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IDENTIFICATION AND CHARACTERIZATION OF ISLET AMYLOID POLYPEPTIDE IN MAMMALIAN GASTROINTESTINAL TRACT

Mikiya MIYAZATO[1], Masamitsu NAKAZATO[1§], Kazutaka SHIOMI[1], Junko ABURAYA[1], Hirotaka TOSHIMORI[1], Kenji KANGAWA[2], Hisayuki MATSUO[3] and Shigeru MATSUKURA[1]

[1]Third Department of Internal Medicine and [2]Department of Biochemistry, Miyazaki Medical College, Kiyotake, Miyazaki 889-16, Japan

[3]National Cardiovascular Center Research Institute, Suita, Osaka 565, Japan

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SUMMARY: We identified and determined the content and molecular form of islet amyloid polypeptide (IAPP/amylin) in the gastrointestinal (GI) tract of human, rat, mouse and cat. IAPP was isolated by anti-IAPP-IgG immunoaffinity chromatography and reverse-phase high performance liquid chromatography coupled with radioimmunoassays for human and rat/mouse IAPPs. Human IAPP[1-37], [17-37] and [24-37] were identified in human stomach with IAPP[1-37] being the major molecular form. In the GI tract of rat, mouse and cat, IAPP[1-37] and IAPP[19-37] were identified with the latter being the major molecular form. IAPP is present from stomach to colon with the highest concentration being observed in pyloric antrum of stomach. IAPP content in rat antrum fell to 69% of control after 4 days of fasting, with the molar ratio of IAPP[19-37] to IAPP[1-37] increasing from 1.4 in controls to 2.9 in fasted rats. Identification of IAPP and characteristic morphology of IAPP- cells in the GI tract indicate a possible biological funtion of IAPP as a gastrointestinal peptide.

Islet amyloid polypeptide (IAPP) (1), also designated amylin (2), is a 37-amino acid peptide which was first isolated from insulinoma amyloid and islet amyloid of patients with non-insulin-dependent diabetes mellitus. IAPP has been shown to be a normal pancreatic peptide, being co-localized with insulin in B cell secretory granules and to be secreted into the bloodstream along with insulin in response to glucose, arginine and glucagon (3, 4). IAPP is thought to serve as a pancreatic hormone to modulate carbohydrate metabolism by inhibiting glycogen synthesis in skeletal muscle (5), reversing insulin-mediated inhibition of hepatic glucose production (6), and reducing glucose uptake due to inhibition of glucose phosphorylation (7). We isolated human IAPP[1-37], IAPP[17-37] and IAPP[24-37] from non-diabetic human pancreas (8), and IAPP[1-37] and IAPP[19-37] from normal pancreata of

§ To whom correspondence should be addressed.

Abbreviations: IAPP, islet amyloid polypeptide; HPLC, high performance liquid chromatography; RIA, radioimmunoassay.

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rat, mouse and cat (8, 9). Other pancreatic hormones such as glucagon, somatostatin and pancreatic polypeptide are also known to be present in the gastrointestinal (GI) tract of human and rodent (10), and to act as GI hormones affecting secretory and motor functions of the GI tract.

In this study, we identified IAPP in the GI tract of human, rat, mouse and cat using reverse-phase high performance liquid chromatography (HPLC) coupled with radioimmunoassays (RIAs). Changes in IAPP content and its molecular form in rat stomach in response to feeding and fasting were studied in order to elucidate the effects of nutrients on metabolism of IAPP in the GI tract.

MATERIALS AND METHODS

Isolation and identification of IAPP in GI tract

Ⅰ. Human stomach:

Human pyloric antra (1.6 g, 3.3 g and 2.7 g wet weight) were collected from non-diabetic 61-, 65- and 75-year-old subjects with gastric cancer. No antra had any macroscopic or microscopic change. Tissue was immediately heated at 95-100 ℃ for 10 min in 10 vol water to inactivate proteases. After cooling to 4 ℃, CH_3COOH and HCl were added to make final concentrations of 1M and 20mM, respectively. Tissue was homogenized and then centrifuged at 8,000 x g for 30 min. Two vol acetone was gradually added to the resulting supernatant under stirring and further stirred overnight at 4 ℃. After removing precipitate by centrifugation at 12,000 x g for 30 min, the supernatant was evaporated with a rotary evaporator and filtered through a GF/B filter (Whatman). The sample was pumped up to an octadesylsilica column (15 ml, Chemcosorb LC-SORB SPW-C-ODS), washed with 0.5M acetic acid then 0.1% trifluoroacetic acid (TFA) solution. The adsorbed materials were eluted with a 60% acetonitrile (CH_3CN) solution containing 0.1% TFA. The eluate was evaporated and loaded on an SP-Sephadex C-25 column (H^+- form, 2 ml, Pharmacia), pre-equilibrated with 1M acetic acid. The column was washed with 1M acetic acid (SP-Ⅰ fraction), then eluted successively with 2M pyridine (SP-Ⅱ fraction) and 2M pyridine-acetate (pH 5.0) (SP-Ⅲ fraction). The SP-Ⅲ fraction was evaporated repeatedly to remove pyridine, then lyophilized. The dried material was dissolved in 0.1M sodium phosphate buffer (pH 7.4) containing 0.05% Triton X-100, and subjected to immunoaffinity chromatogaphy on an anti-human IAPP IgG-Affi-Gel 10 column prepared as previously reported (11). After washing the immunoaffinity column with the above phosphate buffer, adsorbed peptides were eluted with a solution of 1M acetic acid containing 10% CH_3CN and 0.005% Triton X-100. The column eluate was subjected to reverse-phase HPLC on a TSK ODS SIL 120A column (4.6×150 mm, Tosoh). Chromatographic conditions are described in Fig. 1 legend. All fractions were monitored by RIA for human IAPP (12). Elution positions of immunoreactive fractions were compared with those of human pancreatic extract and of authentic human IAPP[1-37], [17-37] and [24-37].

Ⅱ. Rat and mouse stomach:

Pyloric antra were obtained after decapitation from 3 male Sprague-Dawley rats weighing 260-280 g and 3 male ddy mice weighing 40 g. Each antrum was immediately boiled, homogenized and centrifuged as described above. Tissue extracts from 100 mg wet weight were applied to a Sep-Pak C-18 cartridge (Waters) and adsorbed materials were eluted with 60% CH_3CN solution containing 0.1% TFA. Column eluate was evaporated, dissolved in 0.1M sodium phosphate buffer (pH 7.4) containing 0.05% Triton X-100, and subjected to immunoaffinity chromatography on an anti-rat IAPP IgG-Affi-Gel 10 column prepared as previously reported (13). Immunoreactive IAPP was eluted with a solution of 1M acetic acid containing 10% CH_3CN and 0.005% Triton X-100. The column eluate was subjected to reverse-phase HPLC (Fig. 2) under conditions described in Fig. 1 legend. Authentic rat/mouse IAPP[1-37] and IAPP[19-37] were chromatographed with the HPLC system used above. All fractions were monitored by RIA for rat/mouse IAPP (13).

Ⅲ. Cat stomach:

Feline pyloric antra (15 g wet weight) were obtained from 3 male cats after anesthesia with pentobarbital sodium and extracted as described above. Each extract was applied to a Sep-Pak C-18 cartridge then to an SP-Sephadex C-25 column. The SP-Ⅲ fraction was subjected to a human IAPP immunoaffinity column since an antiserum for human IAPP exhibited 6.3% cross-reactivity with both cat IAPP[1-37] and cat IAPP[19-37]. The column eluate was analyzed by HPLC and elution positions of immunoreactive IAPPs were compared with those of authentic cat IAPP[1-37] purchased from Peninsula Laboratories (Belmont, CA) and of cat IAPP[19-37].

Ⅳ. Rat intestine:

Duodenum, jejunum, ileum and colon were extracted from 3 male Sprague-Dawley rats. An extract from 200 mg of each tissue was applied to a Sep-Pak C-18 cartridge and a rat IAPP immunoaffinity column, and chromatographed with the same HPLC system used above. Each fraction was monitored by RIA.

Changes in content and molecular forms of antral IAPP in fed and fasted rats

Male Sprague-Dawley rats weighing 250-300 g were divided into 2 groups: 1) fed ad lib (n=3) and 2) fasted for 4 days (n=3). An extract of pyloric antrum from 200 mg wet weight was applied to a Sep-Pak C-18 cartridge then to rat IAPP immunoaffinity column. The eluate from 50 mg wet weight was lyophilized and reconstituted with RIA buffer for submission to RIA for determination of tissue content. The eluate from 100 mg wet weight was analysed by reverse-phase HPLC as shown in Fig. 2.

RESULTS

Identification of IAPP in GI tract

Fig. 1A is an HPLC chromatogram of immunoreactive IAPPs in human antrum. All immunoreactive IAPPs detected in antrum were observed in the chromatogram prepared from human pancreas (Fig. 1B), however, ratio was different in the two tissues. Although IAPP [1-37] was the major molecular form in both two tissues, it accounted for 50-62% of IAPP-

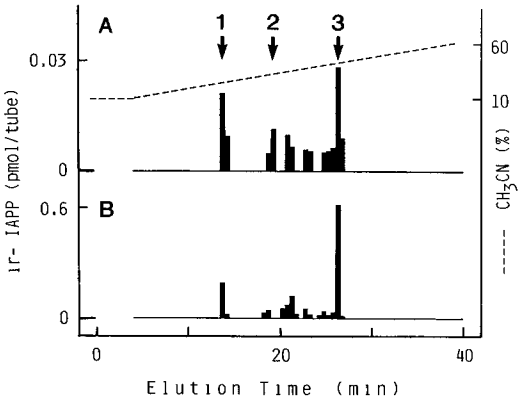

Fig. 1. Representative reverse-phase HPLC of human immunoreactive IAPP fraction isolated by anti-human IAPP IgG immunoaffinity chromatography.
Sample: (A) pyloric antrum (7.6 g wet weight), (B) pancreas (10 mg wet weight).
Column: TSK ODS SIL 120A (4.6 x 150 mm, Tosoh Co. Ltd.). Flow rate: 1.0 ml/min.
Linear gradient from 10% CH3CN to 60% CH3CN in 0.1% TFA for 40 min was employed.
Black bars represent IAPP-immunoreactivity. Arrows indicate elution positions of authentic (1) human IAPP[24-37], (2) human IAPP[17-37] and (3) human IAPP[1-37].

immunoreactivity in pancreas and only 30% in antrum. IAPP[17-37] and IAPP[24-37] comprised 13% and 24% of IAPP-immunoreactivity in antrum, respectively, which was approximately double the ratio of these peptides in pancreas. At positions 5, 3.5 and 1 min earlier than that of IAPP[1-37], 14%, 10% and 9% of antral IAPP-immunoreactivity was detected, respectively, as was the case with pancreatic IAPP. These peaks are thought to represent IAPP molecules whose conformation were changed or denatured, or IAPPs bound to other substances since the amino acid sequence of the IAPP eluted 5 min earlier was identical to that of IAPP[1-37].

Figs. 2A and 2B are HPLC chromatograms from pyloric antra of rat and mouse fed ad lib. Two immunoreactive peaks were eluted at positions identical to those of rat/mouse IAPP[19-37] and IAPP[1-37] in a manner similar to rat pancreatic IAPP as shown in Fig. 2C. IAPP[19-37] and IAPP[1-37] accounted for 50% and 35% of IAPP-immunoreactivity in rat antrum, and 60% and 40% in mouse antrum, respectively. A minor component of IAPP comprising 15% of total IAPP-immunoreactivity was observed at the elution position between IAPP[19-37] and IAPP[1-37] in rat antrum. IAPP[19-37], IAPP[1-37] and one minor IAPP-related peptide were also identified in rat intestine with IAPP[19-37] being the major molecular form as shown in Figs. 2D and 2E.

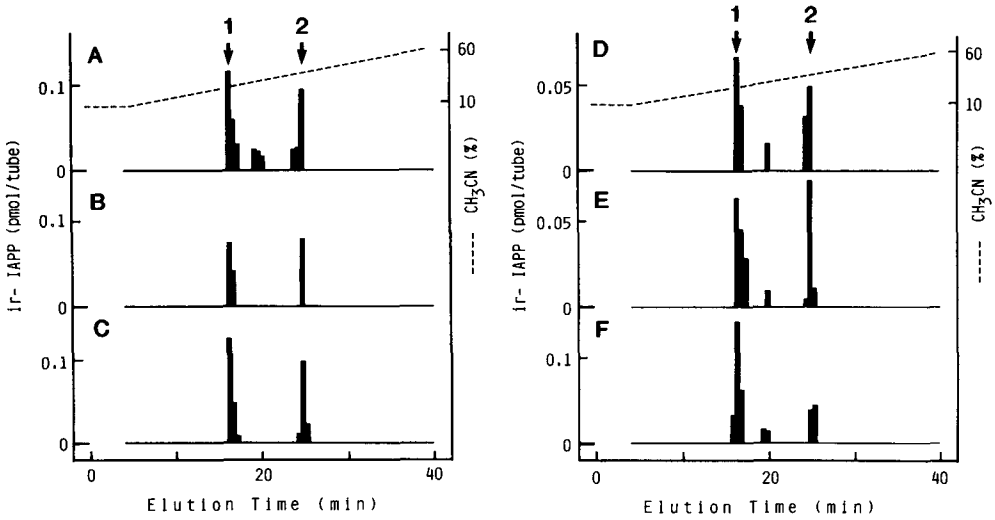

Fig. 2. Representative reverse-phase HPLC of rat and mouse immunoreactive IAPP fraction isolated by immunoaffinity chromatography.
Sample: (A) rat antrum, (B) mouse antrum, (C) rat pancreas, (D) rat jejunum, (E) rat colon, (F) antrum of rat fasted for 4 d.
Samples (A)~(E) were obtained from animals fed ad lib. All samples were from 100 mg wet weight. Column and chromatographic conditions are the same as in Fig. 1. Black bars represent IAPP-immunoreactivity. Arrows indicate elution positions of authentic (1) rat/mouse IAPP[19-37] and (2) rat/mouse IAPP[1-37].

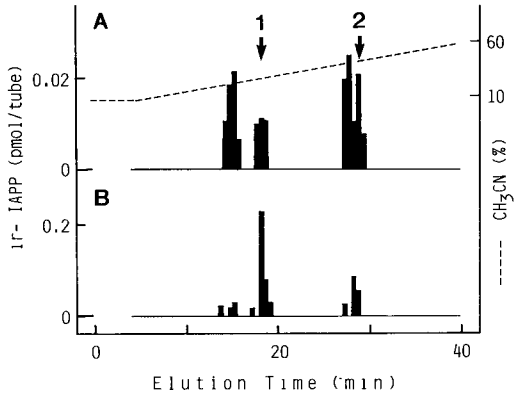

Fig. 3. Representative reverse-phase HPLC of cat immunoreactive IAPP fraction isolated by immunoaffinity chromatography.
Sample: (A) pyloric antrum (15 g wet weight), (B) pancreas (5 mg wet weight).
Column and chromatographic conditions are the same as in Fig. 1. Black bars represent IAPP-immunoreactivity. Arrows indicate elution positions of authentic (1) cat IAPP[19-37] and (2) cat IAPP[1-37].

Also in cat antrum, IAPP[19-37] and IAPP[1-37] were identified (Fig. 3A), with the HPLC chromatogram of antrum being considerably different from that of pancreas (Fig. 3B). One large and broad immunoreactive peak was detected at elution positions 3 min and 1 min earlier than those of IAPP[19-37] and IAPP[1-37], respectively. These peptides were minor components in cat pancreas (Fig. 3B), apparently being IAPP-related peptides as they were also present in the human IAPP molecule (Fig. 1).

IAPP content in the GI tract is represented in Table 1. The highest IAPP content in rat GI tract was noted in pyloric antrum at 1.0% of the level of pancreatic IAPP (328.5 ± 25.0 pmol/g

Table 1. IAPP content in gastrointestinal tract

species	tissue		pmol/g wet weight	species	tissue	pmol/g wet weight
rat	pyloric antrum	fed	3.36 ± 0.09	rat	duodenum	0.22 ± 0.21
		fasted 4 d.	2.33 ± 0.54		jejunum	0.44 ± 0.03
mouse	pyloric antrum		1.70 ± 0.20		ileum	0.43 ± 0.12
cat	pyloric antrum		0.19 ± 0.06		colon	1.21 ± 0.21
human	pyloric antrum		0.03			

Results are expressed as mean ± SD. Organs other than rat and human pyloric antra were obtained from animals fed ad lib.

wet weight) (13). In man and cat, IAPP content in antrum was markedly lower than that in rodent, although pancreatic IAPP contents in all 4 mammals were approximately equivalent (8).

IAPP was not detected by RIA and immunohistochemical studies in non-glandular sites of rat stomach where the epithelium is composed of squamous cells.

Changes in content and molecular form of rat IAPP in response to fasting

IAPP content in antrum was studied in fed and fasted rats. IAPP content fell to 69% of controls after 4 day fasting as shown in Table 1. Molecular forms of antral IAPP from 3 fasted rats were compared with those of controls by reverse-phase HPLC. The mean ratio of IAPP [19-37] to IAPP[1-37] was 1.4 in fed rats, increasing to 2.9 in fasted rats as shown in Fig. 2F.

DISCUSSION

The present study clarified that IAPP is present in mammalian GI tract from pyloric antrum of stomach to colon, chiefly in pyloric antrum. IAPP mRNA has been detected in stomach, duodenum, jejunum, ileum, caecum and colon of rat with the highest concentration being observed in stomach (14). The concentration of IAPP mRNA in rat stomach is 5% of that in pancreas (14), whereas in the present study, IAPP content in stomach is as little as 1.0% of that in pancreas. These results indicate that translational efficiency or turnover rate of IAPP mRNA and/or post-translational regulatory mechanisms of IAPP would be different in these two tissues. IAPP is co-localized with insulin in B cells, however, only the IAPP gene is expressed in the GI tract (14). All molecular forms of IAPPs identified in the GI tract of human, cat and mouse were also detected in their pancreata, indicating that processing of IAPP in the GI tract and pancreas does not differ. However, one IAPP-related peptide which is thought to be a minor intermediate product between IAPP[19-37] and IAPP[1-37] was found in rat GI tract but not in pancreas. Processing of rat IAPP might be slightly different in the two tissues.

Caloric deprivation for 4 days caused a 54% reduction in IAPP content of rat pancreas compared to control (13), and in the present study, a 69% reduction in rat antrum. IAPP[19-37]/IAPP[1-37] molar ratio was increased in fasted rat antrum, similar to that of pancreatic IAPP whose molar ratio was increased from 1.3 in controls to 2.4 in fasted rats (13). The increase of IAPP[19-37] under conditions in which synthesis and secretion of IAPP were suppressed may imply that IAPP[19-37] is associated with intracellular catabolism of IAPP[1-37] or a possible antagonistic action of IAPP[19-37] to IAPP[1-37], as was observed in calcitonin gene-related peptide (CGRP) (15).

We have immunohistochemically demonstrated that IAPP-containing cells were localized in basal layers of the mucosa in the GI tract of human and rat (16). IAPP-immunoreactive cytoplasmic processes reached the surface of the lumen. This topological location suggests that IAPP-cells are exposed to high glucose levels during digestion and absorption. IAPP in the

mucosa might be secreted into the bloodstream to modulate glucose metabolism. Fragments of immunoreactive cytoplasmic processes were often seen next to non-IAPP cells at the base of the glands (16). Gastrointestinal IAPP may also have a trophic paracrine action on surrounding tissues. The distribution of IAPP-cells in the GI tract is similar to that of gastrin-cells or somatostatin- cells, but co-localization of IAPP and gastrin or somatostatin in the same cell was not observed (16).

Glucagon, somatostatin and pancreatic polypeptide present in the mucosa of the GI tract affect the mobility and secretory function of the GI tract as gastrointestinal hormones, inhibit pancreatic exocrine secretion, and also serve as pancreatic hormones. CGRP, a member of the IAPP gene family, has an inhibitory effect on gastric secretion and appetite, relaxes smooth muscle contraction of the GI tract, and inhibits pancreatic exocrine secretion (17). Existence of IAPP and the characteristic morphology of IAPP-cells in the GI tract indicate that IAPP possibly plays a paracrine role in the tract as well as functions as a pancreatic hormone to modulate carbohydrate metabolism. Further investigation is needed to clarify the physiological function and the pathophysiological significance of IAPP in the GI tract.

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