilization of intracellular Ca^{2+} stores.

We observed that the increases in cytosolic free Ca^{2+} induced by forskolin were inhibited by somatostatin. However, somatostatin in maximal doses did not suppress the increases in cytosolic free Ca^{2+} evoked by dibutyryl cAMP. This is consistent with the thesis that somatostatin acts by suppressing adenylate cyclase activity (i.e., the production of cAMP).

Note that somatostatin inhibits forskolin-induced increases in cytosolic free-Ca^{2+} levels to the same extent whether Ca^{2+} is present or absent in the extracellular medium. The inference is that both intracellular mobilization and Ca^{2+} influx are affected to some extent by somatostatin.

The precise mechanism of somatostatin action is not completely understood. In pancreatic islets, somatostatin binding to its cell surface receptor is followed by the inhibition of cAMP generation (13–15), a decrease in membrane permeability to Ca^{2+} (17–19), and disruption of the electrical activity associated with glucose-induced insulin release (46). Several investigators reported that somatostatin is capable of inhibiting cAMP-dependent protein kinase activity (13,16).

Recently, somatostatin has been shown to inhibit secretagogue-induced increases in cytosolic free Ca^{2+} (17–19). This effect has been suggested to be independent of cAMP (17) and to extend beyond voltage-dependent Ca^{2+} channels (18). Previous data describing somatostatin action on Ca^{2+} fluxes in pancreatic islets are contradictory (14,47–49), and somatostatin effects on cytosolic free Ca^{2+} in islet cells have not been investigated. The results of our study are consistent with somatostatin action via inhibition of cAMP formation and Ca^{2+} transport across the cell membrane. The concentrations of somatostatin that produced maximal and half-maximal inhibition of glucose-induced augmentation in intracellular [Ca^{2+}] are compatible with somatostatin-binding constants (herein) and concentrations of somatostatin needed to inhibit insulin release (48).

Finally, the method of calculation of the cytosolic free [Ca^{2+}] with fura 2 seems to be affected by the tissue viscosity (22,23). Because increased viscosity enhances the fluorescence of fura 2 disproportionately at longer wavelengths (23), R_{min} and R_{max} are shifted downward, resulting in lower values of cytosolic free [Ca^{2+}] obtained with the originally described method and formulas (20). Viscosity does not change the effective K_d of Ca^{2+} and fura 2 binding. In PtK_2 cells, the corrected values of R_{min} and R_{max} were reported as 0.85 times those obtained in nonviscous calibration solutions (23).

In islet cells, the tissue viscosity seems to affect the absolute values of cytosolic free [Ca^{2+}] but not the relative changes elicited by various secretagogues. It is conceivable that the processes of intracellular Ca^{2+} mobilization and changes in Ca^{2+} fluxes are not separable, isolated processes. The various stimulatory and inhibitory steps in this process are probably closely related and need to be further elucidated.

ACKNOWLEDGMENTS

We are grateful for the secretarial assistance of Evelyn Fitzgerald and Gloria Smith.

This research was supported by the Medical Research Service, the Veterans Administration, NIH Grant AM-32006, and the Diabetes Research Foundation of Colorado. B.D. is a recipient of the Clinical Investigator Award from the Veterans Administration.

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