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

FENG WANG, THOMAS E. ADRIAN, GUNILLA T. WESTERMARK, XIANZHONG DING, THOMAS GASSLANDER, AND JOHAN PERMERT

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 antisera 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 antisera, IAPP-(8—37), and somatostatin antisera, respectively. Arginine-stimulated somatostatin secretion was dose dependently potentiated by IAPP antisera. Insulin secretion induced by 16.7 mM glucose was enhanced by IAPP antisera and IAPP-(8—37), respectively. A combination of somatostatin antisera with IAPP antisera 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.

Amylin; somatostatin; insulin; glucagon

Islet amyloid polypeptide (IAPP, or amylin) is a 37-amino acid peptide sharing ~50% amino acid homology with calcitonin gene-related peptide (CGRP) (8, 28). IAPP is synthesized in pancreatic β-cells (8, 28) and normally cosecreted with insulin (7). As shown in Table 1, the physiological function of IAPP in pancreatic islets is uncertain and highly controversial (26, 32, 33, 30, 31, 32, 33).

The paracrine effects of IAPP have also been investigated with specific antagonists such as IAPP-(8—37), CGRP-(8—37), and salmon calcitonin-(8—32). IAPP-(8—37) and CGRP-(8—37) increase arginine-stimulated insulin secretion in anesthetized rats (4, 29). In perfused rat pancreatic islets, IAPP-(8—37) increases insulin secretion stimulated by glucose and carbachol (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 antisera, IAPP-(8—37), and somatostatin antisera to investigate the effects of IAPP and somatostatin on hormone secretion of the islets.

MATERIALS AND METHODS

Chemicals. Rabbit antisera against synthetic human somatostatin was purchased from Dakopatts (Glostrup, Denmark). The somatostatin antisera (code no. A0566, lot no. 015A) showed a selective reactivity with somatostatin in a large number of mammalian species. The rabbit antisera against rat IAPP has been described elsewhere (27). The IAPP antisera 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 (KRB) buffer was prepared for islet incubation. The KRB buffer consisted of (in mM) 144 NaCl, 4.4 KCl, 1.4 MgSO4, 25.5 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 antisera, IAPP-(8—37), or somatostatin antisera were investigated, the three reagents were used separately in different KRB buffers. IAPP or somatostatin antisera was added to the test buffers at final concentrations of 0.02, 0.1, and 0.5%. The incubation buffers with <0.5% antisera had additional amounts of nonimmunized rabbit serum added to bring the final serum...
Table 1. Regulatory effects of exogenous IAPP on \( \beta \)-, \( \alpha \)-, and \( \delta \)-cell secretion

<table>
<thead>
<tr>
<th>Regulatory Effect</th>
<th>( \beta )-Cells</th>
<th>( \alpha )-Cells</th>
<th>( \delta )-Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body mammals</td>
<td>([13]_{-2,5,17} [14])</td>
<td>([13]_{-5})</td>
<td>([13]_{-5})</td>
</tr>
<tr>
<td>Isolated rat pancreas</td>
<td>([9]_{-2,12,16})</td>
<td>([10]_{-12})</td>
<td>([10]_{-12,16})</td>
</tr>
<tr>
<td>Isolated pancreatic islets</td>
<td>([14]_{-6,17})</td>
<td>([14]_{-6,17})</td>
<td>([13])</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.

concentration to 0.5%. IAPP-(8—37) was used at final concentrations of 0.5, 5, or 50 µM, together with 0.5% nonimmunized rabbit serum. The control buffers contained 0.5% nonimmunized rabbit serum only.

In a subset of experiments, 0.5% somatostatin antiserum was combined with 0.5% IAPP antiserum or 50 µM IAPP-(8—37) in KRB buffers containing 5.6 mM glucose or 5.6 mM glucose + 15 mM L-arginine. Control buffers contained 1% nonimmunized rabbit serum only. Buffers containing 0.5% IAPP antiserum, 50 µM IAPP-(8—37), or 0.5% somatostatin antiserum were also prepared in the arginine-containing buffer. Nonimmunized rabbit serum was added, if necessary, to bring the final serum concentration to 1% in all of the groups.

Islet incubation in test media. Islet incubation was performed in a similar manner in different subsets of experiments. Preincubated pancreatic islets were rinsed with KRB buffer containing 5.6 mM glucose. In 96-well plates, batches of three islets (150–250 µm in diameter) were incubated with 300 µl of different test media. The islets were matched so that the total islet volumes were comparable among the different wells. In each experiment, the islet incubation was performed in triplicate for each group. After a 90-min incubation, 200-µl aliquots were collected into chilled tubes containing 50 µl of benzamidine (300 mM)-EDTA (30 mM) solution. The samples were stored at \(-20^\circ\)C for subsequent radioimmunoassay.

Efficiency of immunoneutralization. Immunoneutralization of IAPP and somatostatin by respective antisera was assessed after islet incubation. An aliquot of incubation buffer (100 µl) from each culture well was mixed with 10 µl (1.5 nCi) of monoiodinated IAPP or somatostatin. The bound fraction (100 µl) from each culture well was mixed with 10 µl (1.5 nCi) of respective antisera in excess.

The binding ability of each dilution of antiserum was measured with radioimmunoassay kits for rat insulin and glucagon (Linco Research, St. Charles, MO). For the somatostatin assay, the antiserum against somatostatin was used at a final concentration of 1:25,000 (1). Synthetic somatostatin was used as standard and \(^{125}\)I-labeled somatostatin (Amersham, Arlington Heights, IL) as tracer. The assay buffer (pH 7.4) consisted of (in mM) 10 KH2PO4, 60 Na2HPO4, 10 EDTA, 7.6 sodium azide, and 0.3% BSA. The assays were incubated for 7 days at 4°C, and bound radioactivity was separated and counted as described in the previous paragraph.

Statistics. The analyses were carried out by the Instat computer program (version 1.12, Graph Pad, San Diego, CA) with analysis of variance (ANOVA) with the Bonferroni post test for multiple comparisons. The islet incubations presented in each figure were performed simultaneously. The data are presented as means ± SE with n representing the number of experiments performed. A P value of <0.05 was considered significant.

RESULTS

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 antiserum or by 0.5% somatostatin antiserum, respectively. The lower concentrations of antisera were only able to partially bind their respective tracers.

Insulin secretion. Basal insulin secretion from the isolated islets was unchanged in the presence of IAPP antiserum, IAPP-(8—37), or somatostatin antiserum at the concentrations investigated (Fig. 1, A–C). Arginine-stimulated insulin secretion was significantly increased by 0.1–0.5% IAPP antiserum (P < 0.05; Fig. 1A) or by 5–50 µM IAPP-(8—37) (P < 0.01; Fig. 1B) compared with control. Arginine-stimulated insulin secretion was also enhanced by 0.5% somatostatin antiserum compared with control (P < 0.01) or with the group containing 0.02% somatostatin antiserum (P < 0.05; Fig. 1C). 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 insulin secretion (5.6 mM glucose) (data not shown) but significantly increased the arginine-stimulated insulin secretion compared with control (P < 0.001) or with the groups where the blocking reagents were used separately (P < 0.05; Fig. 2). Insulin secretion induced by 16.7 mM glucose was significantly enhanced by 0.1% IAPP antiserum and 50 µM IAPP-(8—37), respectively (Fig. 3).

Glucagon secretion. Basal glucagon secretion from the isolated islets was unchanged in the presence of IAPP antiserum, IAPP-(8—37), or somatostatin antiserum at the concentrations investigated (Fig. 4, A–C). Arginine-stimulated glucagon secretion was signifi-

Table 2. Efficiency of immunoneutralization

<table>
<thead>
<tr>
<th>Specific Antiserum</th>
<th>Tracer Bound By</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAPP antiserum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.02%</td>
<td>40 ± 5</td>
<td></td>
</tr>
<tr>
<td>0.1%</td>
<td>94 ± 3</td>
<td></td>
</tr>
<tr>
<td>0.5%</td>
<td>96 ± 2</td>
<td></td>
</tr>
<tr>
<td>Somatostatin antiserum</td>
<td>14 ± 1</td>
<td></td>
</tr>
<tr>
<td>0.02%</td>
<td>28 ± 3</td>
<td></td>
</tr>
<tr>
<td>0.5%</td>
<td>91 ± 8</td>
<td></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 antiserum. After incubation, radioactive IAPP or somatostatin was mixed with an aliquot of incubation buffer containing respective antiserum. Bound fraction was counted. Binding ability of each dilution of antiserum was calculated as percentage of total binding seen with antiserum in excess.
sificantly 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 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.

Fig. 4. Isolated islets were incubated with IAPP antiserum (A), IAPP-(8—37) (B), or SMS antiserum (C) in KRB buffers containing 5.6 mM glucose (open bars) or 5.6 mM glucose + 15 mM L-arginine (filled bars). Ninety-minute glucagon secretion was measured in incubation media. Data are means ± SE; n = 6. *P < 0.05; **P < 0.01.

Fig. 5. Isolated islets were incubated with IAPP antiserum, IAPP-(8—37), SMS antiserum, and SMS antiserum in combination with IAPP antiserum or IAPP-(8—37), respectively, in KRB buffer containing 5.6 mM glucose + 15 mM L-arginine. Ninety-minute glucagon secretion was measured in incubation media. Data are means ± SE; n = 9. *P < 0.05; **P < 0.01; ***P < 0.001.

Fig. 6. Isolated islets were incubated with IAPP antiserum in KRB buffer containing 5.6 mM glucose + 15 mM L-arginine. Ninety-minute somatostatin secretion was measured in incubation media. Data are means ± SE; n = 6. *P < 0.05; **P < 0.01.
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.

We express our sincere gratitude to Dr. P. Westermark for constructive comments on the present study.

This work was supported by grants from the Swedish Medical Research Council (PROD no. 5941), the Swedish Diabetes Association, the Swedish Cancer Society (2870-B95–05XBB and 2870-B96–06XAC), Novo Nordisk Insulin Fund, and the State of Nebraska Cancer and Smoking-Related Disease Program (LB595).

Address for reprint requests: F. Wang, Arvid Wretlind Laboratory, Clinical Research Center, Novum, Karolinska Institute at Huddinge Univ. Hospital, Huddinge 14186, Sweden.

Received 16 june 1998; accepted in final form 1 September 1998.

REFERENCES


E24 PARACRINE FUNCTION OF ISLET AMYLOID POLYPEPTIDE


