Effect of insulin on glucose- and arginine-stimulated somatostatin secretion from the isolated perfused rat pancreas

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Abstract

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Reference


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Effect of Insulin on Glucose- and Arginine-Stimulated Somatostatin Secretion from the Isolated Perfused Rat Pancreas*

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ABSTRACT. The effects of exogenous insulin on somatostatin secretion from the isolated perfused rat pancreas have been investigated in the presence of 5.6 mM glucose and when somatostatin secretion was stimulated by either glucose (16.7 mM) or arginine (20 mM). Insulin (15 mU/ml) significantly and rapidly suppressed glucose- and arginine-stimulated somatostatin release. However, at 5.6 mM glucose and, in the absence of other stimulators of somatostatin release, insulin had no effect on the somatostatin secretion rate. (Endocrinology 109: 279, 1981)

THE PANCREATIC hormones, insulin, glucagon, and somatostatin, appear to play a role in controlling nutrient homeostasis (1). In addition to the effects of pancreatic hormones on target tissues, an intraislet action of the hormones is possible. Thus, glucagon is known to stimulate the release of insulin (2-4) and pancreatic somatostatin (5, 6), somatostatin to inhibit both insulin (7-9) and glucagon (10-13) secretion, and insulin to suppress glucagon secretion (14, 15). In these studies, higher concentrations of the hormones than those found in peripheral blood were employed. This may be necessary for the assessment of paracrine effects, since the concentrations of the hormones to which the pancreatic endocrine cells are exposed are not precisely known, but they are certainly higher than the circulating levels. The increased plasma concentrations of glucagon and somatostatin in dogs with experimental insulinopenic diabetes may suggest an inhibitory action of insulin on the pancreatic Delta-cells (16) similar to that exerted on the alpha-cells (16, 17). However, all efforts to demonstrate a direct action of insulin on somatostatin secretion from mammalian pancreas preparations have so far given negative results (6, 18-20). In the present study, we investigated the effect of insulin on somatostatin release from the isolated perfused rat pancreas under basal conditions and during stimulation with glucose or arginine.

Materials and Methods

The isolated pancreas was prepared from overnight fasted male Wistar rats, weighing 200–250 g, by the method of Penhos et al. (21) with minor modifications. The perfusate was modified Krebs-Ringer bicarbonate buffer containing 1 mM Ca++, 40 mg/ml dextran, 2 mg/ml human serum albumin, and 400 kIU/ml aprotinin. The buffer was continuously gassed with a mixture of 95% O2 and 5% CO2; the partial pressure of oxygen in the buffer entering the pancreas was 450 mm Hg, and the perfusion rate was 4 ml/min. Previous studies (22) have shown that steady basal concentrations of somatostatin are achieved in the effluent after the first 20 min of perfusion. Baseline samples were accordingly obtained 28, 29, and 30 min after starting the perfusion (designated -2, -1, and 0 min). The experimental variables were introduced at 0 min (i.e. 30 min after the perfusion had begun) according to protocols that are explained in the text. The somatostatin RIA used has been described in detail previously (22); its sensitivity was 15-20 pg/ml. Statistical analysis was made by Student’s t test.

The following substances were used: Dextran T-40 (Pharmacia Fine Chemicals AB, Uppsala, Sweden); human serum albumin (Swiss Red Cross, Bern, Switzerland); aprotinin (Trasylol) kindly provided by Prof. G. L. Haberland (Bayer A. G., Wuppertal, West Germany); cyclic somatostatin and N-tyrosylated somatostatin for standard and iodinated tracer, respectively (Serono, Freiburg, West Germany); somatostatin antiserum, a gift from Dr. J. Ardill (Queen’s University, Belfast, United Kingdom); and pork insulin (Actrapid; Novo Industri A/S, Copenhagen, Denmark).

Results

Throughout the experiments shown in Fig. 1, the glucose concentration was kept constant at 5.6 mM. After 30
min of equilibration and the recording of three baseline somatostatin secretion rates (−2, −1, and 0 min), insulin was infused for 30 min (0−30 min) at a concentration of 15 mU/ml (0.63 μg/ml). Sampling continued for 20 min after insulin was withdrawn (30−50 min). The somatostatin secretion rate did not vary significantly from the initial basal rate, either during the period of insulin infusion or subsequently. At this nonstimulatory glucose concentration, therefore, somatostatin secretion was not affected by insulin.

The next experiments were designed to evaluate further whether insulin affects somatostatin secretion by adding insulin after first inducing stimulation of somatostatin secretion by glucose or arginine. The basal perfusate contained 5.6 mM glucose (−30 to 0 min). Somatostatin secretion was then stimulated, either by increasing glucose to 16.7 mM or by adding 20 mM arginine (in the presence of 5.6 mM glucose) during the period from 0−40 min. After 20 min of stimulation with either glucose or arginine, insulin was infused into a side-arm to give a final concentration of 15 mU/ml insulin in the perfusate during the second half of the stimulatory period (20−40 min) without modifying the concentration of any other constituent. As shown in Fig. 2, 16.7 mM glucose caused a significant increase in the somatostatin secretion rate at all time points at which it was measured. The subsequent addition of insulin resulted in an inhibition of this glucose-induced somatostatin release. At 8 of 12 time points during the 20 min of insulin infusion, the somatostatin secretion rate was significantly less than the mean somatostatin secretion rate for the previous 20 min when 16.7 mM glucose was infused in the absence of insulin. Furthermore, the somatostatin secretion rate during insulin infusion with 16.7 mM glucose never significantly exceeded that during the initial baseline period when 5.6 mM glucose was infused alone.

Arginine (20 mM) added to 5.6 mM glucose resulted in an increase in somatostatin secretion (Fig. 3). The further
addition of insulin caused a rapid inhibition of somatostatin secretion, so that the secretion rate during insulin infusion was significantly less than the mean secretion rate during the previous 20 min of arginine infusion at all time points measured. In control experiments where 16.7 mM glucose or 20 mM arginine plus 5.6 mM glucose was infused for the period from 0–20 min (without the addition of insulin at 20–40 min), the somatostatin secretion rate during the second 20-min period of stimulation (20–40 min) was similar to that during the first 20-min period (0–20 min; Table 1). In addition, the results of all the experiments have been expressed as integrated incremental somatostatin secretion for the period from 0–40 min (Table 2). In control experiments, when glucose was infused for 40 min without insulin, the integrated incremental somatostatin secretion from 0–20 min was similar to that for the subsequent 20–40 min (Table 2A). By contrast, in the experiments where insulin was added during the period from 20–40 min, the integrated incremental somatostatin secretion was markedly reduced compared to that during the 0–20 min period of the same experiment ($P < 0.05$, paired statistics) and compared to

![Graph showing the effect of insulin on somatostatin secretion.](image)

**FIG. 3.** Effect of exogenous insulin (15 mU/ml) on arginine-stimulated somatostatin (SRIF) secretion from the isolated perfused pancreas. Arginine-stimulated SRIF secretion ($P < 0.005$ at 1 min and $P < 0.05$ at 3, 5, 6, 7, 10 and 20 min, compared with the basal secretion rate). The effect of insulin on stimulated SRIF release was significant at all time points ($P < 0.02$). G, Glucose.

**Table 1.** Somatostatin secretion rates during stimulation (0-40 min) with either glucose (16.7 mM) or arginine (20 mM). Values are the mean ± SEM expressed as picograms per min.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Glucose (16.7 mM), $n = 6$</th>
<th>Arginine (20 mM), $n = 6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>81.4 ± 3.4</td>
<td>77.0 ± 2.7</td>
</tr>
<tr>
<td>2</td>
<td>63.3 ± 6.2</td>
<td>56.0 ± 2.1</td>
</tr>
<tr>
<td>4</td>
<td>44.6 ± 3.9</td>
<td>41.4 ± 2.2</td>
</tr>
<tr>
<td>6</td>
<td>33.9 ± 3.5</td>
<td>32.3 ± 2.5</td>
</tr>
<tr>
<td>8</td>
<td>30.0 ± 2.0</td>
<td>23.6 ± 1.5</td>
</tr>
<tr>
<td>10</td>
<td>21.7 ± 1.9</td>
<td>16.1 ± 1.3</td>
</tr>
<tr>
<td>15</td>
<td>12.4 ± 1.1</td>
<td>9.8 ± 0.9</td>
</tr>
<tr>
<td>20</td>
<td>9.0 ± 0.7</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td>25</td>
<td>6.0 ± 0.5</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>30</td>
<td>4.0 ± 0.4</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>35</td>
<td>2.0 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>40</td>
<td>1.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>45</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>50</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>
TABLE 2. Integrated incremental somatostatin secretion during stimulation with 16.7 mM glucose or 20 mM arginine in the presence or absence of insulin during the second part of the stimulatory period (picograms per 20 min; mean ± SEM).

<table>
<thead>
<tr>
<th>Condition</th>
<th>0–20 min</th>
<th>P</th>
<th>20–40 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Glucose (16.7 mM) with insulin</td>
<td>2293 ± 353</td>
<td>NS</td>
<td>3318 ± 454</td>
</tr>
<tr>
<td>B. Glucose (16.7 mM) with insulin (20–40 min)</td>
<td>2283 ± 479</td>
<td>&lt;0.001</td>
<td>185 ± 661</td>
</tr>
<tr>
<td>C. Arginine (20 mM) without insulin</td>
<td>3708 ± 776</td>
<td>NS</td>
<td>2854 ± 497</td>
</tr>
<tr>
<td>D. Arginine (20 mM) with insulin (20–40 mM)</td>
<td>2064 ± 678</td>
<td>&lt;0.01</td>
<td>−746 ± 413</td>
</tr>
</tbody>
</table>

A + C, n = 4; B, n = 6; D, n = 7.

Discussion

The results presented here demonstrate that somatostatin secretion stimulated by either glucose or arginine can be inhibited by an increase in the insulin concentration surrounding the pancreatic D-cells. Basal somatostatin release (i.e. 5.6 mM glucose without added stimuli) was unaffected by insulin. This finding under basal conditions confirms previous work in the rat (18, 19) and dog (6).

With respect to the inhibition by insulin of glucose-stimulated somatostatin release, our results appear at variance with those of Hermansen et al. (20), who failed to observe any effect of 25 mU/ml insulin on somatostatin release. The reason for this difference is unclear; apart from the species difference, the composition of the perfusate was also different (20). In the isolated perfused chicken pancreas, Honey and Weir (23) observed a stimulation of somatostatin secretion by 20 mU/ml insulin. While this finding contrasts markedly with the results reported here, the interaction between hormones in the pancreas of birds may be different from that occurring in mammals. Indeed, in the duck, the infusion of somatostatin in vivo resulted in a stimulation of glucagon secretion rather than the expected inhibition (24).

The use of high concentrations of pancreatic hormones is necessary when investigating their possible paracrine effects. When insulin secretion was stimulated by 16.7 mM glucose, the insulin concentration measured in the mixed effluent of the isolated perfused pancreas preparation was 1 mU/ml (22). This concentration can be increased by a factor of 10 under maximal stimulatory conditions. It appears reasonable, therefore, to use a concentration of insulin large enough to permit the D-cells to recognize an increase.

Suppression of pancreatic somatostatin secretion by insulin under physiological conditions may explain the findings of increased somatostatin secretion in experimental insulinopenic diabetes in dogs (16) and rats (25). In addition, insulin treatment of alloxan-diabetic dogs partially normalized the increased somatostatin and glucagon levels measured in peripheral blood (16). In acute in vivo experiments, insulin infused iv inhibited meal-stimulated pancreatic somatostatin secretion; it also inhibited stimulated somatostatin release from the gastric antrum but not from the fundus (26). However, it is not possible to determine from in vivo studies whether insulin has a direct effect on the pancreatic D-cell. In in vitro experiments using the perfused rat stomach, 5.6 mM glucose, after a delay, caused a stimulation of somatostatin secretion, which could be prevented by simultaneous insulin infusion (27).

In conclusion, the results presented here suggest that insulin can exert a direct inhibitory influence on somatostatin secretion under certain conditions. It would seem that each of the three principal hormones of the pancreatic islet, insulin, glucagon, and somatostatin, may affect the secretion of the other two within the pancreatic islet. The concentration of one of the hormones in the pancreatic effluent may thus depend on the secretory activity of the other hormone-secreting cells in the islets. With regard to pancreatic polypeptide, knowledge is as yet insufficient to show whether it may be similarly involved in reciprocal paracrine regulation of pancreatic hormone secretion.

Acknowledgment

The authors acknowledge with gratitude the expert technical assistance of Jarmila Slesinger.

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