Somatostatin inhibition of pancreatic glucagon release from monolayer cultures and interactions with calcium

WOLLHEIM, Claes, et al.

Abstract
The effects of somatostatin (SRIF) on glucagon release have been studied in the monolayer culture of newborn rat pancreas. It was found that SRIF inhibited glucagon release rapidly and in a dose dependent manner at concentrations of 1-1000 ng/ml. SRIF inhibited glucagon release under basal conditions and after stimulation by arginine, 3-isobutyl-1-methylxanthine (IBMX), high Ca++ concentrations, ionophore A23187 and Ca++, and Ba++. SRIF inhibited ionophore-induced glucagon release over 60 min when a low concentration of A23187 was used (0.1 microgram/ml) but not when a high concentration (10 microgram/ml) was used. The stimulant effect of 10 microgram/ml A23187 was, however, inhibited by SRIF during short periods of incubation. The per cent inhibition of arginine-stimulated glucagon release due to SRIF remained unchanged when the Ca++ concentration in the medium was varied from 1-10 mM. It is concluded that SRIF promptly inhibits glucagon release under basal conditions or when stimulated by a variety of agents. Thus, the action of SRIF appears to be basic to the granule release process and not specifically antagonistic to […]

Reference

DOI : 10.1210/endo-101-3-911
PMID : 330154

Available at: http://archive-ouverte.unige.ch/unige:40546

Disclaimer: layout of this document may differ from the published version.
Somatostatin Inhibition of Pancreatic Glucagon Release from Monolayer Cultures and Interactions with Calcium

CLAES B. WOLLHEIM, BENIGNA BLONDEL, ALBERT E. RENOLD, AND GEOFFREY W. G. SHARP

Institut de Biochimie Clinique, and Institut d'Histologie et d'Embryologie, Université de Genève, Geneva, Switzerland

ABSTRACT. The effects of somatostatin (SRIF) on glucagon release have been studied in the monolayer culture of newborn rat pancreas. It was found that SRIF inhibited glucagon release rapidly and in a dose dependent manner at concentrations of 1–1000 ng/ml. SRIF inhibited glucagon release under basal conditions and after stimulation by arginine, 3-isobutyl-1-methylxanthine (IBMX), high Ca++ concentrations, ionophore A23187 and Ca++, and Ba++. SRIF inhibited ionophore-induced glucagon release over 60 min when a low concentration of A23187 was used (0.1 μg/ml) but not when a high concentration (10 μg/ml) was used. The stimulant effect of 10 μg/ml A23187 was, however, inhibited by SRIF during short periods of incubation. The per cent inhibition of arginine-stimulated glucagon release due to SRIF remained unchanged when the Ca++ concentration in the medium was varied from 1–10 mM.

It is concluded that SRIF promptly inhibits glucagon release under basal conditions or when stimulated by a variety of agents. Thus, the action of SRIF appears to be basic to the granule release process and not specifically antagonistic to any particular stimulants. Further, as SRIF inhibits release due to raised cytosol Ca++ (e.g., ionophore-Ca++ or high Ca++ experiments) the action is probably at a late point in the release mechanism. (Endocrinology 101: 911, 1977)

SOMATOSTATIN (Somatotropin-release-inhibitory factor, SRIF), originally isolated from hypothalamus (1) and thought to be a specific inhibitor of growth hormone release, has been reported to influence the secretion of a wide variety of polypeptide hormones. These include TSH (2), insulin (3,4), glucagon (5,6), gut glucagon-like immunoreactivity (7), gastrin (8), secretin (9), and renin (10). In addition, SRIF has been reported to inhibit gastric acid and pepsin secretion (11,12) and pancreatic exocrine secretion (8).

The release of glucagon in vitro has been shown to be inhibited by SRIF in the perfused pancreas of either the rat (13) or the dog (6), and in pancreatic monolayer cultures (14), whereas SRIF failed to inhibit glucagon release from isolated rat islets (15).

The mode of action of SRIF remains unclear. Release of polypeptide hormones when stimulated by a variety of agents is inhibited by SRIF. Accordingly, SRIF inhibited pancreatic glucagon release evoked by arginine (15–18), by the phosphodiesterase inhibitor, theophylline (17), by the catecholamines epinephrine (13,19) and isoproterenol (17), and by acetylcholine (19). Glucagon release is inhibited also in the presence of low or high (suppressing) concentrations of glucose (6). It has therefore been assumed that SRIF interferes with a step common to many hormone releasing cells. Since Ca++ ions play an important role in hormone secretion, SRIF might act upon Ca++ dynamics in the susceptible cells. Thus, it has been reported that increasing the Ca++ concentration in the medium could partially reverse the inhibitory effect of SRIF on glucose-induced insulin release by perfused rat pancreas (18,20) and by isolated rat islets (21). Since SRIF is the most potent known inhibitor of pancreatic glucagon release, a study designed to examine the effect of SRIF on glucagon release with special reference to the interaction of SRIF and calcium appeared warranted.

The divalent cation ionophore A23187, which acts as a carrier for Ca++ across
biological membranes, was employed to raise cytosolic Ca\(^{++}\) concentrations in the A-cell. As in our earlier study on glucagon release and the effects of A23187 (22), a monolayer culture of newborn rat pancreas was used. The preparation responds to modulators of glucagon release (23,24) and is particularly well suited for studies with ionophores because of the near-maximal exposure of the cellular plasma membranes. In addition to the ionophore, four other stimulators of glucagon release were employed: high Ca\(^{++}\) concentration, barium, arginine and the potent phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX).

**Materials and Methods**

The detailed technique for the culture of newborn rat pancreas in monolayer has been described elsewhere (23,25). The following modifications were made. The cell suspension obtained from the pancreases of 1–3 day old Wistar rats was plated in 94 mm diameter plastic petri dishes. Sixteen hours later, when a major part of the fibroblastoid cells had attached to the bottom of the dish, the unattached cells were pooled and redistributed into 35 mm diameter plastic petri dishes. One newborn rat pancreas was used for each small dish required. Forty-four hours after the initial plating, when the endocrine cells had formed cell clusters of varying size, the cultures were washed extensively. Fresh culture medium 199 was added. The glucose concentration until the time of washing was 16.7 mM and was decreased to 5.6 mM for the last 20–22 h prior to the experiments. Culture medium 199 was supplemented with 14 mM NaHCO\(_3\), 10% heat-inactivated calf serum, 400 IU/ml sodium penicillin G and 200 μg/ml streptomycin sulfate. The experiments reported here were carried out on the third day of culture.

Short incubations were performed in Krebs-Ringer bicarbonate buffer (KRB) containing 0.5% dialyzed bovine serum albumin, 250 KIU/ml Trasylol and either 2.8 or 16.7 mM glucose. The ionic composition of the standard KRB-buffer was the following: NaCl 118.6 mM, KCl 4.8 mM, KH\(_2\)PO\(_4\) 1.2 mM, NaHCO\(_3\) 24.6 mM, MgSO\(_4\) 1.2 mM, and CaCl\(_2\) (when not otherwise stated) 1.0 mM. Calcium-depleted media were obtained by omission of CaCl\(_2\) from the buffer. In experiments where the Ca\(^{++}\) concentration was increased to 10 and 30 mM, the NaCl concentration was decreased to maintain ionicosmolarity of the buffer and the concentration of H\(_2\)PO\(_4\)^– was also decreased to 0.1 mM. When BaCl\(_2\) was to be used in the medium, MgSO\(_4\) was replaced by MgCl\(_2\). These modifications of basal medium did not themselves affect glucagon release. SRIF was dissolved in sterile water and kept as a stock solution at 4°C. The ionophore A23187 was dissolved in dimethyl sulfoxide (DMSO) before addition to the test media. The final DMSO concentration was 1% at which concentration no effects on glucagon release were observed. However, DMSO was always added to control media in experiments with the ionophore.

Before the incubation experiments the petri dishes were washed three times and then 1 ml of KRB buffer was added. When not otherwise stated incubations were carried out over 1 h at 37°C. Samples taken after the incubation were centrifuged for 15 min at 3500 × g, decanted and stored at −20°C until assay. Immunoreactive glucagon (IRG) was measured by the method of Unger et al. (26) using pork glucagon as standard and 30K anti-pork glucagon serum. At the concentrations tested neither SRIF nor A23187 interfered with the radioimmunoassay.

Results are expressed as means ± SEM and statistical analysis was by Student’s t test. The chemicals employed and their sources were as follows: trypsin (Difco Laboratories, Detroit, Mich.), collagenase ( Worthington Biochemical Corp., Freehold, N.J.), medium 199 (Grand Island Biochemical Co., Grand Island, N.Y.), sodium- penicillin G (Chas. Pfizer and Co., Inc., New York, N.Y.), streptomycin sulfate (Novo Industri A.S., Copenhagen, Denmark), petri dishes (Falcon Plastics, Oxnard, Calif.), bovine serum albumin (Behringwerke A.G., Marburg, FRG), Trasylol (kindly provided by Dr. H. Rüff, Bayer Pharma A. G., Zurich, Switzerland), SRIF linear (kindly provided by Dr. R. Guillemin, the Salk Institute, La Jolla, California), A23187 (kindly provided by Dr. O. Behrens, Eli Lilly, Indianapolis, Ind.), dimethyl sulfoxide (Merck A.G., Darmstadt, FRG), glucagon antiserum 30K (generously provided by Dr. R. H. Unger, University of Texas, Southwestern Medical School, Dallas, Texas), 3-isobutyl-1-methylxanthine.
Fig. 1. Effect of different concentrations of SRIF on glucagon release in the presence of 2.8 mM glucose and 10 mM arginine. Vertical lines represent ± SEM. Number of observations in parentheses.

(Aldrich Chemical Company Inc., Milwaukee, Wis.).

Results

The effect of SRIF

1. In the presence of arginine. In Fig. 1 are shown the results of experiments in which the cultured cells were incubated for 1 h in the presence of 10 mM arginine and several different concentrations of SRIF. A dose-related inhibition of glucagon release by SRIF was observed. The inhibition was 14, 20, 42 and 52% with 1, 10, 100 and 1000 ng/ml SRIF, respectively. Per cent inhibition is calculated on the basis of total glucagon release in the presence of the stimulator because SRIF inhibits both stimulated and basal glucagon release (see Table 1).

In a separate series of experiments, the results of which are shown in Table 1, SRIF at 100 ng/ml was tested for its inhibitory activity under conditions of low glucose concentration alone and in the presence of 20 mM arginine over only 15 min of incubation. Inhibition of glucagon release occurred under both conditions. In the presence of glucose alone inhibition was 41%, and in the presence of arginine, 67%. Thus, SRIF has a rapid onset of action. As expected, arginine stimulated release significantly ($P < 0.001$).

2. In the presence of IBMX. The effect of SRIF on glucagon release stimulated by the phosphodiesterase inhibitor IBMX was tested in the presence of 2.8 mM glucose. As is shown in Table 2, 1 mM IBMX caused a highly significant stimulation of glucagon release ($P < 0.001$) which was completely abolished by 100 ng/ml SRIF.

3. In the presence of high glucose. Because of the suppressing effect of glucose on glucagon release, SRIF was tested at either low (2.8 mM) or high (16.7 mM) glucose concentration. As expected, 16.7 mM glucose suppressed glucagon release relative to 2.8 mM glucose ($P < 0.05$). SRIF at 100 ng/ml inhibited the release at both glucose concentrations (Table 3).

<table>
<thead>
<tr>
<th>Arginine (mM)</th>
<th>Control (SRIF 100 ng/ml)</th>
<th>Test (SRIF 100 ng/ml)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>71 ± 5 (5)</td>
<td>42 ± 6 (4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>20</td>
<td>187 ± 19 (5)</td>
<td>62 ± 8 (5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Number of observations in parentheses.

<table>
<thead>
<tr>
<th>IBMX (mM)</th>
<th>Control (SRIF 100 ng/ml)</th>
<th>Test (SRIF 100 ng/ml)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>193 ± 14</td>
<td>156 ± 16</td>
<td>&lt;0.20</td>
</tr>
<tr>
<td>1.0</td>
<td>377 ± 38</td>
<td>162 ± 24</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>
Table 3. Effect of SRIF on glucagon release suppressed by high glucose concentration (n = 11)

<table>
<thead>
<tr>
<th>Glucose (mM)</th>
<th>Control</th>
<th>Test (SRIF 100 ng/ml)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.8</td>
<td>136 ± 14</td>
<td>88 ± 9</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>16.7</td>
<td>100 ± 4</td>
<td>73 ± 9</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

4. In the presence of Ca++ and ionophore A23187. Because of the complex concentration and time-dependent characteristics of the ionophore-Ca++ action on glucagon release (22) experiments were performed with either a low (0.1 μg/ml) or a high (10 μg/ml) concentration of A23187 in the presence of 2.8 mM glucose. As is shown in Table 4(A), 0.1 μg/ml A23187 and 1 mM Ca++ significantly stimulated glucagon release over 60 min. SRIF at 100 ng/ml abolished the enhanced glucagon release.

When the higher ionophore dose was tested over 60 min the Ca++ concentration was reduced to 0.3 mM to optimize the stimulation conditions (22). A23187 enhanced glucagon release and inhibition by SRIF was not statistically significant. In the presence of 2.8 mM glucose alone the expected inhibitory effect of SRIF was seen (Table 4(B)). To examine whether this failure of SRIF to inhibit release was due to prolonged exposure of the cells to high ionophore concentrations, the experiments were repeated except that glucagon release was measured over only 10 min. Over this time A23187 10 μg/ml was used with 1.0 mM Ca++, again to optimize release, and caused a significant release of glucagon. Also SRIF inhibited the release evoked by the ionophore and the inhibition in absolute terms was more marked than the inhibition of non-stimulated release Table 4(C)). Thus, SRIF can inhibit glucagon release caused by low or high concentrations of the ionophore, but with prolonged exposure to high ionophore concentrations an escape from SRIF inhibition occurs.

Table 4. Effect of SRIF on glucagon release stimulated by ionophore A23187 and Ca++ (glucose = 2.8 mM)

<table>
<thead>
<tr>
<th>Ca++ (mM)</th>
<th>A23187 (μg/ml)</th>
<th>Control</th>
<th>Test SRIF (100 ng/ml)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0</td>
<td></td>
<td>222 ± 21 (6)</td>
<td>—</td>
</tr>
<tr>
<td>1.0</td>
<td>0.1</td>
<td>315 ± 32 (6)</td>
<td>187 ± 21 (5)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(P &lt; 0.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>0</td>
<td>262 ± 28 (12)</td>
<td>167 ± 10 (12)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>0.3</td>
<td>10.0</td>
<td>332 ± 35 (12)</td>
<td>293 ± 22 (12)</td>
<td>&lt;0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(P &lt; 0.005)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
</tr>
<tr>
<td>1.0</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

5. In the presence of 30 mM Ca++. Very high Ca++ concentrations have been shown to stimulate glucagon release even in the absence of ionophore (22). In the experiments shown in Fig. 2, the effect of 100 ng/ml SRIF was examined at 1 or 30 mM Ca++ in the presence of 2.8 mM glucose. Thirty millimolar Ca++ markedly stimulated glucagon release (P < 0.001) and SRIF inhibited both the Ca++ induced glucagon release and the release under basal conditions.

6. In the presence of Ba++. The purpose of the following experiments was two-fold: firstly, to examine the effect of SRIF on glucagon release stimulated by Ba++, and secondly, to compare the effectiveness of SRIF in the presence or absence of Ca++ in the bathing medium. As can be seen in Fig. 3, 1 mM Ba++ stimulated glucagon release in the presence of 1 mM Ca++, as well as in the absence of Ca++. The stimulant effect of Ba++ was greater in the absence of Ca++ (P < 0.05). SRIF at 100 ng/ml inhibited the Ba++ stimulated release to approximately the same release rate regardless of whether
Ca++ was present or absent. In absolute terms, the inhibition was greater at 0 Ca++ than at 1 mM Ca++. Under basal conditions, as already shown, SRIF inhibited glucagon release in the presence of 1 mM Ca++ alone, but in the absence of Ca++ the small inhibition observed was not statistically significant. When examining the results shown in Fig. 3, it can be seen that glucagon release is slightly enhanced by the omission of Ca++ ($P < 0.02$) (comparing the first open bar with the first hatched bar in Fig. 3).

7. In the absence of Ca++. To examine further the effect of SRIF in the absence of Ca++, a larger series of experiments was performed (Table 5). As already shown in Fig. 3, omission of Ca++ from the medium stimulated glucagon release (relative to 1 mM Ca++). In this larger series of experiments SRIF caused a small but significant inhibition of glucagon release in the absence of Ca++, although the inhibition was less than that obtained in the presence of Ca++.

8. In the presence of arginine and different Ca++ concentrations. The effect of SRIF on glucagon release was tested in the presence of arginine and different concentrations of Ca++ from 1 to 30 mM. The results are shown in Fig. 4. In the presence of 2.8 mM glucose alone, glucagon release was increased by 10 mM Ca++ and further increased, although not significantly, by 30
TABLE 5. Effect of SRIF on glucagon release in the presence or absence of Ca++ in the medium (n = 18)

<table>
<thead>
<tr>
<th>Ca++ (mM)</th>
<th>Control</th>
<th>Test (SRIF)</th>
<th>Δ</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>185 ± 10</td>
<td>122 ± 11</td>
<td>63 ± 13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0</td>
<td>244 ± 19</td>
<td>203 ± 18</td>
<td>41 ± 13</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(P &lt; 0.02)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

mM Ca++. Arginine-induced glucagon release was also influenced by the Ca++ concentration. The optimal effect was observed with 10 mM Ca++ (P < 0.05 at 1 mM Ca++, P < 0.001 at 3 and 10 mM Ca++). With 30 mM Ca++ arginine failed to stimulate the release relative to control (release with 30 mM Ca++). SRIF (100 ng/ml) inhibited glucagon release in the presence of arginine at all Ca++ concentrations. When the effect of SRIF was expressed as per cent inhibition of the arginine stimulated values, the following results were obtained: 53 ± 4.8% at 1 mM Ca++, 54 ± 5.7% at 3 mM Ca++, 54 ± 3.0% at 10 mM Ca++ and 36 ± 2.4% (mean ± SEM) at 30 mM Ca++. The latter inhibition was significantly less compared to the effect of SRIF on arginine stimulated release at 1 mM Ca++ (P < 0.01) and 10 mM Ca++ (P < 0.001). The inhibitory effect of SRIF was thus unaffected by increasing the Ca++ concentration from 1 to 10 mM. In the presence of very high Ca++ (30 mM) the effect of SRIF was attenuated, but under the same experimental conditions arginine failed to increase the already elevated glucagon release.

Discussion

In this study it has been shown that SRIF inhibits glucagon release from monolayer culture of the endocrine pancreas. Both basal and stimulated release was inhibited. The concentration-dependence of SRIF-induced inhibition of arginine-induced release was similar to that observed in the isolated perfused pancreas for inhibition of glucagon release (17,18). Inhibition of release due to several agents was seen. Thus, arginine-, IBMX-, Ba++, high Ca++ and Ca++ and ionophore A23187-induced release were all inhibited. Similarly, inhibition of glucagon release could be demonstrated in the presence of both low and high glucose concentrations; i.e., even in the presence of the suppressive effect of high glucose on glucagon release.

The activity of SRIF in the presence of different Ca++ concentrations has been examined by others with respect to insulin release (18,20,21) and with regard to glucagon release (18). Both high or intermediate calcium concentrations (22,27–29) and, paradoxically, calcium-deprivation (22,30, 31) enhance glucagon release. Also Ca++ and ionophore A23187 have been shown to stimulate release (22,32). In view of the latter finding and the wide acceptance of a role for increased cytosol Ca++ in the release mechanisms for granule secretion,
it seems likely that glucagon release, due to high Ca++ in the medium or due to Ca++ entry facilitated by the ionophore, mimics the physiological release process whereas the stimulation of glucagon release by Ca++ deprivation may represent a non-physiological mechanism. It was found that when Ca++ was removed from the medium, glucagon release was stimulated and SRIF inhibited release under these conditions. However, the inhibition achieved (Table 5) was somewhat less than the effect of SRIF on glucagon release under normal (1 mM Ca++) conditions. In contrast, in all other cases of stimulated release the inhibitory effect of SRIF was greater than the inhibition of basal release. Thus, it is possible that glucagon release specifically due to low Ca++ may not be inhibited by SRIF, a conclusion which would further support the concept that Ca++ deprivation leads to release of glucagon by a non-physiological mechanism.

Studies on glucagon release with A23187 and Ca++ have shown that different results are obtained when different concentrations of A23187 and Ca++, and when different periods of exposure to the agents, are used (22). The conclusion of that report was that with high ionophore and Ca++ concentrations excess entry of Ca++ and inhibition of release would occur after lengthy incubation periods. Conversely, with a short incubation period (e.g., 10 min) stimulation of release is seen. With low ionophore and Ca++ concentrations a sustained release of glucagon is achieved. Thus, it is important to be aware of the concentration and timing of ionophore and Ca++ application when studying the interaction with other agents. In the results reported here, release stimulated by 0.1 μg/ml ionophore and 1 mM Ca++ was inhibited by SRIF over 60 min. The release due to 10 μg/ml A23187 was not inhibited by SRIF over 60 min. However, the effect of the high concentration of ionophore was inhibited by SRIF when the incubation period was reduced to 10 min. Thus, SRIF does inhibit the ionophore and Ca++-induced release, and the failure to inhibit under high ionophore concentrations and long exposure may be due to an impaired integrity of the cells, for instance, damage to SRIF receptors.

Fujimoto and Ensink (27) reported that glucagon release in the presence of ionophore A23187 at 10 μg/ml and 0.9 mM Ca++ was not inhibited by SRIF over a 60 min incubation with a pancreatic monolayer culture. In this respect the results are similar to those of the high dose ionophore experiments reported here, except that they also report a paradoxical enhancement of the hormone release. It is of interest that Bicknell and Schofield (33) were able to show that SRIF inhibited growth hormone release induced by A23187 (10 μg/ml) from bovine pituitary cells.

Since it has been suggested that increasing Ca++ concentrations could partially antagonize the inhibitory effects of SRIF on insulin (18,20,21) and glucagon (18) secretion, the question was examined with respect to glucagon release by two series of experiments. In the first series of experiments, a comparison was made between the inhibitory effects of SRIF in the presence of 1 and 30 mM Ca++. In the presence of 1 mM Ca++ SRIF inhibited glucagon release by 53%. In the presence of 30 mM Ca++ the release was inhibited by 34%. Thus, while the indications are for an interference with the inhibitory effect of SRIF by high concentrations of Ca++ it should be noted that the absolute decrement at 30 mM Ca++ was greater than at 1 mM (see Fig. 2), and that 30 mM Ca++ might itself be detrimental and fail to fulfill its potential for glucagon secretion. This can be seen in the experiments in which the effect of SRIF was tested against arginine stimulated release at 1, 3, 10 and 30 mM Ca++.. In these experiments no effect of Ca++ was observed on the inhibitory action of SRIF between 1 and 10 mM Ca++. At 30 mM Ca++ the inhibitory effect of SRIF was less, but the extent of stimulation by arginine was also much reduced and not significantly greater than
with 30 mM Ca\(^{++}\) alone. Thus, it may not be possible to use results obtained in the presence of 30 mM Ca\(^{++}\) as evidence for interference by Ca\(^{++}\) on the action of SRIF.

In conclusion, SRIF inhibits glucagon release under a variety of conditions and in the presence of several different stimulators. This, and inhibitory effects on other secretory cells, supports an action of SRIF at a point late in the control of granule secretion. Stimulation of glucagon release either by ionophore A23187 and Ca\(^{++}\) treatment or by increasing the Ca\(^{++}\) concentration in the medium was inhibited by SRIF. The situation with regard to Ca\(^{++}\) interference with the action of SRIF was not clear. No evidence of interference was obtained with Ca\(^{++}\) concentrations up to 10 mM. At the very high level of 30 mM Ca\(^{++}\), relative inhibition of glucagon release by SRIF was reduced while the absolute decrement in release was greater. It seems highly unlikely that Ca\(^{++}\) has any inhibitory effect on SRIF in vivo.

Acknowledgments

The authors are grateful to Mrs. Theres Cuche, Miss Marie-France Blanc and Mrs. Marie-Paule Barrillat for their skilled technical assistance.

References


