

Somatostatin: Mechanism of Action in Pancreatic Islet β -Cells

CAROLINE S. PACE AND JOHN T. TARVIN

SUMMARY

The widespread role of somatostatin (SRIF) as a mediator of function in the brain and gut has stimulated interest in its mechanism of action. We have examined the mode of action of SRIF in stimulus-secretion coupling in the pancreatic islet β -cell to determine whether SRIF antagonizes the glucose-induced decrease in K^+ permeability (P_K). The influence of SRIF on ^{86}Rb fluxes and insulin release in cultured rat islet cells, and also the electrical events recorded from cultured islets and microdissected mouse islets, was examined. In cultured islets, 100 ng/ml SRIF in the presence of 16.7 mM glucose inhibited the incidence of spike activity and evoked hyperpolarization. This effect was counteracted by 0.1 mM quinine and 20 mM tetraethylammonium (TEA), drugs that inhibit the Ca^{2+} -sensitive or voltage-sensitive increase in P_K , respectively. These agents also counteracted the inhibitory influence of SRIF on glucose-induced insulin release in cultured islets. SRIF disrupted the typical glucose-induced oscillatory pattern of electrical activity (burst activity) during continuous microelectrode recordings in mouse β -cells, resulting in a transient 5 mV hyperpolarization and a decrease in the frequency of generation of burst activity. The presence of 20 mM TEA prevented the influence of SRIF on the electrical activity. SRIF had no effect on the accumulation of ^{86}Rb into islet cells obtained in the presence of 16.7 mM glucose. However, SRIF enhanced the rate of ^{86}Rb efflux from cells exposed to glucose. SRIF-induced enhancement of ^{86}Rb efflux was antagonized by TEA or quinine. These results indicate that SRIF may activate P_K as its primary mode of action, an event that may be sufficient to reduce the accumulation of intracellular Ca^{2+} thereby disrupting glucose-induced stimulus-secretion coupling. *DIABETES* 30:836–842, October 1981.

The widespread role of somatostatin (SRIF, somatostatin release inhibiting factor) as a modulator of neural activity as well as endocrine and exocrine secretion has stimulated interest in its mechanism of action. Most of the available evidence concerning the

mechanism of action of SRIF indicates that its primary effect resides in disrupting the transduction of receptor information at the level of the plasma membrane.¹ SRIF inhibits the generation of cyclic AMP,^{2–5} decreases the membrane permeability to Ca^{2+} ,^{4,6,7} and disrupts the electrical activity associated with glucose-induced insulin secretion.⁸ Alterations in the membrane potential and in the oscillatory nature of the spike activity induced by high glucose appear to be primarily due to changes in K^+ permeability (P_K).^{9–14} The ability of SRIF to antagonize glucose activation of the electrical events in the β -cell plasma membrane may be due to a countermodulatory action of SRIF on P_K .

The present study was designed to clarify the influence of SRIF on ionic mechanisms in relation to secretory changes. Specifically, the effects of SRIF on ^{86}Rb fluxes and insulin release in cultured rat islet cells, and on the electrical events recorded from cultured islets and microdissected mouse islets, were determined.

METHODS

Animals, isolation, and culture of islets. Islets of Langerhans were isolated from fed male Sprague-Dawley rats (250–300 g) by the collagenase technique¹⁵ in combination with a Ficoll gradient.¹⁶ Islets were cultured from 3–4 days in RPMI-1640 with the addition of 10% fetal calf serum (heat-inactivated), 100 U/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin sulfate. Cultured rat islets were used in all experiments, except for the electrical studies in which microdissected mouse islets were used for continuous electrode recordings to assess the action of SRIF on a single β -cell. The islets were isolated from fed male CBA/J mice obtained from Jackson Laboratories (Bar Harbor, Maine).

Electrophysiologic studies. Within 3–4 days of culture, the islets attached to the plastic culture dish. The culture me-

From the Department of Physiology and Biophysics, and Department of Physics, University of Alabama, Birmingham, Alabama.
Address reprint requests to Dr. Caroline S. Pace, Diabetes Hospital, 1808 Seventh Avenue South, University of Alabama, Birmingham, Alabama 35294.
Received for publication 30 December 1980 and in revised form 1 June 1981.

dium was replaced by a Krebs-Ringer bicarbonate-buffered medium containing, in addition, 25 mM Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid). The medium was gassed with 95% O₂ and 5% CO₂, and maintained at 37°C. The electrophysiologic setup and procedures were as described previously.⁸ Several islet cells were impaled during exposure to each medium containing the desired additions (see Table 2 for details). The membrane potential and presence of electrical activity were obtained from individual islet cells that portrayed a stable potential after at least 1 min of impalement with a microelectrode. In each experiment, 4 to 6 islet cells were successfully impaled during a 15-min period.

Microdissected mouse islets were used to ascertain the dynamic influence of SRIF on glucose-induced electrical activity recorded from a single β -cell. Briefly, islets were partially dissected from a mouse pancreas, pinned by attached blood vessels in a 0.5-ml perfusion chamber, and subjected to a flow rate of 2 ml/min. The preparation was perfused with a Krebs-Ringer bicarbonate-buffered solution maintained at 37°C, gassed with 95% O₂ and 5% CO₂, and stabilized at pH 7.4 by the addition of 25 mM Hepes. The β -cells were impaled with glass microelectrodes with tip resistances of 175–300 M Ω . The electrical events were recorded using standard electrophysiologic techniques.¹⁷ Initial impalements were made routinely at a level of 11.1 mM glucose to identify β -cells by their characteristic pattern of electrical activity.^{12,18}

⁸⁶Rb accumulation and efflux. Rat islets were cultured for 3–4 days, after which groups of 10–15 were placed in microcentrifuge tubes, each containing 0.2 ml of Krebs-Ringer solution with the addition of 2.8 mM glucose. After 30 min of preincubation at 37°C in an atmosphere of 95% O₂ and 5% CO₂, the tubes were centrifuged and the medium was withdrawn. A second aliquot of medium containing, in addition, 0.2 mM ⁸⁶RbCl (5–15 mCi/mmol) and 0.1 mM 6,6'-n³H sucrose (26 mCi/mmol) was added to each tube. The islets were preloaded with the radioisotopes for 90 min for the efflux studies. The medium was subsequently removed, the islets were washed briefly, and 0.5 ml of nonradioactive medium containing the desired additions was added to each tube. The accumulation of ⁸⁶Rb into the islet cells was determined by adding 0.2 ml of medium containing ³H-sucrose, ⁸⁶Rb, and the desired agents after the preincubation period. For both the accumulation and efflux studies, each tube was centrifuged at selected intervals in a Beckman microfuge (Beckman Instruments, Fullerton, California) to deposit the islets in the tip of the tube. Di-n-butyl phthalate was then layered on top of the medium, and a second centrifugation separated the medium from the islet pellet.¹⁰ The bottom of the tube containing the islet pellet was cut off and placed in a scintillation vial. Hyamine was placed on top of the islets and the vials were incubated for 2 h at 37°C. The islets were mixed with a neutralizing cocktail and examined for their ⁸⁶Rb and ³H content. Appropriate corrections were made for 3% spillover of ⁸⁶Rb counts to the ³H-channel; the spillover of the ³H-counts to the ⁸⁶Rb-channel was negligible. The ⁸⁶Rb remaining in the islets was corrected for the amount of ⁸⁶Rb residing in the extracellular space occupied by ³H-sucrose. Samples of incubation media were used as external standards. Blanks without islets did not differ from the background of the counter.

Insulin release. For the secretory studies, groups of 10–15 rat islets, cultured for 3–4 days, were preincubated in an atmosphere of 95% O₂ and 5% CO₂ for 30 min at 37°C in Krebs-Ringer medium containing 0.3% (w/v) bovine serum albumin and 2.8 mM glucose. The islets were subsequently incubated in fresh medium containing the desired agents for 30 min, after which the medium was withdrawn and stored at –20°C until assayed for insulin content.¹⁹

Chemicals. Somatostatin was obtained from Pierce (Rockford, Illinois); tetraethylammonium chloride from Aldrich Chemical Company (Milwaukee, Wisconsin); quinine chloride and collagenase from Sigma Chemical Company (St. Louis, Missouri); ⁸⁶RbCl and 6,6'-n³H sucrose from Amersham (Arlington Heights, Illinois); and Hepes from Calbiochem (La Jolla, California). All other chemicals were of analytic grade or the highest grade available.

Statistics. Statistical significance of data was determined by Student's *t* test, either paired or unpaired, as appropriate.

RESULTS

INSULIN RELEASE

Quinine and TEA have been used to inhibit the Ca²⁺-dependent or voltage-dependent increase in P_K, respectively.^{11,13} Both agents potentiate the secretory response to glucose in rat islets.^{20,21} These agents were used in conjunction with SRIF to examine the possibility that SRIF activates the mechanism(s) controlling the increase in P_K in the β -cell membrane, thereby antagonizing the glucose-induced reduction in P_K.^{22–24} Addition of 20 mM TEA reversed the inhibitory influence of 100 ng/ml SRIF on the glucose-induced secretory response of cultured islets (Table 1). The addition of 0.1 mM quinine was also an antagonist of the inhibitory influence of SRIF on glucose-induced insulin release (Table 1).

ELECTRICAL ACTIVITY

Cultured rat islets. In previously reported electrophysiologic studies, we used islets cultured in medium CMRL-1066 containing 5.6 mM glucose.⁸ We subsequently found that islet cells maintained in medium containing 14 mM glucose exhibited more negative membrane potentials when examined in acute experiments.²⁵ In view of this we reexamined the effect of SRIF on glucose-induced electrical activity in islets cultured in RPMI-1640 containing 11 mM glucose. After 3–4 days of culture, the medium was exchanged for a buffered salt solution. The membrane potential measured in the absence of glucose was -35 ± 2 mV (M \pm SE; N = 24), and in the presence of 16.7 mM glucose, it was -26 ± 2 mV (N = 19). The decrease in potential due to glucose was accompanied by an increase in the incidence of spike activity from $8 \pm 2\%$ to $56 \pm 7\%$ of the total number of cells impaled. The addition of 100 ng/ml SRIF to a medium containing 16.7 mM glucose resulted in a rapid increase in the average membrane potential (hyperpolarization) and a substantial decrease in the incidence of spike activity (Table 2). The addition of 20 mM TEA or 0.1 mM quinine almost completely antagonized the influence of SRIF on glucose-induced electrical activity (Table 2). SRIF still produced a small but significant hyperpolarization of the membrane despite the addition of TEA or quinine.

TABLE 1
Influence of quinine and TEA on the secretory response of islet cells: reversal of the inhibitory effect of somatostatin*

Experiment	TEA (20 mM)	Glucose (16.7 mM)	SRIF (100 ng/ml)	Insulin release (μ U/islet/30 min)
A				
1	-	+	-	25.3 \pm 4.1
2	-	+	+	8.8 \pm 2.3
3	+	+	-	33.6 \pm 3.1
4	+	+	+	22.0 \pm 3.0
Quinine (0.1 mM)				
B				
1	-	+	-	28.0 \pm 3.7
2	-	+	+	9.7 \pm 1.5
3	+	+	-	34.5 \pm 4.2
4	+	+	+	19.3 \pm 2.5

* Each value represents the mean \pm SE of at least eight observations. The following are P values indicating the statistical significance of the secretory response in relation to a control value. NS indicates not significant.

<0.001 B ₂ vs. B ₁	<0.002 A ₂ vs. A ₃	<0.005 A ₂ vs. A ₁	<0.02 B ₄ vs. B ₃ B ₄ vs. B ₂ A ₄ vs. A ₃	NS A ₃ vs. A ₁ A ₄ vs. A ₁ B ₃ vs. B ₁ B ₄ vs. B ₁
-----------------------------------------------	-----------------------------------------------	-----------------------------------------------	------------------------------------------------------------------------------------------------------------------------	--------------------------------------------------------------------------------------------------------------------------------------------------------

TABLE 2
Influence of somatostatin on glucose-induced electrical activity in cultured islets: antagonistic action of TEA and quinine*

	Membrane potential (mV)			Cells exhibiting spike activity (%)		
	0-15 min	15-30 min	P value	0-15 min	15-30 min	P value
A	Glucose (-28 \pm 3)	Glucose + SRIF (-52 \pm 5)	<0.001	Glucose (50 \pm 6)	Glucose + SRIF (18 \pm 5)	<0.001
B	Glucose + TEA (-23 \pm 3)	Glucose + TEA + SRIF (-30 \pm 3)		NS	(60 \pm 6)	
C	Glucose + quinine (-18 \pm 4)	Glucose + quinine + SRIF (-25 \pm 3)	NS	(62 \pm 8)	Glucose + quinine + SRIF (56 \pm 5)	NS

* Each value represents the mean \pm SE of a total of at least 12 cells impaled in three separate experiments. The concentrations of the agents are glucose, 16.7 mM; SRIF, 100 ng/ml; TEA, 20 mM; and quinine, 0.1 mM.

TABLE 3
Influence of somatostatin on glucose-induced electrical activity

Experiment	Silent phase* (s)			Active phase* (s)			Hyperpolarization† (mV)
	Glucose A	Glucose + SRIF B	B/A	Glucose A	Glucose + SRIF B	B/A	
1	14	29	2.1	11	15	1.4	6
2	14	19	1.4	11	9	0.8	4
3	8	21	2.6	7	4	0.6	6
4	9	20	2.2	6	3	0.5	6
5	12	20	1.7	5	4	1.0	4
(mean \pm SE)	11 \pm 1	22 \pm 2‡	2.0 \pm 0.2	8 \pm 1	7 \pm 2§	0.9 \pm 0.2	5.2 \pm 0.4

* Averaged over a period of 3 min before (A) or after (B) SRIF was added to the preparation. (The electrical activity recorded during the period of chamber mixing was not quantitated for this evaluation.)

† Maximum change in membrane potential (hyperpolarization) following addition of 100 ng/ml SRIF to the medium. Each β -cell was initially impaled in 11.1 mM glucose after which 100 ng/ml SRIF was added to the medium.

‡ P < 0.001 for the statistical significance of B vs. A.

§ There is no statistically significant difference for B vs. A.

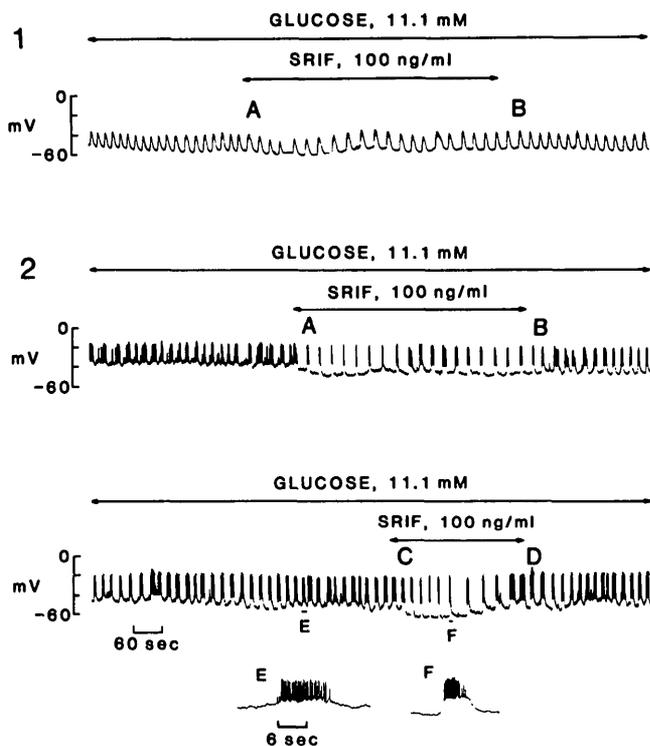
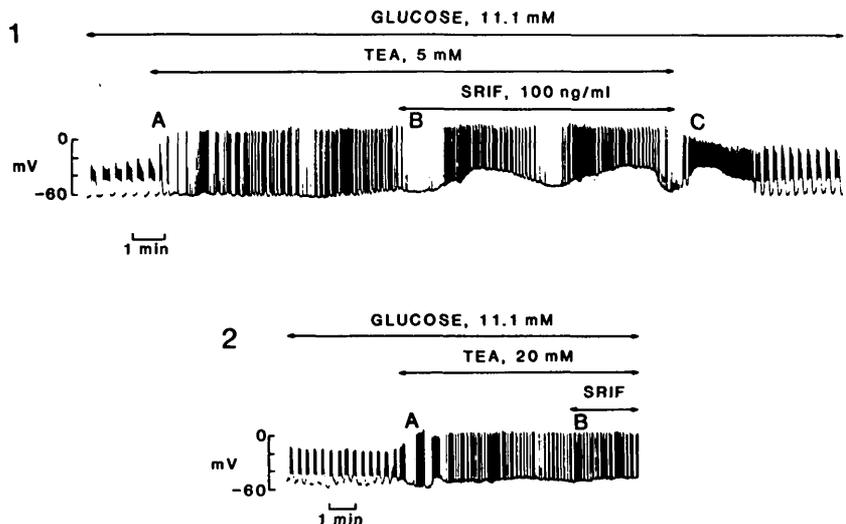


FIGURE 1. Influence of SRIF on glucose-induced electrical activity during continuous recording from individual β -cells located in a microdissected mouse islet. Glucose was present throughout the recordings obtained from cells 1 and 2. In both cells, SRIF was added to the preparation at A and withdrawn at B. In cell 2, SRIF was added for a second period at C and withdrawn at D. Record E or F shows a burst of electrical activity at fast record speed.

Microdissected mouse islets. Figure 1 shows the influence of SRIF on the electrical activity of two different β -cells. SRIF rapidly evoked a slight lengthening and increased the extent of hyperpolarization of the silent phase. The effect was transient, lasting for about 3–4 min. Following the removal of SRIF from the solution, cell 1 returned to its control activity. Cell 2 did not return to its control activity, but continued to show hyperpolarization during the silent phase, although not to the extent seen when SRIF was present. A second exposure of cell 2 to SRIF elicited a transient increase in the duration of the silent phase and hyperpolarization.

FIGURE 2. Influence of SRIF on electrical activity due to glucose and TEA during continuous recordings in mouse islet β -cells. Glucose was present throughout the recordings. With both cells 1 and 2 TEA was added at A and SRIF was added at B. Both TEA and SRIF were withdrawn from cell 1 at C.



On the average, SRIF doubled the duration of the silent phase, had no effect on the duration of the active phase, and provoked a transient hyperpolarization (Table 3). After about 3 min in the presence of SRIF, the increase in both the duration of the silent phase and the membrane potential gradually returned to pre-SRIF values (data not shown). However, the overall pattern of electrical activity was often quite irregular when compared with the patterns observed either immediately before or after the addition of SRIF.

In Figure 2, 5 mM TEA is shown to abolish the oscillatory pattern of electrical activity observed in the presence of 11.1 mM glucose.^{13,26} After TEA, SRIF introduction to the preparation rapidly induced a transient loss of spike activity. In the intervals between the silent phases there was depolarization accompanied by fast spikes. After the removal of both TEA and SRIF, the cell exhibited a biphasic pattern of electrical activity. SRIF (100 ng/ml) had no effect on the electrical events elicited by 20 mM TEA (Figure 2). In additional experiments with 20 mM TEA, SRIF up to 500 ng/ml had no effect on the electrical activity (data not shown).

⁸⁶Rb ACCUMULATION AND EFFLUX

SRIF had no apparent influence on the uptake of ⁸⁶Rb into islet cells over a 30-min period as compared with that obtained in the presence of 16.7 mM glucose alone (Figure 3). However, SRIF significantly enhanced the rate of ⁸⁶Rb efflux from cells exposed to 16.7 mM glucose (Figure 4). In agreement with the results of other investigators,^{22–24} glucose decreased the rate of ⁸⁶Rb efflux from islet cells (Figure 4). The addition of SRIF to medium containing 5.6 mM glucose led to a 1.4-fold increase in the rate constant for ⁸⁶Rb efflux, whereas with 16.7 mM glucose SRIF provoked a 1.8-fold increase in the rate of ⁸⁶Rb efflux. The glucose-induced reduction of ⁸⁶Rb efflux was not further reduced by the addition of 20 mM TEA (Figure 5). However, the enhancement of the rate of ⁸⁶Rb efflux due to SRIF was substantially reduced by the simultaneous presence of TEA. In addition, 0.1 mM quinine partially reversed the SRIF-induced increase in the rate of ⁸⁶Rb efflux (Table 4).

An increase in Ca²⁺ from 2.5 to 5.0 mM or the addition of SRIF to a medium containing 16.7 mM glucose produced a similar increment in the efflux rate of ⁸⁶Rb when acting separately; when working together, there was an additional aug-

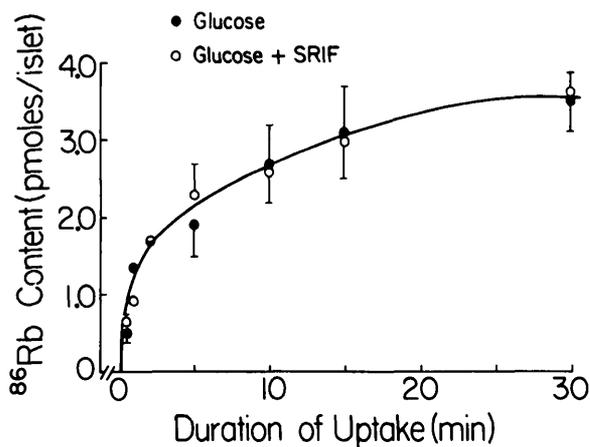


FIGURE 3. Effect of SRIF in the presence of glucose on ^{86}Rb accumulation in cultured rat islet cells. The uptake of ^{86}Rb in excess of the ^3H -sucrose space was determined in the presence of 16.7 mM glucose (—●—) or 16.7 mM glucose plus 100 ng/ml SRIF (—○—). Each point and the vertical bar represents the mean \pm SE of 3–5 individual observations.

mentation of the increase in ^{86}Rb efflux (Table 4). Raising K^+ from 5 to 13 mM also led to an increase in ^{86}Rb efflux, but the addition of SRIF to a medium containing excess K^+ did not produce a further increase in the efflux rate (Table 4).

DISCUSSION

The study of the mode of action of SRIF provides an excellent opportunity to establish the relative importance of selected cellular phenomena in contributing to the physiologic regulation of insulin release from the β -cell. The primary effect of SRIF may be to disrupt the transduction of molecular information at the level of the plasma membrane via the activity of adenylate cyclase and components controlling Ca^{2+} and K^+ permeabilities.¹

The suggestion that SRIF disrupts plasma membrane activation of Ca^{2+} permeability has stemmed from evidence showing that elevation of the concentration of extracellular Ca^{2+} or K^+ antagonizes the inhibitory action of SRIF.^{1,6,8,27–31} The antagonistic action of high K^+ on SRIF inhibition of glucose-induced insulin release may be due to depolarization and subsequent activation of voltage-dependent Ca^{2+} chan-

FIGURE 4. Influence of SRIF on the rate of ^{86}Rb efflux from cultured rat islet cells exposed to low or high glucose. The data represent the ^{86}Rb content of the islets in excess of the ^3H -sucrose space. Each value with the vertical bar represents the mean \pm SE of at least 10 observations. The solid lines are single exponential curve fits obtained by the method of least squares.

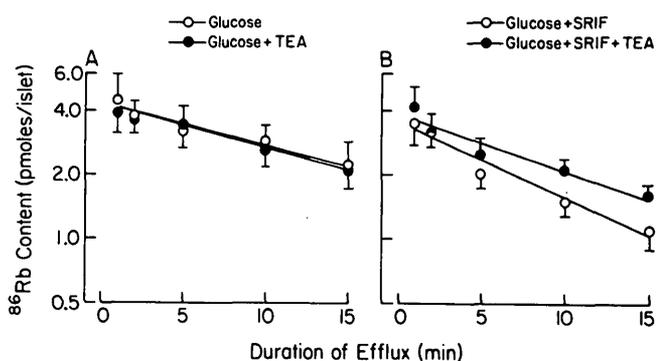
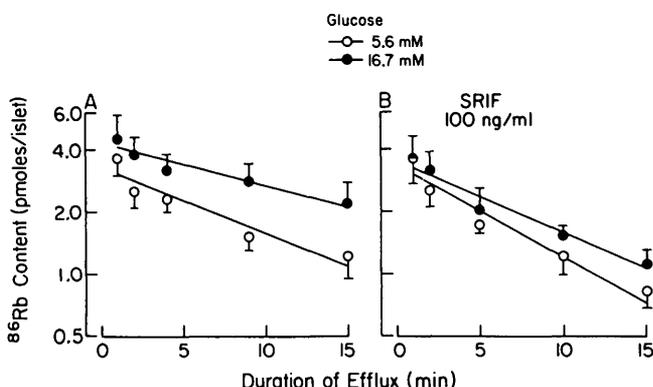


FIGURE 5. Effect of TEA on the rate of ^{86}Rb efflux from cultured rat islets in the presence of glucose and SRIF. The data represent the ^{86}Rb content of the islets in excess of the ^3H -sucrose space. Glucose (16.7 mM) was present in all experiments shown in A and B, and 20 mM TEA and/or 100 ng/ml SRIF were added as indicated. Each value with the vertical bar represents the mean \pm SE of at least 10 observations. The solid lines are single exponential curve fits obtained by the method of least squares.

nels.^{1,32,33} Because an increase in extracellular Ca^{2+} in the presence of high glucose is associated with an increase in $^{45}\text{Ca}^{2+}$ uptake,³⁴ it seems reasonable to conclude that the antagonistic influence of excess Ca^{2+} on the action of SRIF is primarily due to an increase in Ca^{2+} uptake, as has been documented by several investigators.^{4,6,7} This suggests that SRIF interferes with stimulation of Ca^{2+} entry into the β -cell, rather than blocking a Ca^{2+} -dependent site distal to plasma membrane associated processes, a concept that is further supported by the observation that SRIF inhibits glucose-induced electrical activity in islet cells. It is generally accepted that the spike activity in β -cells is due mostly to voltage-dependent Ca^{2+} influx,^{32,33,35,36} although this point is still under investigation.³⁷ Partial reversal of the inhibitory action of SRIF on glucose-induced electrical and secretory events occurs by adding excess Ca^{2+} or K^+ ,^{1,8} or the drugs TEA or quinine, suggesting that the primary action of SRIF may be to increase the P_{K} of the plasma membrane, thus shifting the membrane potential toward the K^+ equilibrium potential. The membrane may then hyperpolarize to a level beyond the threshold for elicitation of voltage-dependent Ca^{2+} spikes. The depolarization of the β -cell membrane associated with glucose-induced insulin release appears to be primarily due to a reduction in P_{K} .^{10,11,13,14,20–22,26,38} The regulation of P_{K} has an important role in the maintenance of the oscillatory pattern of electrical activity evoked by glucose.^{9,11,13,39} Prevention of an increase in P_{K} inhibits repolarization to the silent phase, producing continuous spike activity.

It is not clear as to the mechanism by which SRIF, in the presence of TEA and glucose, elicits silent phases followed by depolarization with superimposed spikes (Figure 2, cell 1). A possible explanation may be that SRIF by activation of P_{K} counteracts, in a phasic manner, the inhibitory influence of TEA on P_{K} . However, we have often observed that SRIF induces instability in the membrane potential and pattern of spike activity, and this suggests that the hormone alters ionic control mechanisms subserving the oscillatory nature of the spike activity.

The results of the ^{86}Rb accumulation studies indicate that SRIF may not hyperpolarize the plasma membrane via activation of a Na-K ATPase, since there is no difference ob-

TABLE 4
Influence of quinine, Ca²⁺, and K⁺ on rate of ⁸⁶Rb efflux in the presence of glucose and/or somatostatin*

Experiment	Addition	Glucose (16.7 mM)	SRIF (100 ng/ml)	⁸⁶ Rb content after 15 min (pmol/islet)
A 1	Control	+	-	2.1 ± 0.28 (13)
2		+	+	1.1 ± 0.17 (13)
B 1	Quinine, 0.1 mM	+	-	2.4 ± 0.25 (6)
2		+	+	1.7 ± 0.09 (4)
C 1	Ca ²⁺ , 5 mM	+	-	0.9 ± 0.07 (8)
2		+	+	0.7 ± 0.05 (6)
D 1	K ⁺ , 13 mM	+	-	1.6 ± 0.13 (12)
2		+	+	1.7 ± 0.18 (5)

* The values represent the ⁸⁶Rb content of islets, preloaded with ⁸⁶Rb and ³H-sucrose, after 15 min of exposure to radioisotope-free medium containing the indicated agents. Each value is the mean ± SE of the number of experiments indicated in parenthesis. The following are P values indicating the statistical significance of the secretory response in relation to a control value. NS indicates not significant.

<0.005
A₂ vs. A₁

<0.01
B₂ vs. A₂

<0.03
B₂ vs. B₁
D₂ vs. A₂

NS
B₁ vs. A₁
B₂ vs. B₁
C₂ vs. C₁
D₁ vs. A₁
D₂ vs. D₁
D₂ vs. A₁

served in the rate of ⁸⁶Rb uptake in the presence of glucose vs. glucose plus SRIF. It is clearly evident from the ⁸⁶Rb efflux studies that SRIF enhances P_K, thereby antagonizing the glucose-induced decrease in P_K. Both antagonists of P_K activation, TEA or quinine, effectively counteracted the SRIF-induced augmentation of ⁸⁶Rb efflux, lending further supporting evidence to a possible direct action of SRIF on P_K.

The definitive effects of SRIF on the inhibition of Ca²⁺ uptake on one hand,^{4,6,7} and the augmentation of ⁸⁶Rb efflux on the other, suggest that one or both of these phenomena prevent normal regulation of a Ca²⁺-sensitive P_K system. The influence of excess Ca²⁺ on ⁸⁶Rb efflux is consistent with electrophysiologic studies that revealed the dynamic relationship between changes in Ca²⁺ and K⁺ permeabilities.⁹ These studies showed that an increase in the Ca²⁺ level from 2.5 to 5.0 mM in the presence of 11.1 mM glucose elicited prolonged silent phases between bursts of spikes at a potential more negative than that obtained in the presence of 2.5 mM Ca²⁺. We have observed a similar effect of excess Ca²⁺ on the membrane potential in previous studies in which cultured rat islet cells were used.¹ Although excess Ca²⁺ led to hyperpolarization and a decrease in the level of spike activity in the cultured islet cells, the addition of SRIF led to no further change in these parameters. The inability of excess Ca²⁺ to reverse the influence of SRIF on either the membrane potential or the rate of ⁸⁶Rb efflux is probably due to activation of a Ca²⁺-sensitive P_K. Other investigators have found that excess K⁺ increases the rate of ⁸⁶Rb efflux in the presence of high glucose.²³ The increase in ⁸⁶Rb efflux with high K⁺ may be due to activation of a Ca²⁺-sensitive P_K resulting from a K⁺-induced increase in Ca²⁺ uptake.⁴⁰

The ability of SRIF to provoke only a transient hyperpolarization and a decrease in the frequency of the generation of bursts of electrical activity in microdissected mouse islets contrasts with the sustained inhibitory influence of SRIF on

electrical events in cultured rat islet cells.¹ This may be due to a greater sensitivity of cultured rat islet cells to SRIF, or to the alteration in the topography of the islet cells and, thereby, impairment of the paracrine influence of intraislet hormonal release.

In view of the hypothesis that a signal stemming from glucose metabolism serves as a trigger for both the membrane and the secretory events,⁴¹ it is important to determine if the action of SRIF could be mediated by inhibition of glucose metabolism. Our previously reported findings¹ are in agreement with other studies in which SRIF was found not to affect the metabolism of glucose through the glycolytic pathway,² the Krebs cycle,^{5,42} or the pentose monophosphate shunt.^{2,5} In light of these results, it is apparent that SRIF can influence processes involved in the transduction of glucose activation without the mediating influence of glucose metabolism.

In summary, evidence from electrophysiologic and cationic flux studies indicates that SRIF-induced hyperpolarization is most likely due to an increase in P_K to an extent that counteracts the decrease in P_K elicited by high glucose. Consequently, in the absence of adequate depolarization, the influx of Ca²⁺ via voltage-sensitive Ca²⁺ channels would be inhibited as indicated by the inhibition of spike activity and ⁴⁵Ca uptake. The ability of TEA or quinine to antagonize the influence of SRIF on electrical events, insulin release, and ⁸⁶Rb efflux supports the postulate that the peptide activates P_K as its primary mode of action. It may be concluded that SRIF inhibits the transduction of secretory information at the level of the cell membrane by increasing and decreasing K⁺ and Ca²⁺ permeabilities, respectively, and directly or indirectly (via reduction of {Ca²⁺}_i) inhibiting the activity of adenylate cyclase.¹ This would interrupt the coupling of the recognition process to activation of the secretory complex. This postulated mechanism of action is attractive in view of the widespread inhibitory action of SRIF in its role as a regulatory chemical messenger for a wide variety of secretory cells.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the skilled technical assistance of Ann Neighbors and Douglas Swain.

This study was supported by a grant from the National Institutes of Health, AM21973. Caroline S. Pace is the recipient of an NIH Research Career Development Award, AM00499. John T. Tarvin is the recipient of a fellowship from the Juvenile Diabetes Foundation.

REFERENCES

- ¹ Pace, C. S.: Somatostatin: control of stimulus-secretion coupling in pancreatic islet cells. *In* Peptides: Integrators of Cell and Tissue Function. Bloom, F. E., Ed. New York, Raven Press, pp. 163-95.
- ² Bent-Hansen, L., Capito, K., and Hedekov, C. J.: The effect of calcium on somatostatin inhibition of insulin release and cyclic AMP production in mouse pancreatic islets. *Biochim. Biophys. Acta* 585:240-49, 1979.
- ³ Claro, A., Grill, V., Efendic, S., and Luft, R.: Studies on the mechanisms of somatostatin action on insulin release. IV. Effect of somatostatin on cyclic AMP levels and phosphodiesterase activity in isolated rat pancreatic islets. *Acta Endocrinol. (Kbh)* 85:379-88, 1977.
- ⁴ Katada, T., and Ui, M.: Islet-activating protein. Enhanced insulin secretion and cyclic AMP accumulation in pancreatic islets due to activation of native calcium ionophores. *J. Biol. Chem.* 254:469-79, 1979.
- ⁵ Oliver, J. R., Wright, P. H., and Ashmore, J.: The effect of somatostatin on glucose stimulated adenosine 3':5'-monophosphate accumulation. *Proc. Soc. Exp. Biol. Med.* 158:458-61, 1978.
- ⁶ Bhathena, S. J., Perrino, P. V., Voyles, N. R., Smith, S. S., Wilkins, S. D., Coy, D. H., Schally, A. V., and Recant, L.: Reversal of somatostatin inhibition of insulin and glucagon secretion. *Diabetes* 25:1031-40, 1976.
- ⁷ Oliver, J. R.: Inhibition of calcium uptake by somatostatin in isolated rat islets of Langerhans. *Endocrinology* 99:910-13, 1976.
- ⁸ Pace, C. S., Murphy, M., Conant, S., and Lacy, P. E.: Somatostatin inhibition of glucose-induced electrical activity in cultured rat islet cells. *Am. J. Physiol.* 233:C164-71, 1977.
- ⁹ Ribalet, B., and Beigelman, P. M.: Cyclic variation of K^+ conductance in pancreatic β -cells: Ca^{2+} and voltage dependence. *Am. J. Physiol.* 237(3):C137-46, 1979.
- ¹⁰ Malaisse, W. J., Boschero, A. C., Kawazu, S., and Hutton, J. C.: The stimulus secretion coupling of glucose-induced insulin release. XXVII. Effect of glucose on K^+ fluxes in isolated islets. *Pfluegers Arch.* 373:237-42, 1978.
- ¹¹ Atwater, I., Dawson, C. M., Ribalet, B., and Rojas, E.: Potassium permeability activated by intracellular calcium ion concentration in the pancreatic β -cell. *J. Physiol. (Lond.)* 288:575-88, 1979.
- ¹² Atwater, I., Ribalet, B., and Rojas, E.: Cyclic changes in potential and resistance of the β -cell membrane induced by glucose in islets of Langerhans from mouse. *J. Physiol. (Lond.)* 278:117-39, 1978.
- ¹³ Atwater, I., Ribalet, B., and Rojas, E.: Mouse pancreatic β -cells: tetraethylammonium blockage of the potassium permeability increase induced by depolarization. *J. Physiol. (Lond.)* 288:561-74, 1979.
- ¹⁴ Henquin, J. C.: D-Glucose inhibits potassium efflux from pancreatic islet cells. *Nature* 271:271-73, 1978.
- ¹⁵ Lacy, P. E., and Kostianovsky, M.: Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 16:35-39, 1967.
- ¹⁶ Kostianovsky, M., McDaniel, M. L., Still, M. F., Codilla, R. C., and Lacy, P. E.: Monolayer cell culture of adult rat islets of Langerhans. *Diabetologia* 10:337-44, 1974.
- ¹⁷ Tarvin, J. T., and Pace, C. S.: Glucose-induced electrical activity in the pancreatic β -cell: effect of veratridine. *Am. J. Physiol.* 240:C127-34, 1981.
- ¹⁸ Meissner, H. P.: Electrical characteristics of the beta-cells in pancreatic islets. *J. Physiol. (Paris)* 72:757-67, 1976.
- ¹⁹ Wright, P. H., Makulu, D. R., Vichick, D., and Sussman, K. E.: Insulin immunoassay by back-titration: some characteristics of the technic and the insulin precipitant action of alcohol. *Diabetes* 20:33-45, 1971.
- ²⁰ Henquin, J. C.: Tetraethylammonium potentiation of insulin release and inhibition of rubidium efflux in pancreatic islets. *Biochem. Biophys. Res. Commun.* 77:551-56, 1977.
- ²¹ Henquin, J. C., Horemans, B., Nenquin, M., Verniers, J., and Lambert, A. E.: Quinine induced modifications of insulin release and glucose metabolism by isolated pancreatic islets. *FEBS Lett.* 57:280-84, 1975.
- ²² Sehlin, J., and Taljedal, I.-B.: Transport of rubidium and sodium in pancreatic islets. *J. Physiol. (Lond.)* 242:505-15, 1974.
- ²³ Boschero, A. C., and Malaisse, W. J.: Stimulus-secretion coupling of glucose-induced insulin release. XXIX. Regulation of $^{86}Rb^+$ efflux from perfused islets. *Am. J. Physiol.* 236:E139-46, 1979.
- ²⁴ Henquin, J. C.: Opposite effects of intracellular Ca^{2+} and glucose on K^+ permeability of pancreatic islet cells. *Nature* 280:66-68, 1979.
- ²⁵ Pace, C. S., Conant, S., and Lacy, P. E.: Electrical responses of rat islets maintained in culture with varying levels of glucose. *Endocrinology* 102:1350-55, 1978.
- ²⁶ Henquin, J. C., Meissner, H. P., and Preissler, M.: 9-amino-acridine- and tetraethylammonium-induced reduction of the potassium permeability in pancreatic β -cells. *Biochim. Biophys. Acta* 587:579-92, 1979.
- ²⁷ Curry, D. L., and Bennett, L. L.: Reversal of somatostatin inhibition of insulin secretion by calcium. *Biochem. Biophys. Res. Commun.* 60:1015-19, 1974.
- ²⁸ Curry, D. L., and Bennett, L. L.: Does somatostatin inhibition of insulin secretion involve two mechanisms of action? *Proc. Natl. Acad. Sci. USA* 73:248-51, 1976.
- ²⁹ Taminato, T., Seino, Y., Goto, Y., and Imura, H.: Interaction of somatostatin and calcium in regulating insulin release from isolated pancreatic islets of rats. *Biochem. Biophys. Res. Commun.* 66:298-34, 1975.
- ³⁰ Fujimoto, W. Y.: Somatostatin inhibition of glucose-, tol-butamide theophylline-, cytochalasin B-, and calcium-stimulated insulin release in monolayer cultures of rat endocrine pancreas. *Endocrinology* 97:1494-1500, 1975.
- ³¹ Basabe, J. C., Cresto, J. C., and Aparicio, N.: Studies on the mode of action of somatostatin on insulin secretion. *Endocrinology* 101:1436-43, 1977.
- ³² Matthews, E. K., and Sakamoto, Y.: Electrical characteristics of pancreatic islet cells. *J. Physiol. (Lond.)* 246:421-37, 1975.
- ³³ Matthews, E. K., and Sakamoto, Y.: Pancreatic islet cells: electrogenic and electrodiffusional control of membrane potential. *J. Physiol. (Lond.)* 246:439-57, 1975.
- ³⁴ Malaisse, W. J., Hutton, J. C., Sener, A., Levy, J., Herchuelz, A., Devis, G., and Somers, G.: Calcium antagonists and islet function. VII. Effect of calcium deprivation. *J. Membr. Biol.* 38:193-208, 1978.
- ³⁵ Ribalet, B., and Beigelman, P. M.: Calcium action potentials and potassium permeability activation in pancreatic β -cells. *Am. J. Physiol.* 239:C124-33, 1980.
- ³⁶ Atwater, I.: Control mechanisms for glucose induced changes in the membrane potential of mouse pancreatic β -cell. *Ciênc. Biol. (Portugal)* 5:299-14, 1980.
- ³⁷ Meissner, H. P., and Preissler, M.: Ionic mechanisms of the glucose-induced membrane potential changes in β -cells. *Horm. Metab. Res. (Suppl.)* 10:91-99, 1980.
- ³⁸ Boschero, A. C., Kawazu, S., Duncan, G., and Malaisse, W. J.: Effect of glucose on K^+ handling by pancreatic islets. *FEBS Lett.* 83:151-54, 1977.
- ³⁹ Atwater, I., and Beigelman, P. M.: Dynamic characteristics of electrical activity in pancreatic beta cells. Effects of calcium and magnesium removal. *J. Physiol. (Paris)* 72:769-86, 1976.
- ⁴⁰ Henquin, J. C., and Lambert, A. E.: Cationic environment and dynamics of insulin release. II. Effect of a high concentration of potassium. *Diabetes* 23:933-42, 1974.
- ⁴¹ Malaisse, W. J., Sener, A., Herchuelz, A., and Hutton, J. C.: Insulin release: the fuel hypothesis. *Metabolism* 28:373-86, 1979.
- ⁴² Lin, B. J.: Effects of somatostatin on insulin biosynthesis, glucose oxidation, and cyclic guanosine monophosphate level. *Metabolism* 27 (Suppl. 1):1295-98, 1978.