Inhibition of insulin release by somatostatin: no evidence for interaction with calcium

WOLLHEIM, Claes, et al.
Inhibition of Insulin Release by Somatostatin: No Evidence for Interaction With Calcium

Claes B. Wollheim, Benigna Blondel, Masatoshi Kikuchi, and Geoffrey W. G. Sharp

**SOMATOSTATIN (SRIF)** inhibits hormone release in response to different stimuli. Because of the well-known importance of Ca++ for hormone release, it has been postulated that the action of SRIF on insulin release is exerted by an inhibitory effect on Ca++ uptake by the pancreatic β cell. The present study was undertaken to examine the effect of a wide range of Ca++ concentrations on SRIF inhibition of glucose- or arginine-stimulated insulin release in pancreatic monolayer cultures. In addition, we studied the effect of SRIF on insulin release stimulated by the ionophore A23187, which acts as a Ca++ carrier in the plasma membrane. Finally, SRIF action on Ba++-stimulated insulin release was investigated. In contrast to the other three stimuli, Ba++ is able to elicit release in the absence of Ca++.  

**MATERIALS AND METHODS**

Cell suspensions from neonatal rat pancreas were prepared and cultured for 3 days as described previously. Insulin release was measured in Krebs-Ringer bicarbonate buffer containing 0.5% bovine serum albumin, 250 KIU/ml Trasylol, 2.8 mM glucose, and the test agents. In experiments with 10 or 30 mM CaCl₂, the concentration of H₃PO₄⁻ was decreased to 0.1 mM and the NaCl content of the buffer was reduced to maintain isosmolality. When BaCl₂ was used, (MgSO₄) was replaced by MgCl₂. Dihydrosomatostatin was provided by Dr. R. Guillemin (La Jolla, Calif.) and A23187 by Dr. O. Behrens (Indianapolis, Ind.). A23187 was dissolved in dimethylsulfoxide (DMSO), the final concentration of which was 1%. DMSO at this concentration did not affect the release of insulin, measured by radioimmunoassay using rat insulin as standard.

**RESULTS**

The effect of different SRIF concentrations on glucose- or arginine-stimulated insulin release was tested. Glucose (16.7 mM)-stimulated release was inhibited by 48%, 57%, 86%, and 93% in the presence of 10, 30, 100, and 300 ng/ml SRIF, respectively. The same concentrations of SRIF inhibited arginine (20 mM)-stimulated release by 38%, 69%, 74%, and 70%, respectively. The basal insulin release in the presence of 2.8 mM glucose and 3 mM Ca++ was not affected by these concentrations of SRIF.

To study the effect of different Ca++ concentrations on the inhibitory effect of SRIF on glucose-stimulated insulin release, 30 ng/ml SRIF was examined at Ca++ concentrations between 1 and 30 mM. As shown in Table 1A insulin release was stimulated by 16.7 mM glucose at all Ca++ concentrations tested. The stimulation was maximal at 10 mM Ca++ and was significantly less at 30 mM.

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Ca++: SRIF inhibited the stimulated release at all Ca++ concentrations. In absolute terms the inhibition was greater at 3, 10, and 30 mM Ca++ relative to 1 mM Ca++, although the decrements were not significantly different from that at 1 mM Ca++. When the results were expressed as percent inhibition of stimulated release, the SRIF effect appeared to be less at 10 mM Ca++ relative to the other Ca++ concentrations.

Similar experiments were performed to study the SRIF inhibition of arginine-stimulated insulin release at different Ca++ concentrations. The results in Table 1B show that insulin release was stimulated by 20 mM arginine at all Ca++ concentrations. The arginine effect was optimal at 10 mM Ca++. SRIF (100 ng/ml) inhibited the arginine-stimulated release at all four Ca++ concentrations. In absolute terms the inhibition was most pronounced at 10 mM Ca++, at which concentration the decrement was 212% of the decrement at 1 mM Ca++ (p < 0.001). The decrements at 3 and 30 mM Ca++ were not different from that at 1 mM Ca++. The expression of these results as percent inhibition of stimulated release shows that SRIF inhibition was most marked at 1 and 30 mM Ca++.

In the experiments shown in Table 2A, Ba++ was used as an insulin secretagogue. Ba++ (1 mM) stimulated insulin release both in the presence and absence of Ca++, although the effect was greater in the absence of Ca++ (p < 0.02). SRIF (100 ng/ml) inhibited Ba++-stimulated release whether Ca++ was present or not. The inhibition was the same in absolute terms but when expressed as percent inhibition, it was 69% in the presence, compared to 46% in the absence, of Ca++.

Finally, the effect of SRIF on ionophore A23187-stimulated release was
**Table 2. Effect of SRIF on Insulin Release Stimulated by Either Ba\(^{++}\) (A) or Ionophore A23187 (B)**

<table>
<thead>
<tr>
<th>A</th>
<th>Ca(^{++}) (mM)</th>
<th>Ba(^{++}) (mM)</th>
<th>Insulin Release (ng/dish/60 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test (SRIF 100 ng/ml)</td>
<td>(p)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>3.5 ± 0.2 (12)</td>
<td>2.7 ± 0.4 (11)</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>26.0 ± 3.3 (11)</td>
<td>10.4 ± 1.5 (12)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>3.8 ± 0.5 (12)</td>
<td>3.9 ± 0.5 (12)</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>38.5 ± 3.6 (12)</td>
<td>22.5 ± 3.2 (12)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B</th>
<th>Ca(^{++}) (mM)</th>
<th>A23187 (µg/ml)</th>
<th>Insulin Release (ng/dish/60 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test (SRIF 100 ng/ml)</td>
<td>(p)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>7.5 ± 0.6 (10)</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>0.1</td>
<td>9.3 ± 0.7 (10)</td>
<td>6.3 ± 0.7 (10)</td>
</tr>
</tbody>
</table>

*Results expressed as mean ± SEM.

The glucose concentration of the buffer was 2.8 mM throughout.
DMSO (1%) was added to the control dishes of the experiments in B.
Statistical analysis by Student’s t test. Number of observations in parentheses.

Examined. As is shown in Table 2B, 0.1 µg/ml A23187 in the presence of 1 mM Ca\(^{++}\) significantly stimulated insulin release (\(p < 0.02\)). The ionophore-stimulated release was completely inhibited by SRIF (100 ng/ml).

**DISCUSSION**

The sensitivity to SRIF inhibition of stimulated insulin release from the cultured cells was comparable to that of the perfused pancreas.\(^{7,8}\) The failure of SRIF to inhibit basal release agrees with a previous report on perfused pancreas\(^7\) but not with another on cultured cells.\(^6\) Since the rate of stimulated insulin release varied with the Ca\(^{++}\) concentrations of the buffer, the relative effect of SRIF at different Ca\(^{++}\) concentrations depended on the method of expression of the results. Accordingly, the absolute decrements of glucose-induced insulin release were similar at 1, 3, 10, and 30 mM Ca\(^{++}\), whereas in percent terms the effect of SRIF decreased at 10 mM and increased again at 30 mM. In the case of arginine-stimulated release, the absolute decrement was greatest at 10 mM, although the percent inhibition was reduced at this Ca\(^{++}\) concentration and most pronounced at 30 mM. The failure of these high Ca\(^{++}\) concentrations to counteract the SRIF inhibition of glucose-induced insulin release disagrees with the reports by Curry and Bennett\(^8\) and Taminato et al.\(^7\) in which elevation of the Ca\(^{++}\) content of the buffer to 5.5 and 6 mM, respectively, attenuated the effect of SRIF. In our opinion, however, the experimental design used in the former study\(^7\) does not allow an evaluation of the relative effectiveness of SRIF under control and high Ca\(^{++}\) conditions. In the latter study,\(^10\) the inhibition of cyclic AMP-stimulated release was unaffected by the change in Ca\(^{++}\) concentration. Another report did not show attenuation of the SRIF effect following an increase of the Ca\(^{++}\) from 0.9 to 3.6 mM.\(^6\) Moreover, following Ca\(^{++}\) elevation during the first phase rather than the second phase of insulin release, Curry and Bennett did not observe attenuation of the SRIF action.\(^8\) With respect to arginine-stimulated release, our results are at
variance with those of Bhathe ena et al., who reported partial reversal of SRIF inhibition when the Ca++ concentration was raised to 7.6 mM. Ba++-stimulated insulin release was most pronounced in the absence of Ca++, probably due to similar handling of the two cations by the islets. SRIF inhibition (expressed as percent), however, was most marked in the presence of Ca++. SRIF abolished insulin release stimulated by the ionophore A23187 (0.1 µg/ml). The ionophore is thought to increase cytosol Ca++ and to bypass the physiological Ca++ uptake mechanism. These results contrast with those of Fujimoto and Ensinck, who reported a paradoxical enhancement of insulin release following simultaneous application of A23187 (10 µg/ml) and SRIF (1 µg/ml). The higher concentrations of the agents used by these authors could explain the different results. It is noteworthy that SRIF has been shown to inhibit the release of glucagon, and growth hormone stimulated by A23187.

When direct measurements of Ca++ fluxes in islets were performed, no inhibitory effects of SRIF were observed on either glucose-evoked initial 45Ca++ uptake or 45Ca++ efflux from preloaded islets despite marked inhibition of simultaneously measured insulin release. The effect of SRIF to reduce glucose-stimulated net 45Ca++ retention by islets after lengthy incubations does not necessarily indicate a direct action of SRIF on Ca++ movements (see Wollheim et al.).

The results presented here and the measurements of Ca++ fluxes in islets of Langerhans do not support the hypothesis that SRIF inhibits insulin release by interfering with Ca++ influx in the β cell.

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