Islet amyloid polypeptide tonally inhibits β-, α-, and δ-cell secretion in isolated rat pancreatic islets

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Department of Surgery, Karolinska Institute at Huddinge University Hospital, Huddinge 14186; Department of Biomedicine and Surgery, Linköping University Hospital, Linköping 58185, Sweden; and Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, Nebraska 68178

Wang, Feng, Thomas E. Adrian, Gunilla T. Westermark, Xianzhong Ding, Thomas Gasslander, and Johan Perment. Islet amyloid polypeptide tonally inhibits β-, α-, and δ-cell secretion in isolated rat pancreatic islets. Am. J. Physiol. Endocrinol. Metab. 39: E19–E24, 1999.—Islet amyloid polypeptide (IAPP, or amylin) is produced in pancreatic β-cells. The intraislet significance of IAPP is still uncertain. In the present study, paracrine effects of endogenous IAPP and somatostatin were investigated in isolated rat pancreatic islets. The intraislet IAPP activity was inhibited with an IAPP antiserum or a specific antagonist [IAPP-(8−37)]. Somatostatin activity was inhibited by immunoneutralization. Basal insulin and glucagon secretion were not affected by the somatostatin and/or IAPP blockade. Arginine-stimulated insulin and glucagon secretion were dose dependently increased by IAPP antiserum, IAPP-(8−37), and somatostatin antiserum, respectively. Arginine-stimulated somatostatin secretion was dose dependently potentiated by IAPP antiserum. Insulin secretion induced by 16.7 mM glucose was enhanced by IAPP antiserum and IAPP-(8−37), respectively. A combination of somatostatin antiserum with IAPP antiserum or IAPP-(8−37) further enhanced the arginine-stimulated insulin and glucagon secretion compared with effects when the blocking reagents were used individually. These results indicate that endogenously produced IAPP tonally inhibits stimulated insulin, glucagon, and somatostatin secretion. Furthermore, the paracrine effects of IAPP and somatostatin are additive.

Materials and Methods

Chemicals. Rabbit antiserum against synthetic human somatostatin was purchased from Dakopatts (Glostrup, Denmark). The somatostatin antiserum (code no. A0566, lot no. 015A) showed a selective reactivity with somatostatin in a large number of mammalian species. The rabbit antiserum against rat IAPP has been described elsewhere (27). The IAPP antiserum showed no cross-reactivity with insulin, glucagon, somatostatin and pancreatic polypeptide and <1% cross-reactivity with CGRP. Rat IAPP-(8−37) [rat IAPP-(8−37)amide] was purchased from Chiron Mimotopes (Clayton, Australia), culture medium (RPMI 1640) from Life Technologies (Paisley, UK), and L-arginine from Sigma (St. Louis, MO).

Islet preparation. Male Sprague-Dawley rats (250–300 g) were purchased from B & K Universal (Stockholm, Sweden) and kept in a 12:12-h light-dark cycle with free access to water and pelleted rat chow. Rat pancreatic islets were isolated as described elsewhere (25). The study was approved by the local animal ethical committee. Islets in each experiment came from two to three pancreata. The isolated islets were preincubated overnight with RPMI 1640 medium (10% fetal calf serum) at 37°C in 95% humidified air and 5% CO2.

Preparation of test media. Modified Krebs-Ringer bicarbonate (KR) buffer was prepared for islet incubation. The KR buffer consisted of (in mM) 114 NaCl, 4.4 KCl, 1 MgSO4, 29.5 NaHCO3, 1.28 CaCl2, 10 HEPES, and 0.1% bovine serum albumin (BSA). The buffer (pH 7.4) was supplemented with (in mM) 5.6 glucose, 16.7 glucose, or 5.6 glucose + 15 L-arginine. When individual effects of IAPP antiserum, IAPP-(8−37), or somatostatin antiserum were investigated, the three reagents were used separately in different KR buffers. IAPP or somatostatin antiserum was added to the test buffers at final concentrations of 0.02, 0.1, and 0.5%. The incubation buffers with <0.5% antiserum had additional amounts of nonimmunized rabbit serum added to bring the final serum choline (26). In perfused rat pancreas, salmon calcitonin-(8–32) enhances glucose-induced insulin secretion (22). The effects of these IAPP antagonists are assumed to result from a competitive displacement of IAPP from its receptor in islet cells (22). In addition, IAPP antagonists are known to inhibit binding of IAPP to CGRP receptor (19). Although there is considerable evidence in favor of a distinct IAPP receptor (19), the IAPP receptor has not been identified to date.

Unlike IAPP antagonist, IAPP antibody can deplete endogenous IAPP from intercellular space before the peptide binds to its putative receptor. In the present study, isolated rat pancreatic islets were incubated with IAPP antiserum, IAPP-(8−37), and somatostatin antiserum to investigate the effects of IAPP and somatostatin on hormone secretion of the islets.
Table 1. Regulatory effects of exogenous IAPP on β-, α-, and δ-cell secretion

<table>
<thead>
<tr>
<th>Regulatory effect</th>
<th>β-Cells</th>
<th>α-Cells</th>
<th>δ-Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated pancreatic islets</td>
<td>[14] (6,17)</td>
<td>[16] (6)</td>
<td>[19] (17)</td>
</tr>
<tr>
<td>Hormonal cell lines</td>
<td>[20] (6)</td>
<td>[21] (13)</td>
<td>[22] (15)</td>
</tr>
</tbody>
</table>

Data summarize protocols of various studies. Nos. in parentheses represent corresponding references: |: Inhibition; —: no effect; |:
stimulation. IAPP, islet amyloid polypeptide. *IAPP had no effect on glucose-induced insulin secretion but inhibited arginine-induced insulin secretion.

Efficiency of immunoneutralization. Table 2 shows the specific binding abilities of IAPP and somatostatin antisera left in the test media after islet incubation. More than 90% of radioactive IAPP and somatostatin was bound by 0.1 and 0.5% IAPP antisera or by 0.5% somatostatin antisera, respectively. The lower concentrations of antisera were only able to partially bind their respective tracers.

Table 2. Efficiency of immunoneutralization

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Tracer Bound By Specific Antiserum, %</th>
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<tbody>
<tr>
<td>IAPP antiserum</td>
<td></td>
</tr>
<tr>
<td>0.02%</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>0.1%</td>
<td>94 ± 3</td>
</tr>
<tr>
<td>0.5%</td>
<td>96 ± 2</td>
</tr>
<tr>
<td>Somatostatin antiserum</td>
<td></td>
</tr>
<tr>
<td>0.02%</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>0.1%</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>0.5%</td>
<td>91 ± 8</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 6. Isolated islets were incubated in Krebs-Ringer bicarbonate buffers with different concentrations of IAPP or somatostatin antisera. After incubation, radioactive IAPP or somatostatin was mixed with an aliquot of incubation buffer containing respective antisera. Bound fraction was counted. Binding ability of each dilution of antisera was calculated as percentage of total binding seen with antisera in excess.
cantly increased by 0.1–0.5% IAPP antiserum (P < 0.01; Fig. 4A), by 5 and 50 µM IAPP-(8—37) (P < 0.05 and P < 0.01, respectively; Fig. 4B), or by 0.1–0.5% somatostatin antiserum (P < 0.05; Fig. 4C) compared with control. The combination of somatostatin antiserum (0.5%) with either IAPP antiserum (0.5%) or IAPP-(8—37) (50 µM) had no significant effect on basal glucagon secretion (5.6 mM glucose) (data not shown) but significantly increased the arginine-stimulated glucagon secretion compared with control (P < 0.001) or with the groups where the blocking reagents were used separately (P < 0.05; Fig. 5).

Somatostatin secretion. Arginine-stimulated somatostatin secretion was significantly enhanced by 0.1 and 0.5% IAPP antiserum compared with control (P < 0.05 and P < 0.01, respectively; Fig. 6). The somatostatin secretion at 0.5% IAPP antiserum was also increased compared with that at 0.02% antiserum (P < 0.01; Fig. 6).

DISCUSSION

Immunoneutralization of one islet hormone can lead to alterations in secretion of other pancreatic hormones (13, 20, 21, 23). The alterations in islet secretion are
believed to reflect the paracrine potential of the neutralized hormone. Immunoneutralization has been used both in perfused pancreas (20) and in batch incubation of isolated islets (13, 21, 23). In the present study, inhibition of intraislet IAPP with either IAPP antiserum or IAPP antagonist potentiated arginine-stimulated insulin and glucagon secretion from isolated rat pancreatic islets. Insulin secretion induced by 16.7 mM glucose was enhanced by the IAPP blockers as well. In addition, arginine-stimulated somatostatin secretion from the islets was also enhanced by IAPP immunoneutralization. To our knowledge, this is the first report on the paracrine effects of IAPP with immunoneutralization.

The enhancement of stimulated insulin secretion by IAPP blockade is consistent with previous studies with IAPP antagonists (4, 22, 26, 29). These results indicate that the depletion of endogenous IAPP activity increases insulin secretion. In previous studies, exogenous IAPP inhibited insulin secretion at concentrations that were much higher (11, 14, 24, 26) than the IAPP levels seen in the systemic circulation (7, 18). Because the extracellular IAPP concentrations in pancreatic islets are uncertain, the demonstration that endogenous IAPP regulates insulin secretion is of considerable importance.

In the present study, the secretion of insulin, glucagon, and somatostatin was measured simultaneously...
in the presence of IAPP antiserum. The effects of IAPP or IAPP antagonist on β-, α-, and δ-cell secretion have been investigated in perfused rat pancreas (10, 22). IAPP stimulates insulin secretion but inhibits glucagon and somatostatin secretion (10). Salmon calcitonin-(8–32) increases insulin secretion but has no effects on glucagon or somatostatin secretion (22). Therefore, different conclusions have been drawn from these studies (10, 22). In the perfused pancreas, intraislet regulation takes place in both the vascular and interstitial compartments in the β-α-δ-cell order (20). Thus hormone secretions from the perfused pancreas reflect an integrated response of the different islet cells. In the present study, the effects of endogenous IAPP on insulin, glucagon, and somatostatin secretion were studied in isolated islets. In these islets, the IAPP blockers reached β-, α-, and δ-cells via the interstitial space of the islets. Under these conditions, the islet cell types are more likely to react directly to the IAPP blockade. Thus the results from the present study may help us to understand the direct reaction of islet cells to IAPP depletion.

In the present study, arginine-stimulated insulin and glucagon secretion was also enhanced by somatostatin antiserum. These results are in agreement with previous reports about the effect of intraislet somatostatin on stimulated insulin and glucagon secretion (13, 21).

From the binding studies, it is evident that a nearly complete immunoneutralization was achieved by the two highest concentrations of IAPP antiserum and by the highest concentration of somatostatin antiserum. However, IAPP and somatostatin blockade, either separately or in combination, had no effects on basal secretion of β- and α-cells. Schatz and Kullek (21) have found that the blockade of intraislet somatostatin does not affect insulin secretion from unstimulated isolated rat pancreatic islets. Similarly, IAPP-(8–37) has no significant effects on basal insulin levels in rat plasma (4). As unstimulated pancreatic islets have a slow rate of hormone release (21), it is not inconceivable that the extracellular concentrations of pancreatic hormones are also low in these islets. Thus the lack of effect of the intraislet blockade on basal insulin and glucagon secretion may be caused by the downregulated intercellular communication in unstimulated islets.

In the present study, combined blockade of somatostatin and IAPP caused a greater increase in arginine-stimulated insulin and glucagon secretion, compared with the separate use of somatostatin and IAPP blockers. These findings suggest that in the stimulated pancreatic islets, IAPP and somatostatin interact to downregulate hormone secretion from β- and α-cells. These results are also consistent with our previous observation that exogenous IAPP enhanced the inhibitory effect of somatostatin on arginine-stimulated insulin secretion from isolated rat pancreatic islets (25). The mechanism underlying this IAPP-somatostatin interaction is unknown.

The results from this study also support the hypothesis that the effect of endogenous IAPP on insulin secretion may mask the effects of exogenous IAPP on β-cells and be at least partially responsible for the divergent insulin response to IAPP administration (26). This hypothesis is based on the observation that islet β-cells show distinct IAPP sensitivity in different experimental models. For instance, insulin secretion is inhibited by 75 pM IAPP in the perfused rat pancreas (9), but 10 µM IAPP is required to achieve the same inhibitory effect in incubated isolated islets (2, 14). The relatively high sensitivity to IAPP in the perfused pancreas is believed to be caused by the continuous washout of endogenous IAPP from the islets (26). This concept is in line with the observation that the IAPP sensitivity of β-cells in isolated islets is improved substantially in a nonrecirculating perfusion system (26). As islet α- and δ-cells are also exposed to the local release of endogenous IAPP, intraislet IAPP may influence the effects of exogenous IAPP on glucagon and somatostatin secretion as well.

In summary, this study demonstrates that intraislet IAPP is a local inhibitor of stimulated β-, α-, and δ-cell secretion in isolated rat pancreatic islets. Induction of the same effects by exogenous IAPP usually requires pharmacological doses of the peptide. Thus the intraislet effect of endogenous IAPP through short-loop feedback control may be of more physiological importance than the possible effect of circulating IAPP through an endocrine mechanism. The downregulation of stimulated β-, α-, and δ-cell secretion by endogenous IAPP may serve as an intraislet feedback control to prevent oversecretion of hormonal products from islet cells.

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