Rapid Publications

Evidence of Cosecretion of Islet Amyloid Polypeptide and Insulin by β-Cells

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Islet amyloid polypeptide (IAPP) has been identified as the major constituent of the pancreatic amyloid of non-insulin-dependent diabetes mellitus (NIDDM) and is also present in normal β-cell secretory granules. To determine whether IAPP is a pancreatic secretory product, we measured the quantity of IAPP-like immunoreactivity (IAPP-LI), insulin, and glucagon released into 5 ml of incubation medium during a 2-h incubation of monolayer cultures (n = 5) of neonatal (3- to 5-day-old) Sprague-Dawley rat pancreases under three conditions: 1.67 mM glucose, 16.7 mM glucose, and 16.7 mM glucose plus 10 mM arginine and 0.1 mM isobutylmethylxanthine (IBMX). The quantity of IAPP-LI, insulin, and glucagon in the cell extract was also determined. Mean ± SE IAPP-LI in the incubation medium increased from 0.041 ± 0.003 pmol in 1.67 mM glucose to 0.168 ± 0.029 pmol in 16.7 mM glucose ($P < 0.05$) and 1.02 ± 0.06 pmol in 16.7 mM glucose plus arginine and IBMX ($P < 0.05$ vs. 1.67 or 16.7 mM glucose). Insulin secretion increased similarly from 4.34 ± 0.27 to 20.2 ± 0.6 pmol ($P < 0.05$) and then to 135 ± 5 pmol ($P < 0.05$ vs. 1.67 or 16.7 mM glucose). Glucagon release tended to decrease with the increase in glucose concentration (0.39 ± 0.01 vs. 0.33 ± 0.02 pmol, $P < 0.1$), whereas with the addition of arginine and IBMX to high glucose, glucagon release increased to 1.32 ± 0.03 pmol ($P < 0.05$ vs. 1.67 or 16.7 mM glucose). Thus, the molar proportion of IAPP-LI to insulin secreted in low glucose was ~1% and did not differ significantly with stimulation (0.95 ± 0.08 vs. 0.84 ± 0.15 vs. 0.76 ± 0.05%). In contrast, there was no constant proportional relationship between the release of IAPP-LI and glucagon (10.6 ± 0.8 vs. 51.3 ± 8.7 vs. 77.5 ± 5.2%). After incubation in 1.67 mM glucose, the extracted cells contained 3.7 ± 0.2 pmol IAPP-LI, 944 ± 25 pmol insulin, and 28.2 ± 1.5 pmol glucagon. After maximal stimulation, the fractional release of IAPP-LI was 26.7 ± 0.7% vs. 14.7 ± 0.6% of insulin and 4.4 ± 0.2% of glucagon. These data indicate that nondiabetic neonatal rat islet cultures contain IAPP-LI and release it after stimulation by glucose and nonglucose secretagogues. Furthermore, the data suggest that IAPP-LI is a product of the β-cell, which coreleases it with insulin in a molar ratio of ~1:100. *Diabetes* 39:634–38, 1990

Hyalinosis of the pancreas was first described in 1900 (1), but it was only 30 yr ago that this material was shown to be amyloid (2). These amyloid deposits have been demonstrated in the islets of Langerhans of healthy humans and animals (2–6) and are also present in excess in the pancreases of individuals with insulinomas (7) and diabetes mellitus (2–4). Recently, a 37–amino acid peptide, which has been termed *islet amyloid polypeptide* (IAPP), *diabetes-associated peptide*, or *amylin*, has been shown to be the major constituent of pancreatic amyloid (8–10). Gene cloning and analyses of the nucleotide sequence of human cDNAs have suggested that IAPP is processed from an 89–amino acid precursor (11–13). The likelihood of IAPP to precipitate as amyloid in humans, nonhuman primates, and cats and not in other species is probably due to a species-specific divergence in amino acid residues 20–29 of the peptide (13), with positions 25 and 26 appearing to be most critical (14).

The role of IAPP in normal physiology and its potential contribution to the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM) is still somewhat speculative and controversial. Leighton and Cooper (15) demonstrated that, in high concentrations, the peptide is capable of impairing glucose utilization in vitro, suggesting that it may be a factor in the insulin resistance observed in this disease. Other workers have evaluated the effect of the IAPP on insulin secretion. Ohsawa et al. (16) used isolated rat islets and found that, at a peptide concentration of 10^{-5} M, glucose-stimulated insulin release was inhibited, whereas Kogire et al. (17) dem-

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onstrated that, in the isolated perfused rat pancreas, 10^{-7} M peptide was capable of inhibiting carbachol-induced insulin secretion but not glucose-stimulated insulin release.

We recently suggested that IAPP may be a normal secretory product of the β-cell and that an abnormality in its processing or release in NIDDM may result in high concentrations of the peptide precipitating in the region of the islet and thus may be a factor in the abnormality of islet function that occurs with NIDDM (18). The possibility that the peptide is a normal secretory product of the islet β-cell is supported by the results of immunostaining, which have shown that IAPP is present in the secretory granules of the β-cell and not the α-cell (19–21). However, definitive proof of this hypothesis requires demonstration of peptide release.

Therefore, to determine whether IAPP is normally synthesized and released by pancreatic islets, we undertook this study with monolayer cultures of neonatal rat pancreases. We found that IAPP-like immunoreactivity (IAPP-LI) is present in rat pancreas and is secreted in response to glucose and nonglucose secretagogues. This immunoreactive substance was released in approximate molar proportion with insulin, consistent with its storage and release from β-cell secretory granules.

RESEARCH DESIGN AND METHODS

Monolayer cultures were established from the pancreases of neonatal (3- to 5-day-old) Sprague-Dawley rats as previously described (22,23). Pancreases were pooled for digestion by trypsin and collagenase, and the resultant cell suspensions were aliquoted into culture dishes. Cultures were grown in an atmosphere of 95% O_2/5% CO_2 in culture medium consisting of 45% NCTC 135/45% medium 199 (vol/vol) and 10% fetal bovine serum supplemented with 16.7 mM glucose and 50 μg/ml gentamicin. After an overnight incubation, unattached cells were collected, and the cell suspension was aliquoted in equal amounts into 100-mm culture dishes. Studies were performed after 3 days of growth. Cultures were preincubated in Krebs-Ringer bicarbonate buffer containing 0.1% bovine serum albumin (KRBB-BSA) supplemented with 1.67 mM glucose for 2 h and were then incubated for 2 h with 5 ml of a test medium. Test medium consisted of KRBB-BSA supplemented with 1.67 mM glucose, 16.7 mM glucose, or 16.7 mM glucose plus 10 mM arginine and 0.1 mM isobutylmethylxanthine (IBMX). Medium was removed after 2 h of incubation, and the cells were then extracted in 3 ml acid-ethanol (500 ml 95% ethanol and 10 ml 1 N HCl). The cell extracts were dried down under N_2 and then reconstituted in 2 ml 0.1 M sodium phosphate buffer containing 0.1% Triton X-100, 0.1% BSA, 0.5 M NaCl, and 0.01% sodium azide. Medium and cell extracts were stored at −20°C until assayed.

Immunoreactive insulin and glucagon were determined by radioimmunoassay as previously described (24,25). IAPP-LI was measured by radioimmunoassay with a commercial kit (Peninsula, Belmont, CA). This radioimmunoassay consisted of a double-antibody technique with a primary polyclonal antiserum raised in rabbits. The antibody binds rat and human IAPP equally but recognizes <0.1% of rat calcitonin gene–related peptide despite its 46% homology with rat IAPP. In addition, the antibody does not recognize insulin in 1000-fold molar excess, pancreastatin, neuropeptide Y, or vasoactive intestinal polypeptide, and the acid-ethanol extraction mixture does not interfere in the assay. The assay was performed in 100 μl 0.1 M sodium phosphate buffer containing 0.1% Triton X-100, 0.1% BSA, 0.05 M NaCl, and 0.01% sodium azide with human IAPP as standard and ^{125}I-Tyr^{37}-labeled IAPP as tracer. Goat anti-rabbit IgG serum was used to separate bound from free peptide with normal rabbit

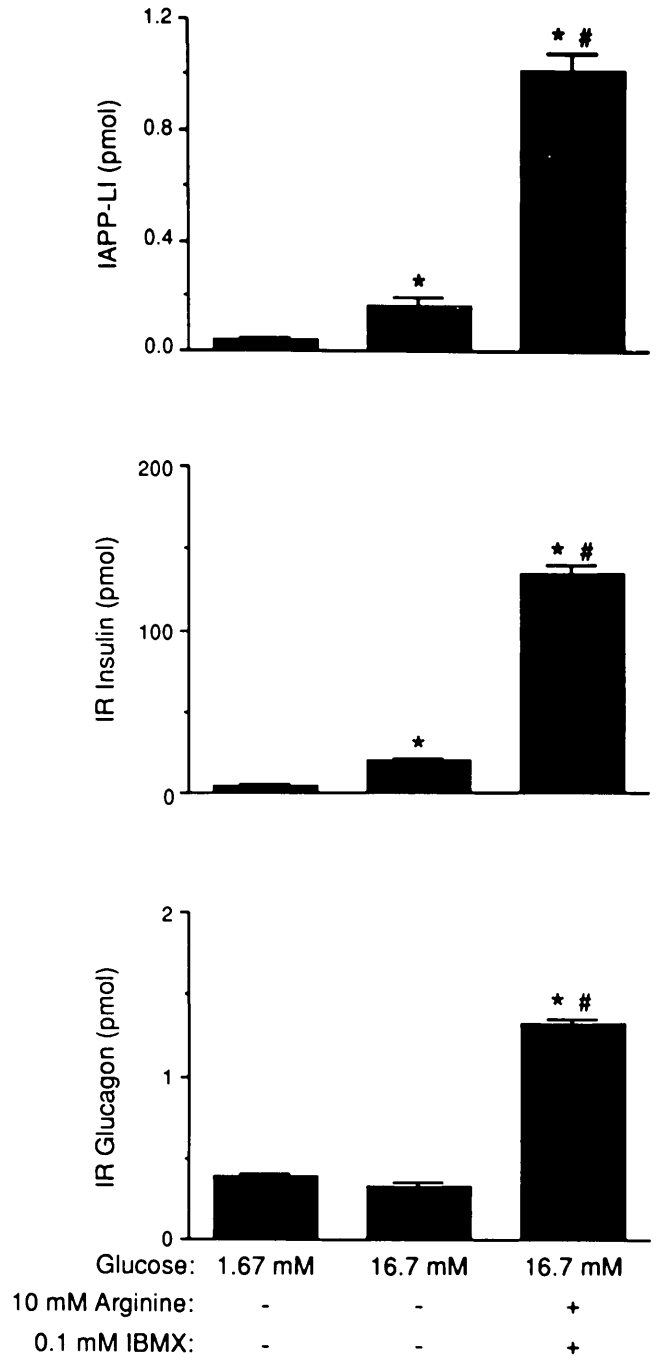

FIG. 1. Quantity of islet amyloid polypeptide–like immunoreactivity (IAPP-LI, *top*), immunoreactive (IR) insulin (*middle*), and IR glucagon (*bottom*) secreted by monolayer cultures of neonatal rat pancreas into 5 ml of incubation medium under 3 test conditions. Note difference in scales for amount of IAPP-LI, IR insulin, and IR glucagon secreted. Values for 5 experiments/condition are expressed as means ± SE. *$P < 0.05$ for peptide release vs. 1.67 mM glucose. #$P < 0.05$ for peptide release in 16.7 mM glucose plus arginine and isobutylmethylxanthine (IBMX) vs. 16.7 mM glucose.

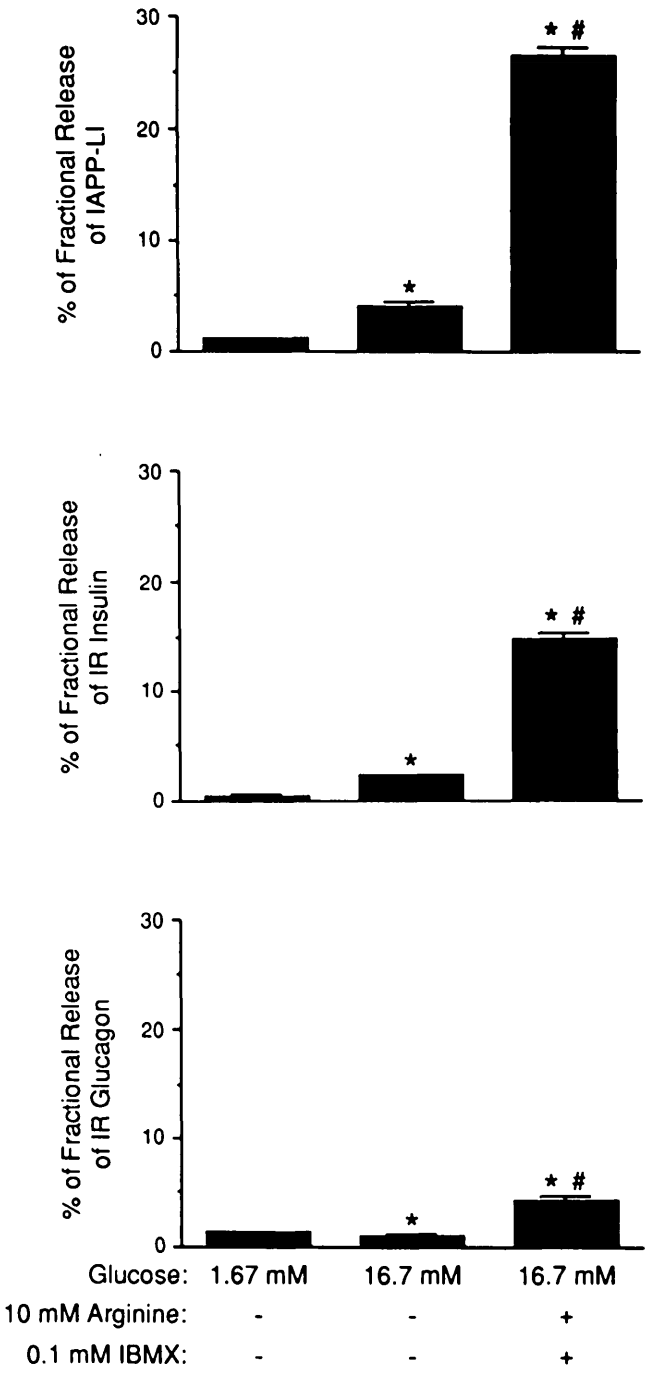

FIG. 2. Percentage of fractional release of islet amyloid polypeptide–like immunoreactivity (IAPP-LI, *top*), immunoreactive (IR) insulin (*middle*), and IR glucagon (*bottom*) from monolayer cultures of neonatal rat pancreas cells under 3 test conditions. Values for 5 experiments/condition are expressed as means ± SE. *$P < 0.05$ for percentage of fractional release vs. 1.67 mM glucose. #$P < 0.05$ for percentage of fractional release in 16.7 mM glucose plus arginine and isobutylmethylxanthine (IBMX) vs. 16.7 mM glucose.

serum added to enhance precipitation. After incubation of IAPP (25.6 pmol/ml) with cells in 1.67 mM glucose for 2 h, recovery of the peptide from the culture medium was 100%.

The quantity of IAPP-LI, insulin, and glucagon released into the medium or present in the extracted cells was calculated, and the data for each experimental group (n = 5/group) were expressed as means ± SE. Peptide release was expressed as percentage of fractional release calculated as 100% [amount of hormone secreted/(hormonal content of cell extract + amount of hormone secreted)]. Insulin, glucagon, and IAPP-LI results were compared by the Mann-Whitney U test with Bonferroni adjustment for multiple comparisons. $P < 0.05$ was considered significant.

RESULTS

The effect of the three different test media on IAPP-LI, insulin, and glucagon release is illustrated in Fig. 1. IAPP-LI secretion increased 4-fold from 0.041 ± 0.003 pmol in 1.67 mM glucose to 0.168 ± 0.029 pmol in 16.7 mM glucose ($P < 0.05$) and was 25-fold higher in 16.7 mM glucose plus arginine and IBMX at 1.02 ± 0.06 pmol ($P < 0.05$ vs. 1.67 or 16.7 mM glucose). Insulin release increased in similarfold increments from 4.34 ± 0.27 pmol in 1.67 mM glucose to 20.2 ± 0.6 pmol in 16.7 mM glucose ($P < 0.05$) and 135 ± 5 pmol in 16.7 mM glucose plus arginine and IBMX ($P < 0.05$ vs. 1.67 or 16.7 mM glucose). However, glucagon secretion tended to decrease with the increase from low to high glucose (0.39 ± 0.01 vs. 0.33 ± 0.02 pmol, $P < 0.1$), whereas with incubation in 16.7 mM glucose plus arginine and IBMX, glucagon secretion increased to 1.32 ± 0.03 pmol ($P < 0.05$ vs. 1.67 or 16.7 mM glucose).

The relationship between the molar quantity of IAPP-LI and that of insulin and glucagon secreted into the medium is shown in Table 1. In all three test media, the molar quantity of IAPP-LI released relative to insulin was constant at ~1%. In contrast, the molar quantity of IAPP-LI relative to glucagon varied significantly with the different incubation conditions.

After incubation in 1.67 mM glucose, cell extracts contained measurable quantities of IAPP-LI, insulin, and glucagon. IAPP-LI content was measured as 3.7 ± 0.2 pmol, insulin content as 944 ± 25 pmol, and glucagon content as 28.2 ± 1.5 pmol. Thus, in molar proportion, the quantity of IAPP-LI extracted from the cells was 0.4% that of insulin and 13% that of glucagon, whereas the molar content of glucagon relative to insulin was 3%.

From the quantity of peptide released into the medium and the cell content, we calculated the percentage of fractional release. The relationship of the percentage of fractional release of IAPP-LI, insulin, and glucagon secreted into the medium under the three conditions is illustrated in Fig. 2. The proportion of IAPP-LI released was 1.17 ± 0.05% in 1.67 mM glucose, increased to 4.1 ± 0.4% in 16.7 mM glucose ($P < 0.05$), and increased further to 26.7 ± 0.7% in 16.7 mM glucose plus arginine and IBMX ($P < 0.05$ vs. 1.67 or 16.7 mM glucose). The proportion of insulin released increased under the same conditions from 0.46 ± 0.03 to 2.3 ± 0.2% ($P < 0.05$) and then to 14.7 ± 0.6% ($P < 0.05$ vs. 1.67 or 16.7 mM glucose). In contrast, the percentage of fractional release of glucagon never increased >5%, being 1.37 ± 0.08% in 1.67 mM glucose, 1.02 ± 0.08% in 16.7 mM glucose ($P < 0.05$), and 4.4 ± 0.2% in 16.7 mM glucose plus arginine and IBMX ($P < 0.05$ vs. 1.67 or 16.7 mM glucose).

DISCUSSION

The results of this study demonstrate that IAPP-LI is present in rat pancreas cells shortly after birth and that it is released in a regulated manner in response to both glucose and non-glucose secretagogues. The fact that this immunoreactivity

TABLE 1
Relationship between quantities of islet amyloid polypeptide–like immunoreactivity (IAPP-LI), immunoreactive (IR) insulin, and IR glucagon secreted from monolayer cultures of neonatal rat pancreas during 2 h of incubation under 3 test conditions

	1.67 mM glucose	16.7 mM glucose	16.7 mM glucose + 10 mM arginine + 0.5 mM IBMX
IAPP-LI/IR Insulin (%)	0.95 ± 0.08	0.84 ± 0.15	0.76 ± 0.05
IAPP-LI/IR Glucagon (%)	10.6 ± 0.8	51.3 ± 8.7*	77.5 ± 5.2*

Values are means ± SE (in %). IBMX, isobutylmethylxanthine.
*$P < 0.05$ vs. 1.67 mM glucose.

is detectable in incubation medium after stimulation with classic islet hormone secretagogues suggests that it is being released from pancreatic endocrine cells. Furthermore, because the pattern of release paralleled that of insulin and the molar proportion that was released relative to insulin remained constant under the different conditions, it would appear that IAPP-LI synthesis and release are occurring from β-cells. This finding is thus compatible with the recent description of IAPP being present in the secretory granule of the β-cell and not in the α-cell (19–21).

Whether the IAPP-LI we measured is all intact IAPP or whether a proportion of the immunoreactivity represents pro-IAPP or conversion intermediates formed during the processing of the peptide has not been determined. A recent report of the presence of pro-IAPP immunoreactivity in human islet amyloid deposits suggests the possibility that all the propeptide may not undergo processing to IAPP and that some conversion intermediates may reside in β-cell secretory granules (26). If the IAPP-LI we measured was partially composed of conversion intermediates, this would be analogous to the situation where insulin is incompletely processed in the β-cell from proinsulin and then stored in secretory granules along with small quantities of proinsulin and its conversion intermediates before they are all either released or undergo crinophagy (27).

The molar ratios of IAPP-LI relative to insulin both in the media and the cell extracts was <1%. This observation of a small quantity of IAPP-LI in islets is compatible with the recent findings of Leffert et al. (28), who, with Northern blot analysis, suggested that the quantity of IAPP in the islet was far less than that of insulin. In summary, we demonstrated that IAPP-LI is present in neonatal rat pancreas and appears to be synthesized and released in a regulated manner by β-cells.

NOTE ADDED IN PROOF

A recent study demonstrated that isolated rat pancreatic islets release IAPP in response to glucose (29).

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