Reversal of Somatostatin Inhibition of Insulin and Glucagon Secretion

S. J. Bhathena, Ph.D., P. V. Perrino, M.D., N. R. Voyles, S. S. Smith, S. D. Wilkins, D. H. Coy, Ph.D., A. V. Schally, Ph.D.,* and L. Recant, M.D., Washington, D. C.*

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/25/11/1031/215217/25-11-1031.pdf by guest on 21 April 2024

SUMMARY

These studies were designed to elucidate the mechanism of inhibitory action of somatostatin (SRIF) on glucagon (IRG) and insulin (IRI) secretion. Studies were carried out in the unrecirculated isolated rat pancreas perfusion with arginine 19.2 mM and glucose 5.5 mM as stimulus primarily for IRG but also IRI secretion. The effects of excess Ca^{++} (15.2 mEq./L.) and excess K^{+} (12.8 mEq./L.) on IRG, IRI, and the SRIF-inhibited pancreas were studied. Ca^{++} excess in five perfusions strikingly stimulated IRG secretion (+92 per cent) but only stabilized IRI secretion compared with control perfusions. K^{+} excess (in seven perfusions) markedly inhibited IRG secretion (−39 per cent) while stimulating IRI secretion (+16 per cent). Restoration of normal concentration of K^{+} resulted in a rebound of IRG to levels 120 per cent that of controls. SRIF, at concentrations from 0.1-20 ng./ml., produced inhibition of both IRG and IRI. In 11 perfusions, with SRIF at 10 ng./ml., IRG decreased more than IRI (−75.2 per cent IRG and −46.9 per cent IRI). In five perfusions, addition of Ca^{++} (15.2 mEq./L.) 10 minutes after SRIF was started resulted in a reversal of IRG inhibition to 69.4 per cent and IRI to 73.2 per cent of the arginine controls. The reversal by Ca^{++} of SRIF effect on IRG was greater at higher concentrations of Ca^{++}, suggesting some form of competition. In four perfusions, excess K^{+} reversed SRIF-induced IRI inhibition to 79.6 per cent that of controls but had no effect on IRG inhibition. Studies in vitro with isolated islets revealed that SRIF (2 μg./ml.) inhibited ^{45}Ca uptake of islets as did epinephrine (10^{-5} M). It was concluded that SRIF-induced inhibition of hormone release appears related to an action on Ca^{++} uptake. DIABETES *25*: **1031-40, November, 1976.**

Somatostatin (SRIF), a hypothalamic peptide[1] named for its capacity to inhibit the secretion of growth hormone[2] from the pituitary, has been demonstrated to inhibit the secretion of numerous hormones, including thyrotropic hormone,[3] adrenocorticotrophic hormone,[4] gastrin,[5] glucagon, and insulin.[6-8] Effects of SRIF on hypophyseal hormone secretion have been demonstrated in vitro with pieces of pituitary,[9] and since the greatest concentration of this peptide is in the brain, it appears reasonable that the action of the peptide is directly upon its target organ from a local site of production.

With regard to the pancreatic and gut hormones, it is of considerable interest that the highest concentrations of SRIF, aside from brain, have been reported in the islets of Langerhans,[10,11] the stomach, and the duodenum[12]—namely, sites of production and secretion of glucagon and insulin and of gastrin. Such findings are suggestive of local effects of SRIF.

Evidence has accumulated that the effects of SRIF on glucagon and insulin are mediated by a direct action on the pancreas. Experiments utilizing SRIF in vitro with isolated rat pancreas perfusions,[6-8,13] and under certain conditions with isolated islets,[14,15] have shown inhibitory effects on islet hormone secretion. Recently Fujimoto and Ensinck[16] reported that in monolayer cultures of newborn rat pancreas, SRIF inhibited hormone release at low calcium concentrations. Calcium ionophore A23187 increased insulin and glucagon secretion and in the presence of SRIF further augmented the hormones' secretion.

The present study was designed to clarify the mechanism of action of SRIF on pancreatic glucagon and insulin release. Curry and Bennett, in a preliminary report, demonstrated that excess calcium ions could partially reverse the SRIF inhibition of insulin release in isolated pancreas perfusions.[17] Therefore, to

Presented at the Thirty-fifth Annual Meeting of the American Diabetes Association, June 1975 (appeared in abstract form in Diabetes *24* (Suppl. 2): 408, 1975).

From the Veterans Administration Hospital and Georgetown University School of Medicine, Washington, D.C.

*Veterans Administration Hospital, New Orleans, Louisiana.

Accepted for publication June 8, 1976.

determine whether SRIF might act by interfering with calcium uptake, we studied the effect of SRIF on ^{45}Ca uptake by isolated rat islets during glucose-stimulated insulin release. Since potassium stimulates insulin release[18-21] by a mechanism thought to involve increased permeability[19] to calcium, we also studied the effects of potassium as well as of excess calcium on SRIF inhibition of insulin and glucagon release from the isolated perfused rat pancreas. Our results provide support for the hypothesis that SRIF effects are intimately related to the calcium ion.

MATERIALS AND METHODS

Studies were conducted in nonrecirculating perfusions of isolated pancreas from fed, male, Sprague-Dawley rats (350-400 gm.) according to the method of Curry et al.[22] The perfusion medium used was a Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 3 per cent dextran T-40 (Pharmacia), 5.5 mM glucose, and 1 per cent salt-poor human serum albumin (Cutter Laboratories). The medium was gassed with a 95 per cent O_2-5 per cent CO_2 mixture for 15 minutes prior to use. Flow rate was maintained constant at between 6 and 8 ml. per minute throughout the perfusion with a pressure of approximately 75 mm. of mercury. Collection of the unrecirculated effluent was made continuously in one-minute fractions in tubes containing 10 mg. EDTA and 1,000 KIU Trasylol (FBA Pharmaceuticals). All tubes were centrifuged to remove any blood cells, and the supernatants were frozen for insulin and glucagon analyses. Insulin was assayed by the double-antibody radioimmunoassay of Morgan and Lazarow,[23] while glucagon was assayed by the radioimmunoassay of Unger[24] with Unger's 30K antiserum. Since the flow rates for these experiments were essentially constant, the insulin levels are reported as μU./ml./min. and the glucagon levels as ng./ml./min. As we were primarily interested in the effects of SRIF and ions on glucagon secretion, the secretory stimulus used was arginine at a concentration of 19.2 mM in the presence of 5.5 mM glucose.

Protocols were designed to investigate the effect of Ca^{++} or K^+ ion excess on (a) arginine-induced insulin and glucagon secretion and (b) the inhibition by SRIF of arginine-induced insulin and glucagon secretion. Synthetic cyclic form of SRIF was obtained from the laboratory of Dr. A. Schally and used at concentrations ranging from 0.1 to 20 ng./ml. Alterations in Ca^{++} and K^+ concentration were obtained through the addition of excess cations in their chloride form. The basal concentrations of Ca^{++} and K^+ in the buffer were 5.05 mEq./L. and 6.4 mEq./L., respectively. The concentrations of other ion components were identical with the buffer utilized by Grodsky and Bennett.[21] The buffer pH was maintained constant at 7.4. All of the test ions and SRIF were introduced during the second phase of the biphasic hormonal response. The experimental design and the ion and SRIF concentrations used in each experiment are detailed in the text. The excess Ca^{++} or K^+ was introduced without altering the basic composition of the perfusion media. In order to clarify whether the effects were due to ions themselves and not due to the change in the osmolarity of the media, three rat pancreases were perfused with additional Na^+ as NaCl to make the perfusion media isotonic with high Ca^{++}- and high K^+-containing media. There was no change in the response of the pancreas for glucagon and insulin release compared with control perfusions (data not shown).

For in-vitro studies of islets, the collagenase method of Lacy and Kostianowsky was used.[25] Islets were incubated in Krebs-Henseleit bicarbonate buffer containing 2 mEq./L. calcium and ^{45}Ca for 90 minutes in the absence or presence of glucose (300 mg./100 ml.). The effects of additions of SRIF (2 μg./ml.) or epinephrine (10^{-5} M) were tested. ^{45}Ca uptake by islets was measured by the method of Malaisse.[26,27]

RESULTS

The classic biphasic response of insulin (IRI) and glucagon (IRG) release to an arginine-glucose stimulus in the isolated rat pancreas is shown in figure 1. The first period (prestimulatory) is a 10-minute equilibration phase with 5.5 mM glucose. The glucose is continued throughout the perfusion. Stimulation of IRI and IRG secretion is initiated by introduction of 19.2 mM arginine after the equilibration phase. The arginine is then also continued throughout the perfusion. Ten minutes after arginine is started, test ions or SRIF are perfused. Thus, all tests are carried out during the second phase of the IRG and IRI responses to arginine. In order to calculate the responses of the perfusions to either test cations or SRIF, set comparisons were utilized. Eleven control perfusions were carried out. IRI and IRG secretions for each 10-minute period were measured, and a mean secretion with S.E.M. was calculated (μU./ml./min. for IRI and ng./ml./min. for IRG). The data are shown in the upper two panels of figure 2. The mean for the 10-20-minute period includes the whole of the first phase and a portion of the second phase of hormone release. The subsequent periods are entirely second-phase. Similar calculations

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/25/11/1031/215217/25-11-1031.pdf by guest on 21 April 2024

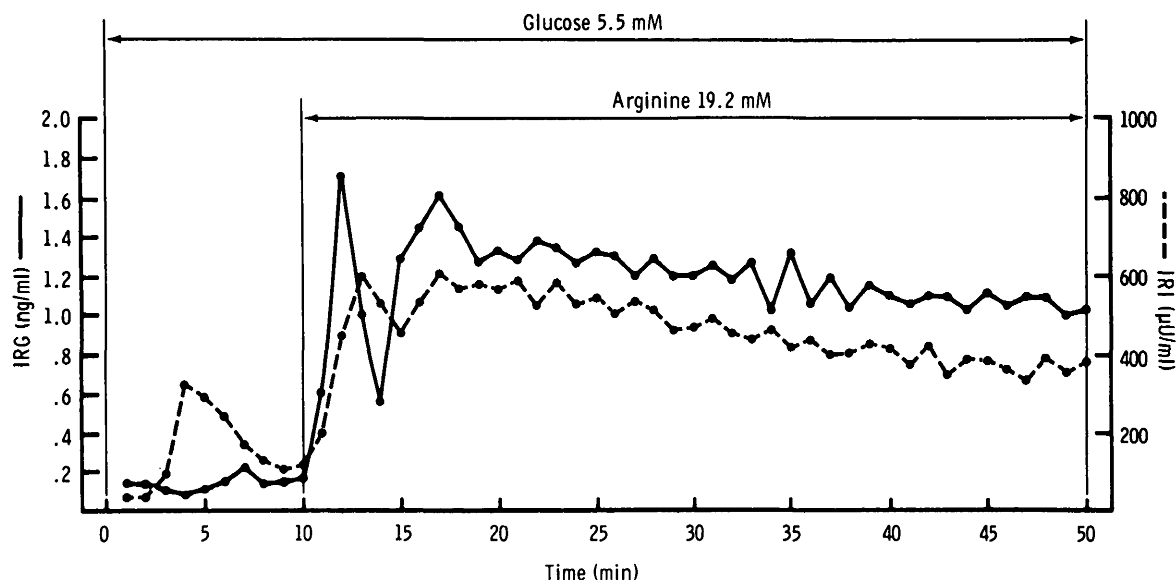

FIG. 1. Control pancreas perfusion illustrating classic biphasic response of IRI and IRG secretion to arginine stimulus.

were made for each 10-minute period in the experimental perfusions with excess cations. It should be noted that in the controls there is gradual falling off of hormone secretion with time (figure 2). The p values were calculated by comparing the mean of each 10-minute period with the preceding 10-minute period.

A. *The Effect of Elevated Levels of Cations on IRI and IRG Secretion*

When elevated levels of Ca^{++} ion (3 × normal concentration, 15.2 mEq./L.) were introduced in five perfusions (figure 2), there was no mean increment in IRI release but rather a stabilization of the arginine-stimulated levels. IRG release increased significantly ($p < 0.01$). These effects are more evident when compared with the same intervals in the control perfusions. Restoration of Ca^{++} ion to normal levels (5.05 mEq./L.) was followed by a fall in IRI and IRG, though IRG remained above the 10-20-minute-period value (152 per cent). Figure 3 shows a single perfusion illustrating the calcium ion effect. Perfusion with K^{+} ion in excess (figure 2) at twice basal buffer concentration (12.8 mEq./L.) in seven perfusions resulted in single-spike elevation of IRI with a significant fall when normal K^{+} was restored. In contrast to the IRI response to elevation of K^{+}, IRG secretion was significantly inhibited and rebounded to levels above the 10-20-minute value (120 per cent) when normal K^{+} levels were reëstablished. Figure 4 shows a single perfusion with two periods of excess K^{+}. A single-spike elevation of IRI is particularly evident in the first period of K^{+} excess. The inhibition of IRG secretion is seen in both periods of K^{+} excess, as are the IRG rebounds with restoration of normal K^{+}.

B. *Effect of SRIF on IRI and IRG Secretion*

Dose-response studies were carried out with four concentrations of SRIF (0.1, 1.0, 10.0, and 20.0 ng./ml.). Figure 5 shows increasing effectiveness of SRIF inhibition of IRI and IRG with increasing concentration. No difference was found between 10 and 20 ng./ml. SRIF (data not shown). All subsequent perfusions were carried out at 10 ng./ml. In figure 6, the upper panels show the response to SRIF in six perfusions where mean ± S.E.M. secretions of IRI and IRG were calculated for each interval. The mean IRG secretion was decreased to 25 per cent of the pre-SRIF arginine-stimulated interval (10-20-minute period), while IRI decreased to only 53 per cent of the prestimulation period.

In figure 7, a single perfusion illustrating the effect of Ca^{++} on SRIF action is shown. The mean ± S.E.M. of five such perfusions is seen in figure 6. When elevated levels of Ca^{++} (15.2 mEq./L.) were introduced 10 minutes after the start of SRIF and continued for 15 minutes, the SRIF-induced inhibition of both IRI and IRG secretion was partially overcome. IRI rose to 73 per cent and IRG to 69 per cent of their respective pre-SRIF arginine-stimulated levels. With restoration of basal Ca^{++} levels, the SRIF inhibition of IRI and IRG reappeared. However, the levels of IRI and IRG were higher than in the control perfusions during the same interval.

To determine if the Ca^{++} effect on SRIF action was

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/25/11/1031/215217/25-11-1031.pdf by guest on 21 April 2024

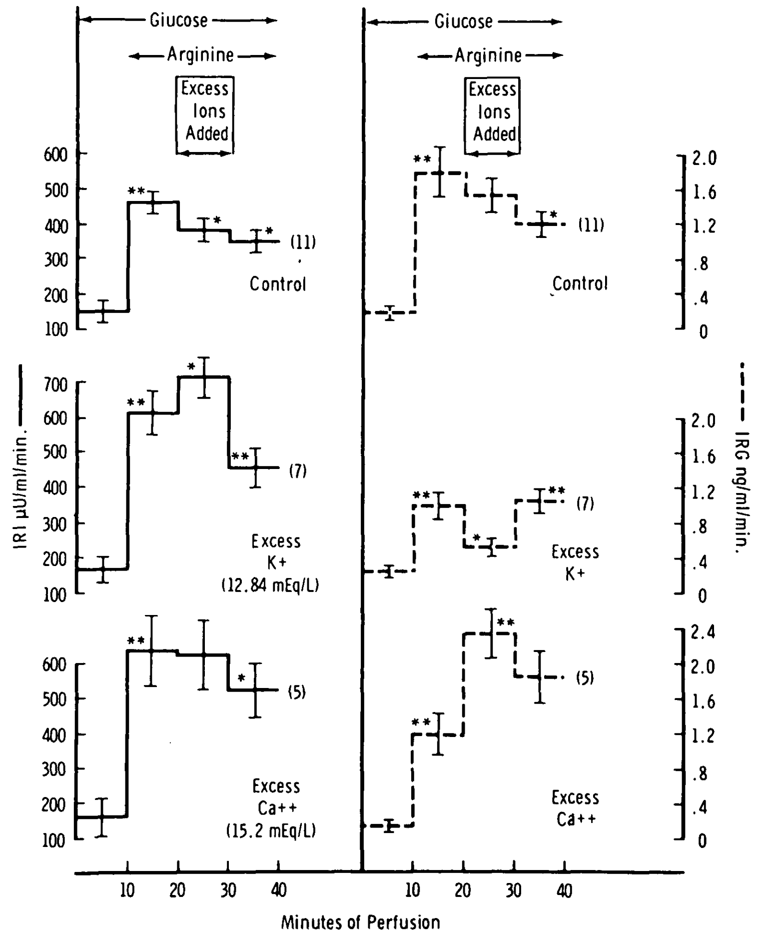

FIGURE 2

Mean ± S.E.M. for IRI and IRG secreted during each 10 minutes of perfusion are plotted for 11 control perfusions—seven with elevated K^+ and five with elevated Ca^{++}. Glucose, 5.5 mM, was present throughout. Arginine, 19.2 mM, was introduced 10 minutes later and continued throughout. Excess Ca^{++} or K^+ were introduced during the 20-30-minute period. Significance (p values) was determined for each 10-minute interval compared with the preceding interval and compared against the values in control perfusions during the same time intervals. *$p < 0.05$; **$p < 0.01$.

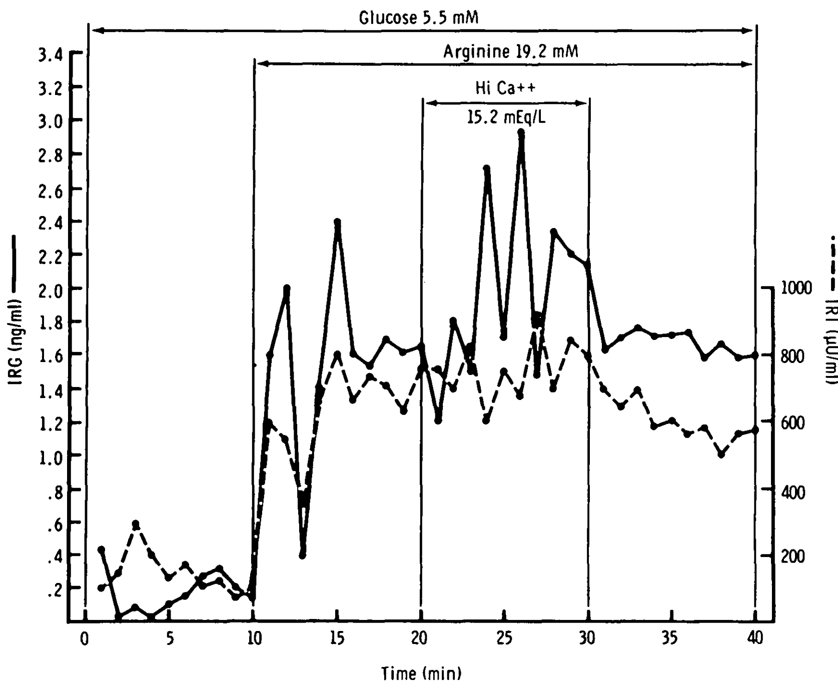

FIGURE 3

A single perfusion demonstrating the effect of elevation of Ca^{++} ion concentration during the second phase of arginine-stimulated hormone secretion.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/25/11/1031/215217/25-11-1031.pdf by guest on 21 April 2024

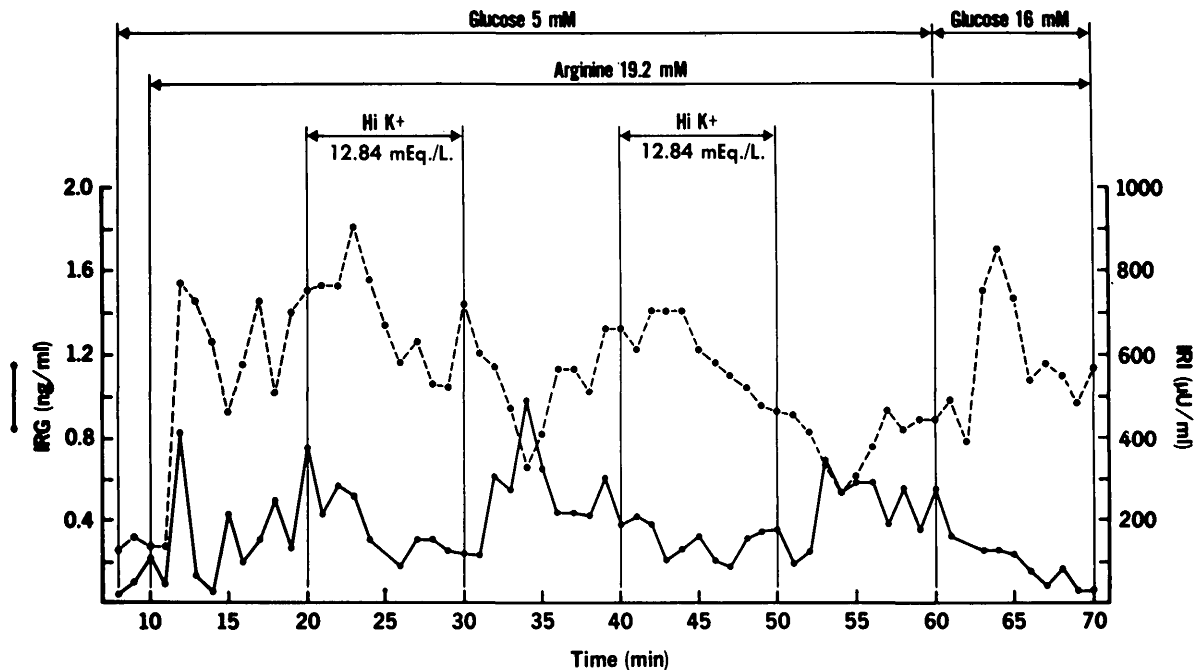

FIG. 4. Effect of elevation of K^+ ion concentration during the second phase of arginine-stimulated hormone secretion. Similar responses to excess K^+ are noted in both periods. The introduction of 16 mM glucose at the end of the perfusion shows that perfusion was intact and responsive after 60 minutes.

dose-dependent, additional perfusions were carried out with Ca^{++}, 5.05, 10.1, and 15.15 mEq./L. (figure 8). In all perfusions, SRIF (10 ng./ml.) caused inhibition of glucagon secretion from a mean of 0.83 ± 0.31 ng./ml./min. to 0.22 ± 0.09 ng./ml./min. With basal Ca^{++} (5.05 mEq./L.), the inhibitory effect of SRIF continued. With Ca^{++} at 10.1 mEq./L. (twice the basal level), the secretion of glucagon was restored to 40 per cent of the control within 10 minutes, and with 15.2 mEq./L. of Ca^{++}, glucagon secretion rose to 69 per cent of the arginine-stimulated control. Concentrations of Ca^{++} up to 25 mEq./L. were no more effective than 15.2 mEq./L.

Increasing K^+ levels from 6.4 to 12.8 mEq./L. 10 minutes after SRIF infusion partially reversed the inhibition of IRI but not of IRG (figure 9). Figure 6 shows mean data from four perfusions. IRI returned to 79.5 per cent of pre-SRIF values, while IRG remained at the inhibited level. Removal of excess K^+ resulted in a fall in IRI though the level of IRI was higher than in control perfusions for that interval. No differences in IRG were noted between control perfusions and perfusions with SRIF plus excess K^+.

C. *Effect of SRIF on ^{45}Calcium Uptake by Isolated Islets of Langerhans*

Table 1 shows the results of experiments measuring ^{45}Ca uptake. The data are presented as cpm/5 islets following a 90-minute exposure to ^{45}Ca. Glucose (300 mg./100 ml.) produces a significant increase in ^{45}Ca uptake. Addition of 2 µg./ml. SRIF had no effect in the absence of glucose. In the presence of glucose, SRIF produced a significant decrease in ^{45}Ca uptake when compared with glucose alone ($p < 0.05$). However, glucose in the presence of SRIF was still able to produce an increase in ^{45}Ca uptake ($p < 0.01$) compared with ^{45}Ca uptake in the absence of glucose.

Epinephrine was found to markedly decrease glucose-associated ^{45}Ca uptake, in confirmation of a previous report.[27]

DISCUSSION

The present investigation confirms the observations that somatostatin is a potent inhibitor of glucagon as well as insulin secretion in the isolated perfused rat pancreas and demonstrates that inhibition of glucagon secretion is greater and more striking than that of insulin.[6] Within 10 minutes of perfusion with 10 ng./ml. of SRIF, insulin secretion decreased 46.9 per cent while glucagon decreased 75.2 per cent. Introduction of excess Ca^{++} (two to three times basal concentration) reversed the inhibition of both glucagon and insulin secretion. The reversal of insulin inhibition by Ca^{++} confirms the observations of Curry and Bennett[17] despite the difference in stimulatory agents.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/25/11/1031/215217/25-11-1031.pdf by guest on 21 April 2024

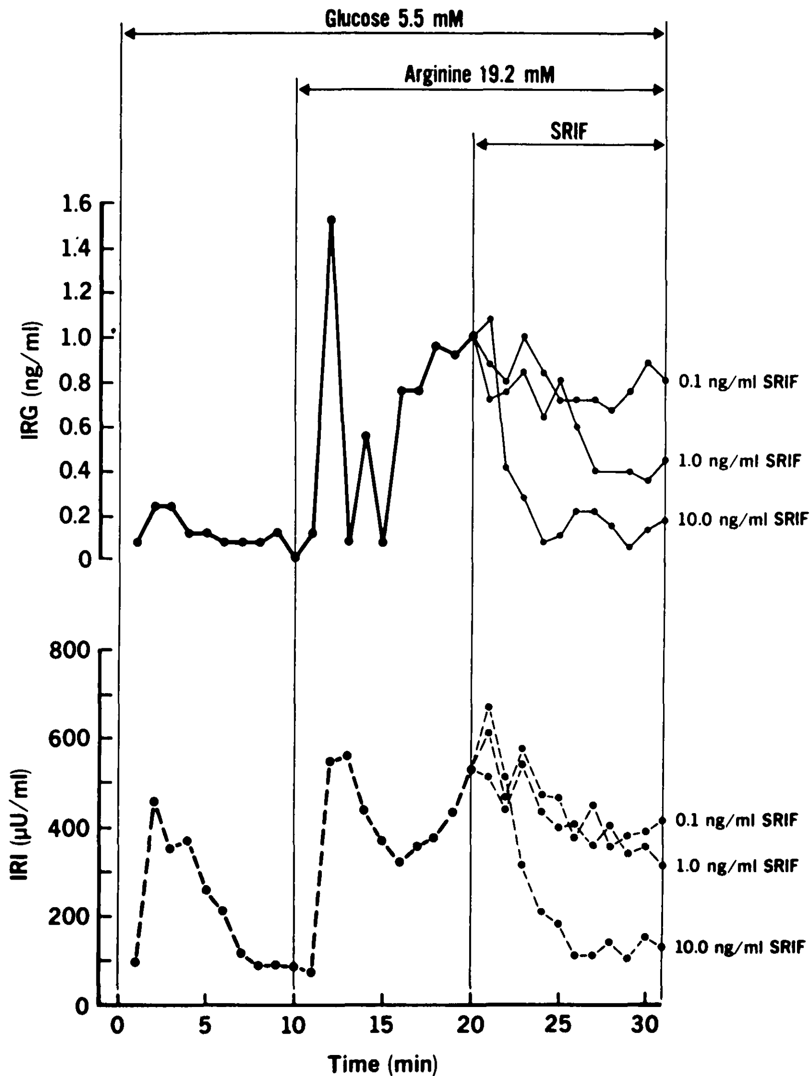

FIGURE 5

Inhibition of arginine-stimulated IRI and IRG secretion by SRIF is dose-dependent. A mean of three perfusions of 0.1 ng./ml., three at 1.0 ng./ml., and 11 at 10 ng./ml. are shown.

The reversal of glucagon inhibition that had not been previously reported was shown to be dependent on Ca^{++} concentration. At a constant perfusion level of SRIF (10 ng./ml.) (figure 8), progressive increase in Ca^{++} ion produced significantly greater reversal of the SRIF inhibition. Although these observations suggest that some form of competition exists between SRIF and Ca^{++} ion, the nature of this competition is not clear. That the effect of Ca^{++} on SRIF action is specific and not merely a function of generally increased secretion of insulin and glucagon is supported by several findings. First, other secretagogues for insulin such as theophylline,[6] tolbutamide,[28] or glucagon[28] fail to overcome the inhibitory effect of SRIF. Second, in our study, excess Ca^{++} in the absence of SRIF failed to cause significant increase in insulin secretion. Grodsky and Bennett[17] and Grodsky,[29] using glucose as a stimulus, reported increasing insulin secretion with increasing elevations of Ca^{++}. This difference may relate to the fact that our stimulus was arginine.

In contrast to the lack of effect of excess Ca^{++} on insulin release, however, perfusion with excess Ca^{++} for 10 minutes dramatically increased glucagon levels to 170 per cent of the 10-20-minute period of arginine-stimulated levels. There was no significant decline in these levels over the 10-minute period, during which time normal Ca^{++} ion concentration was reëstablished. This effect of Ca^{++} on glucagon secretion could be a potentiation of the arginine stimulus or it could indicate a greater sensitivity of α-cells (as against β-cells) to Ca^{++}, which would also explain a more marked effectiveness of SRIF inhibition of glucagon secretion than insulin.

It seemed reasonable as a consequence of the Ca^{++} ion findings to investigate whether other ion(s) could also reverse the action of SRIF. It was pertinent, there-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/25/11/1031/215217/25-11-1031.pdf by guest on 21 April 2024

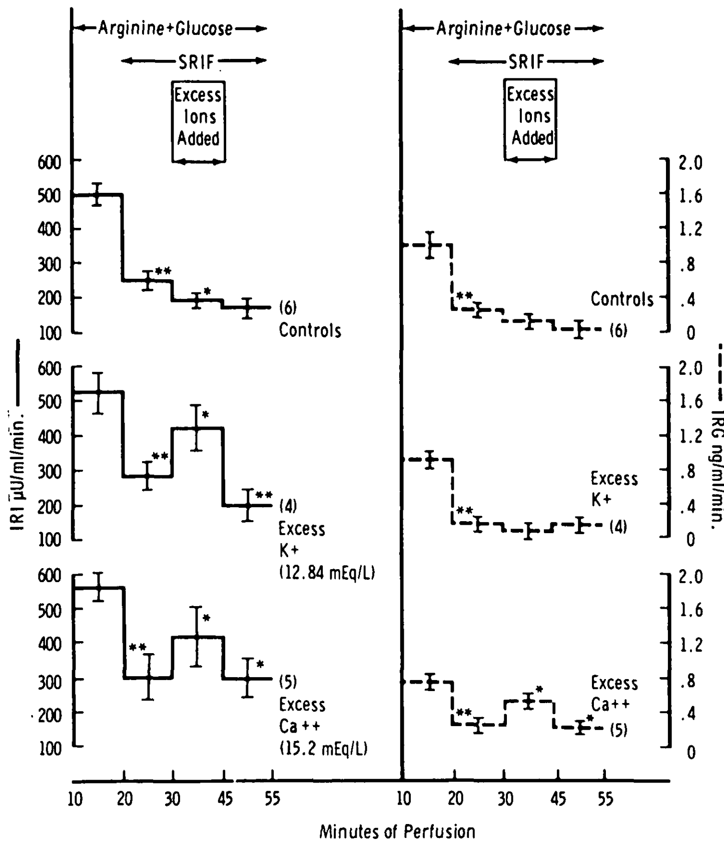

FIGURE 6

Mean ± S.E.M. for IRI and IRG secreted during each 10 minutes of perfusion are plotted for six control perfusions with SRIF (10 ng./ml.), four perfusions with SRIF plus excess K^+, and five perfusions with SRIF plus excess Ca^{++}. SRIF was added 10 minutes after the introduction of arginine, and excess ions were added 10 minutes later and continued for 15 minutes. Significance (p value) was determined for each interval as compared with the preceding period. Set comparisons (between same time intervals for control perfusions and perfusions with excess cations) clearly show the partial reversal of SRIF action on IRI release by Ca^{++} and K^+ and on IRG release by Ca^{++}. * $p < 0.05$; ** $p < 0.01$.

fore, to examine the effect of K^+ ion, since excess K^+ stimulates insulin secretion in vitro[20] and in perfused rat pancreas.[18,19] In fact, excess K^+ can stimulate insulin release even in the complete absence of glucose if Ca^{++} ion is present.[19] In addition, Santeusanio et al.,[30] as well as Kuzuya et al.,[31] reported that K^+ excess increases glucagon secretion in vivo in dogs. Although K^+ at twice basal buffer concentration (12.8

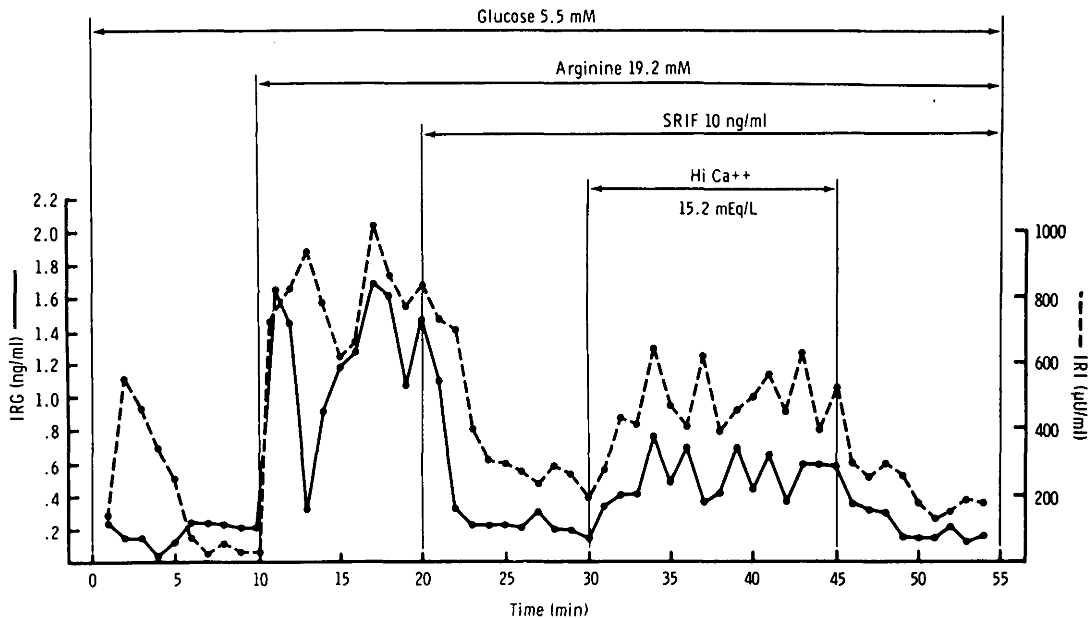

FIG. 7. A single perfusion showing the partial reversal of SRIF-inhibited hormone release by elevated concentration of Ca^{++}.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/25/11/1031/215217/25-11-1031.pdf by guest on 21 April 2024

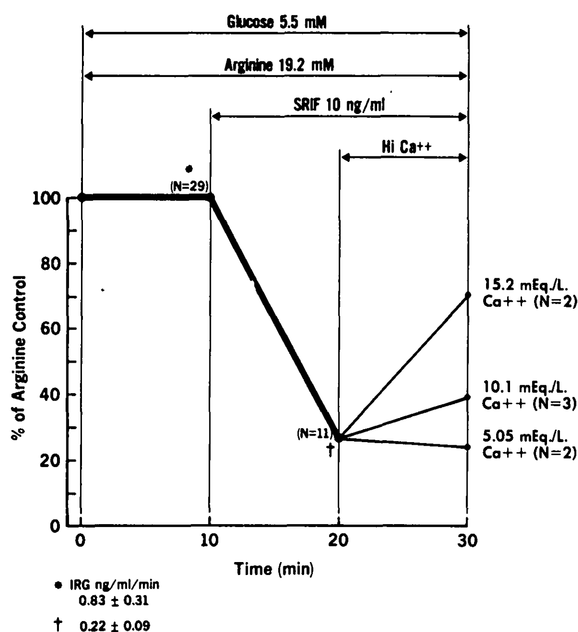

FIG. 8. Effect of various levels of Ca^{++} ion on SRIF-inhibited secretion. Data are presented as per cent of control arginine-stimulated IRG secretion. N = number of perfusions.

mEq./L.) perfused for 10 minutes increased insulin release in our perfusions by 16.0 per cent, it simultaneously produced an unexpected 39.0 per cent inhibition of glucagon release ($p < 0.05$). Further, on withdrawal of the excess K^+ ion, glucagon secretion immediately rebounded to levels as high or higher than those noted prior to the introduction of excess K^+ ion. To our knowledge, this effect of K^+ ion as an inhibitor of

TABLE 1

Effect of SRIF and epinephrine on the uptake of ^{45}Ca by rat islets of Langerhans in the presence and absence of glucose

Glucose mg./100ml.	Basal	SRIF 2 µg./ml.	Epinephrine 10^{-5} M
0	165.3±6.19*	185.0±14.65	169.3±14.50
	(80)†	(39)	(16)
		N.S.‡	N.S.‡
300	410.4±26.50	331.6±16.49	272.1±24.70
	(104)	(55)	(15)
		$p < 0.05$§	$p < 0.05$§
	$p < 0.01$¶	$p < 0.01$¶	$p < 0.01$¶

*Mean ± S.E.M. cpm/5 islets.
†No. of samples.
‡p values compared against basal value without glucose.
§p values compared against basal value with 300 mg./100 ml. glucose.
¶p values between 0 and 300 mg./100 ml. glucose for each test substance.

glucagon secretion has not been previously described. The rapid rebound suggests an effect of K^+ on inhibition of granule release, so that on restoration of normal K^+ ion concentration, the prestored granules are released in a burst. This in-vitro effect of K^+ differs from that reported in vivo[30,31] and may be explained by extrapancreatic effects of hyperkalemia in vivo that could alter the α-cell response. It may also be a consequence of the K^+ levels that were used. In our studies, K^+ levels were consistently higher than those used in vivo. Finally, it may reflect the difference between excess K^+ superimposed on a stimulated α-cell vs. the effect on basal secretion. If, as in other tissues,[32] it is true that with glucose entry there is enhanced transport

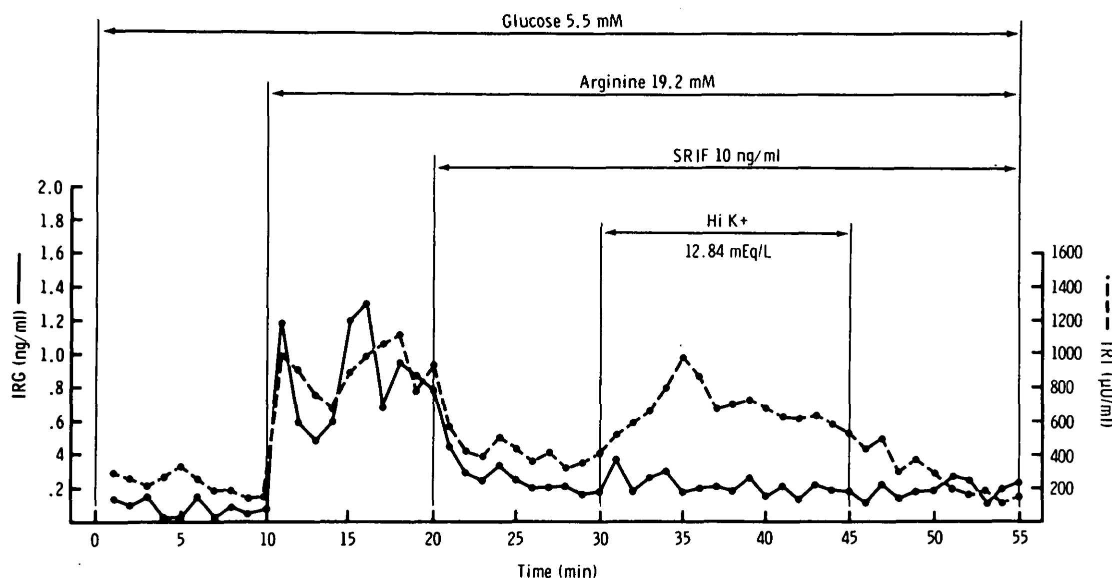

FIG. 9. A single perfusion showing the effect of elevation of K^+ ion concentration on the SRIF-inhibited hormone secretion.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/25/11/1031/215217/25-11-1031.pdf by guest on 21 April 2024

of K^+ into the cell, one might postulate that elevation of intra-islet K^+ may explain in part the effect of glucose as an inhibitor of glucagon release.

The addition of excess K^+ to perfusions inhibited by SRIF resulted in the restoration of insulin release to 79.6 per cent of the pre-SRIF values ($p < 0.05$). However, inhibition of glucagon secretion by SRIF persisted. These data show that the common denominator of SRIF action is undoubtedly not the K^+ ion and suggest that the site of SRIF action, at least on insulin release, is proximal to that of K^+ ion.

Our findings with isolated islets were supportive of SRIF action on Ca^{++}. We observed a significant decrease ($p < 0.05$) of $^{45}Ca^{++}$ uptake by isolated islets when they are incubated with SRIF (2 μg./ml.) in the presence of high glucose. No significant alteration of $^{45}Ca^{++}$ uptake was observed when islets were incubated with SRIF in the absence of glucose. These ^{45}Ca data were very similar to those obtained with epinephrine in vitro.

Though several theories on the action of SRIF have been proposed, the exact mechanism of its action is still unknown. Curry and Bennett[17] postulated thathSRIF acts through binding of Ca^{++}. Schofield et al.[33] suggested that SRIF inhibits exocytosis. Efendic et al.[14] reported that SRIF inhibits insulin secretion by decreasing intracellular levels of cAMP. This contention was in contrast with that of Borgeat et al., who demonstrated that in isolated pituitary fragments, the site of action of SRIF appeared to be beyond the cAMP signal.[9] Further, the observations that insulin secretagogues such as isoproterenol,[6] tolbutamide,[28] glucagon,[28] or theophylline,[6] all of which act in great part by accumulation of cAMP in the β-cell, cannot reverse the action of SRIF on insulin release, leads one to conclude that the action of SRIF is probably distal to that of cAMP. Yet another view was forwarded by Smith et al.,[34] who demonstrated that blocking α-adrenergic receptors reverses SRIF effects on insulin secretion in vivo. Thus, it seems clear that there is no concensus on the mechanism of action of SRIF.

We undertook this investigation in the hope of clarifying certain aspects of SRIF action, at least as regards the effect on insulin and glucagon secretion. As a result of our studies, we would like to suggest that SRIF acts on the islets of Langerhans to impair Ca^{++} ion uptake, and this effect is intimately involved in its inhibitory action on both glucagon and insulin secretion (figure 10). A significant role of metal ions, particularly Ca^{++}, in the action of SRIF appears reasonable in that SRIF has such widespread effects on hormone release that an action on Ca^{++} provides a possible common mechanism.

In the absence of Ca^{++} ion, neither glucagon nor insulin secretion occurs.[35,21] The essential role of Ca^{++} ion, at least in insulin release, presumably entails at least two phases: (1) generation of cAMP, especially when glucose is the stimulus[36] and (2) requirement for the secretory process distal to cAMP action and apparently required for all known insulin secretagogues. Though a similar role for Ca^{++} ion in the secretion of glucagon is debated by some,[37] it may well be that Ca^{++}-dependent cAMP generation as well as Ca^{++}-sensitive distal secretory process are also operative.

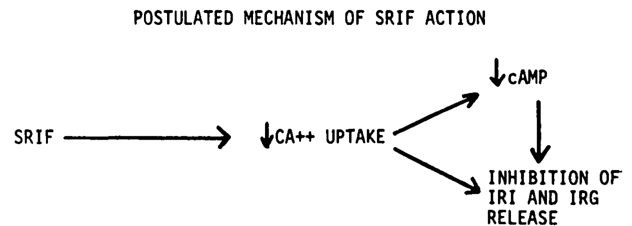

FIG. 10. Schematic representation of the inhibitory effect of SRIF on IRI and IRG secretion.

The recent observations of Dubois[10] and Hokfelt et al.[11] showing the presence of SRIF in D-cells of pancreatic islets and the quantitative data of Arimura et al.[12] showing the highest concentration of SRIF in pancreas has opened yet another vista in thinking of the mechanism of SRIF action. The presence of SRIF in significant amounts within the pancreas clearly leads one to think in terms of intra-islet control of insulin and glucagon secretion. This view gains credence because of the recent demonstration of gap junctions between α- and β-cells.[38] Exactly how this intra-islet control may be taking place is yet unknown and open to speculation.

ACKNOWLEDGMENT

This work was supported in part by NIH grant R01 AM17431.

REFERENCES

[1]Brazeau, P., Vale, W., Burgus, R., Ling, N., Burcher, M., River, J., and Guillemin, R.: Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science *179*:77-79, 1973.

[2]Siler, I. M., Vandenberg, G., and Yen, S. S. C.: Inhibition of growth hormone release in humans by somatostatin. J. Clin. Endocrinol. Metab. *37*:632-34, 1973.

[3]Weeke, J., Prange Hansen, A., and Lundbaek, K.: The inhibition by somatostatin of the thyrotropin response to thyrotropin-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/25/11/1031/21527/25-11-1031.pdf by guest on 21 April 2024

releasing hormone in normal subjects. Scand. J. Clin. Lab. Invest. *33*:101-03, 1974.

[4]Tyrell, J. B., Lorenzi, M., Gerich, J. E., and Forsham, P. H.: Inhibition by somatostatin of ACTH secretion in Nelson's syndrome. J. Clin. Endocrinol. Metab. *40*:1125-27, 1975.

[5]Bloom, S. R., Mortimer, C. M., Thorner, M. O., Besser, G. M., Hall, R., Gomez-Pan, A., Roy, V. M., Russel, R. C. G., Coy, D. H., Kastin, A. J., and Schally, A. V.: Inhibition of gastrin and gastric acid secretion by growth-hormone release-inhibiting hormone. Lancet *2*:1106-09, 1974.

[6]Gerich, J. E., Lovinger, R., and Grodsky, G. M.: Inhibition by somatostatin of glucagon and insulin release from the perfused rat pancreas in response to arginine, isoproterenol and theophylline: Evidence for a preferential effect on glucagon secretion. Endocrinology *96*:749-54, 1975.

[7]Johnson, D. G., Ensinck, J. W., Koerker, D., Palmer, J., and Goodner, C. J.: Inhibition of glucagon and insulin secretion by somatostatin in the rat pancreas perfused in situ. Endocrinology *96*:370-74, 1975.

[8]Curry, D. L., Bennett, L. L., and Li, C. H.: Direct inhibition of insulin secretion by synthetic somatostatin. Biochem. Biophys. Res. Commun. *38*:885-87, 1974.

[9]Borgeat, P., Labrie, F., Drovin, J., Belanger, A., Immer, H., Sestanj, K., Nelson, V., Gotz, M., Schally, A. V., Coy, D. H., and Coy, E. J.: Inhibition of adenosine 3′:5′-monophosphate accumulation in anterior pituitary gland in vitro by growth hormone-release inhibition hormone. Biochem. Biophys. Res. Commun. *56*:1052-59, 1974.

[10]Dubois, M. P.: Immunoreactive somatostatin is present in discrete cells of the endocrine pancreas. Proc. Natl. Acad. Sci. U.S.A. *72*:1340-43, 1975.

[11]Hokfelt, T., Efendic, S., Hellstrom, C., Johannson, O., Luft, R., and Arimura, A.: Cellular localization of somatostatin in endocrine-like cells and neurons of the rat with special references to the A-cells of the pancreatic islets and to the hypothalamus. Acta Endocrinol. *80*(Suppl. 200):1-41, 1975.

[12]Arimura, A., Sato, H., Dupont, A., Nishi, N., and Shally, A. V.: Somatostatin: Abundance of immunoreactive hormone in rat stomach and pancreas. Science *189*:1007-09, 1975.

[13]Efendic, S., Luft, R., and Grill, V.: Effect of somatostatin on glucose induced release in isolated perfused rat pancreas and isolated rat pancreatic islets. F.E.B.S. Lett. *42*:169-72, 1974.

[14]Efendic, S., Grill, V., and Luft, R.: Inhibition by somatostatin of glucose induced 3′:5′-monophosphate (cyclic AMP) accumulation and insulin release in isolated pancreatic islets of the rat. F.E.B.S. Lett. *55*:131-33, 1975.

[15]Turcot-Lemay, L., Lemay, A., and Lacy, P. E.: Somatostatin inhibition of insulin release from freshly isolated and organ cultured rat islets of Langerhans in vitro. Biochem. Biophys. Res. Commun. *63*:1130-38, 1975.

[16]Fujimoto, W. Y., and Ensinck, J. W.: Somatostatin inhibition of insulin and glucagon secretion in rat islet culture: Reversal by ionophore A23187. Endocrinology *98*:259-61, 1976.

[17]Curry, D. L., and Bennett, L. L.: Reversal of somatostatin inhibition of insulin secretion by calcium. Biochem. Biophys. Res. Commun. *60*:1015-19, 1974.

[18]Gomez, M., and Curry, D. L.: Potassium stimulation of insulin release by the perfused rat pancreas. Endocrinology *92*:1126-34, 1973.

[19]Henquin, J. C., and Lambert, A. E.: Cationic environment and dynamics of insulin secretion. II. Effect of high concentration of potassium. Diabetes *23*:933-42, 1974.

[20]Hales, C. N., and Milner, R. D. G.: The role of sodium and potassium in insulin secretion from rabbit pancreas. J. Physiol. *194*:725-43, 1968.

[21]Grodsky, G. M., and Bennett, L. L.: Cation requirements for insulin secretion in isolated perfused pancreas. Diabetes *15*:910-13, 1966.

[22]Curry, D. L., Bennett, L. L., and Grodsky, G. M.: Dynamics of insulin secretion by the perfused rat pancreas. Endocrinology *83*:572-84, 1968.

[23]Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: Two antibody system. Diabetes *12*:115-26, 1963.

[24]Unger, R. H., Eisentraut, A. M., and Madison, L. L.: The effects of total starvation upon the levels of circulating glucagon and insulin in man. J. Clin. Invest. *42*:1031, 1963.

[25]Lacy, P. E., and Kostianowsky, M.: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes *16*:35-39, 1967.

[26]Malaisse, W. J., and Malaisse-Lagae, F.: Uptake of ^{45}calcium by isolated islets of Langerhans. Diabetes *19*:365, 1970.

[27]Malaisse-Lagae, F., and Malaisse, W. J.: The stimulus secretion coupling of glucose induced insulin release. III. Uptake of ^{45}calcium by isolated islets of Langerhans. Endocrinology *88*:72-80, 1971.

[28]Gerich, J. E., Lorenzi, M., Schneider, V., and Forsham, P. H.: Effect of somatostatin on plasma glucose and insulin responses to glucagon and tolbutamide in man. J. Clin. Endocrinol. Metab. *39*:1057-60, 1974.

[29]Grodsky, G. M.: A threshold distribution hypothesis for packet storage of insulin. II. Effect of calcium. Diabetes *21*:584-93, 1972.

[30]Santeusanio, F., Faloona, G. R., Korochel, J. P., and Unger, R. H.: Evidence for a role of endogenous insulin and glucagon in the regulation of potassium homeostasis. J. Lab. Clin. Med. *81*:809-17, 1973.

[31]Kuzuya, T., Kajinuma, H., and Ide, T.: Effect of intrapancreatic injection of potassium and calcium on insulin and glucagon secretion in dogs. Diabetes *23*:55-60, 1974.

[32]West, E. S., and Todd, W. R.: Renal function, acid-base balance, electrolyte and water balance. Textbook of Biochemistry. New York, Macmillan, 1961, p. 652.

[33]Schofield, J. G., Mira, F., and Orci, L.: Somatostatin and growth hormone secretion in vitro: A biochemical and morphological study. Diabetologia *10*:385, 1974.

[34]Smith, P. H., Woods, S. C., and Porte, D., Jr.: Inhibition of insulin secretion by somatostatin. Reversal of the in vivo effect by alpha-adrenergic blockage. Diabetes *24*:408, 1975.

[35]Gerich, J. E., Frankel, B. J., Fanska, R., West, L., Forsham, P. H., and Grodsky, G. M.: Calcium dependency of glucagon secretion from the in vitro perfused rat pancreas. Endocrinology *94*:1381-85, 1974.

[36]Charles, M. A., Lawecki, J., Pictet, R., and Grodsky, G. M.: Insulin secretion. Interrelationships of glucose, cyclic adenosine 3′:5′-monophosphate and calcium. J. Biol. Chem. *250*:6134-40, 1975.

[37]Edwards, J. C., and Howell, S. L.: Effects of vinblastine and colchicine on the secretion of glucagon from isolated guinea pig islets of Langerhans. F.E.B.S. Lett. *30*:89-91, 1973.

[38]Orci, L., Malaisse-Lagae, F., Ravazzola, M., Rouiller, D., Renold, A. E., Perrelet, A., and Unger, R.: A morphological basis for intracellular communication between α and β-cells in the endocrine pancreas. J. Clin. Invest. *56*:1066-70, 1975.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/25/11/1031/215217/25-11-1031.pdf by guest on 21 April 2024