Secretion of islet amyloid polypeptide in response to glucose

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The content of islet amyloid polypeptide (IAPP) in isolated rat pancreatic islets was determined by a radioimmunoassay. Reverse-phase high-performance liquid chromatography analysis revealed that a main peak of IAPP immunoreactivity in the extracts from the islets corresponded to a synthetic rat IAPP. Secretion of IAPP from the cells is regulated by the extracellular glucose concentration. Thus, IAPP may be a novel regulator for glucose homeostasis and changes in the secretion perhaps relate to insular amyloid deposits and impaired glucose tolerance in type 2 diabetes mellitus.

Islet amyloid polypeptide; Pancreatic islet; Secretion; Radioimmunoassay

1. INTRODUCTION

In type 2 (non-insulin dependent) diabetes mellitus, the most significant pathological change in pancreatic islets is amyloid deposits [1]. In diabetic macaques, islet amyloid deposition increases along with the degree of reduction in insulin secretion, accompanied by an increase in glucose intolerance [2]. In human type 2 diabetes mellitus, the extent of islet amyloid deposition increases with increase in the clinical severity [3]. IAPP, also termed amylin, a major component of islet amyloid, is a 37 amino acid peptide [4,5]. IAPP seems to be a natural occurence in pancreatic B-cells, as determined immunohistochemically [6] and by an analysis of human genomic DNA encoding IAPP [7]. Gene analysis using cDNA cloned from a human insulinoma cDNA library revealed that a cDNA encoding IAPP contained an open reading frame encoding precursors with a typical signal peptide [8]. To investigate possible roles of IAPP in functions of the endocrine pancreas and the development of type 2 diabetes mellitus, we measured the content of IAPP in isolated rat pancreatic islets by an RIA which we developed ourselves, analyzed IAPP immunoreactivity of the extracts from the islets by reverse-phase HPLC and examined the mechanisms underlying the secretion of this peptide in the islet cells.

2. MATERIALS AND METHODS

2.1. *Immunogen preparation and immunization*
The hydropathy and antigenicity of human IAPP along with its

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

amino acid sequence was evaluated by computer programs [9-11]. Since the region, Ala8-Val17, has the highest antigenicity value, we proposed to raise an antiserum against IAPP by immunization of oligopeptides consisting of the region. Oligopeptide (R93) was assembled automatically by a solid-phase technique, using a 430A Peptide Synthesizer (Applied Biosystems Inc., Foster City, CA.). Reverse-phase HPLC analysis on a Nucleosil $5C_{18}$ column (4 mm × 15 cm) (M. Nagel GmbH) indicated that R93 had a purity of 100%. R93 was linked by glutaraldehyde through the N-terminus to hemocyanin keyhole limpet (Calbiochem). The conjugate emulsified with complete Freund's adjuvant was given to New Zealand white rabbit. Immunization was repeated every 2 weeks and sera were obtained 7 days after the third, fourth and fifth immunizations.

2.2. *Preparation of the tracer and the RIA procedure*
N-tyrosinated R93 radiolabeled with ^{125}I (New England Nuclear) using lactoperoxidase (Sigma Chemical) and purified on a Sephadex G-25 column was used as a tracer. The method of the RIA was similar to that used for somatostatin [12]. In brief, the diluent for reagents was 0.1% gelatin made up in 0.01 M phosphate/0.14 M NaCl/0.25 M ethylenedinitrilotetraacetic acid disodium, pH 7.4. The reaction mixtures, the antiserum, standard solution or unknown samples, human serum stripped of its peptide by dextran-coated charcoal and tracer (approximately 10 000 cpm), were incubated at 4°C for 48 h and the free and bound peptides were separated by dextran-coated charcoal. Radioactivity of the bound labeled peptide was determined in an autogamma counter. R93, N-tyrosinated R93, human insulin (Actrapid human insulin, Novo Ind.), rat insulin (Novo Ind.), glucagon, somatostatin and human and rat calcitonin gene-related peptide (Peptide Institute, Osaka, Japan) were used to test the cross-reactivity.

2.3. *Measurement of IAPP content in rat pancreatic islets*
Fed male Sprague-Dawley rats weighing 300 g were anesthetized with sodium pentobarbital and the pancreatic islets were isolated by a modification of the method described earlier [13]. The islets were homogenized in 70% formic acid by sonication. The formic acid was removed to near dryness with a vaporizer and the extracts were lyophylized. Just before the assay, the extracts were dissolved in 0.2 N acetic acid, diluted in the assay buffer and adjusted to pH with NaOH. The extracts were measured for IAPP-like substance by the RIA.

2.4. *Analysis of immunoreactive substance by reverse-phase HPLC*
According to the previously described amino acid sequence, rat IAPP was assembled automatically by a solid-phase technique. This

peptide was analyzed by reverse-phase HPLC on a TSK gel ODS-120T column (4.6 mm id × 25cm) (Tosoh, Tokyo, Japan) using a linear gradient from 20 to 80% acetonitrile containing 0.05% trifluroacetic acid throughout and the elution profile was monitored at 220 nm. The extracts of 300 isolated rat pancreatic islets first partially purified by passage through a C_{18} Sep Pak (Waters Associates, Melbourne, Australia) were analyzed by reverse-phase HPLC. The flow rate was 0.5 ml/min and fractions were collected every minute. Each fraction lyophilized and reconstituted was measured for IAPP-like substance, using the RIA.

2.5. Secretion from pancreatic islets

Isolated rat pancreatic islets, 300–400, preincubated in 1 ml Krebs-Ringer bicarbonate buffer, pH 7.4, containing 1 g/l bovine serum albumin (RIA grade fraction V: Sigma) and 500 kallikrein inhibiting units/ml aprotinin (Bayer, Osaka, Japan) and 2.8 mM glucose were incubated for 30 or 60 min in 1 ml of the medium containing 2.8 or 16.7 mM glucose under 95% O_2–5% CO_2 at 37°C [15]. IAPP-like substance in the medium was assayed by the RIA and insulin was measured by RIA using an insulin assay kit (Eiken Chemical, Tokyo, Japan).

Student's t-test was used for statistical analysis.

3. RESULTS AND DISCUSSION

As shown in fig.1a, the binding of the tracer with the antiserum was inhibited by adding R93, and the unlabeled N-tyrosinated R93 and the inhibition was dose-dependent. With the antiserum at a final dilution of 1:8000 and 10 000 cpm of the tracer, the minimum detectable dose was 70 fmol/tube. Intra and interassay coefficients of variation were 4.2% and 5.5%, respectively (assay of identical sample containing 1 pmol/tube in five replicates in one assay). Human and rat insulin, glucagon and somatostatin, and human and rat calcitonin gene-related peptide did not interfere with the binding of the tracer with R93-2, thereby indicating that this radioimmunoassay is specific for rat IAPP.

Since the measured concentrations of IAPP immunoreactivity in extracts from isolated rat pancreatic islets fell linearly with dilutions (fig.1b), the material assayed is indistinguishable immunologically from this peptide. In analysis of the extracts by reverse-phase HPLC, the main peak of IAPP immunoreactivity corresponded to the synthetic rat IAPP (fig.2). These findings strongly suggest that the substance measured by the RIA is identified with rat IAPP. The concentration of IAPP-like substance is 233.6 ± 16.2 fmol/islet ($n = 8$). The presence of IAPP in the islet cells was also confirmed by an immunohistochemical study using the antiserum (data not shown), compatible with a previous report that IAPP immunoreactive cells were present in the islet of cat, dog, mouse and rat. Leffert et al. reported that human and rat IAPP differ in 6 of the 37 residues, all within the 18–29 region [14]. Because the 8–17 region used for production of our antiserum is homologous to the region of rat IAPP, the antiserum recognized rat IAPP.

In the analysis by reverse-phase HPLC, two small peaks of IAPP immunoreactivity were observed and did not correspond to the synthetic rat IAPP. At the
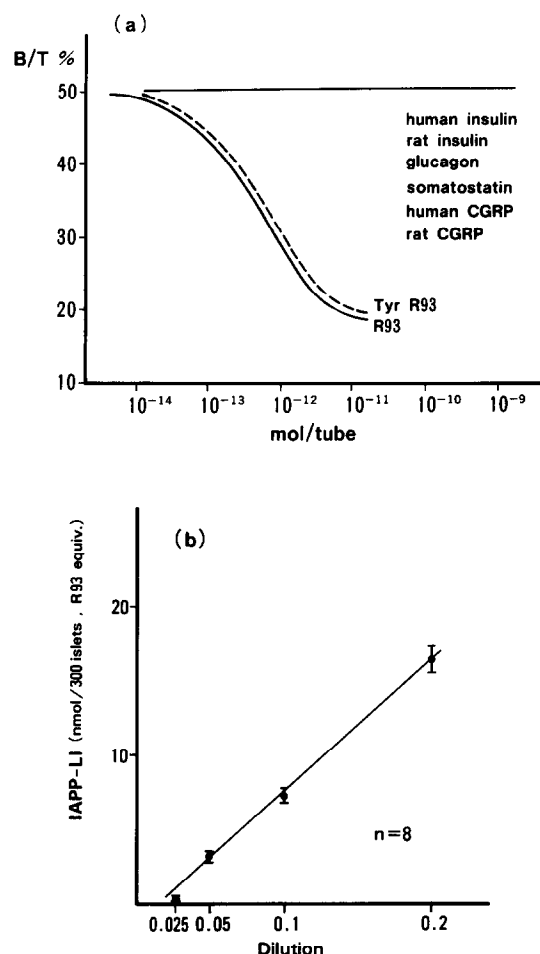
Fig.1. Development of RIA, using a specific antiserum for IAPP and determination of IAPP-like substances in extracts of normal rat pancreatic islets. a: Competitive inhibition of the antiserum tracer binding by various peptides. b: Amounts of IAPP-like substance determined as a function of the dilution of rat pancreatic islet extracts.
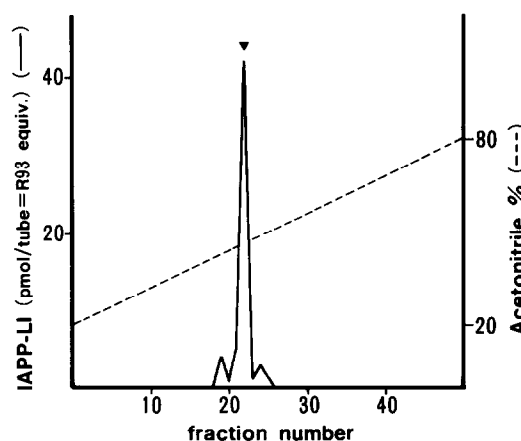
Fig.2. Analysis of the extracts from isolated rat pancreatic islets by reverse-phase HPLC on a TSK gel ODS-120T column. Closed triangle indicates retention time of a synthetic rat IAPP.
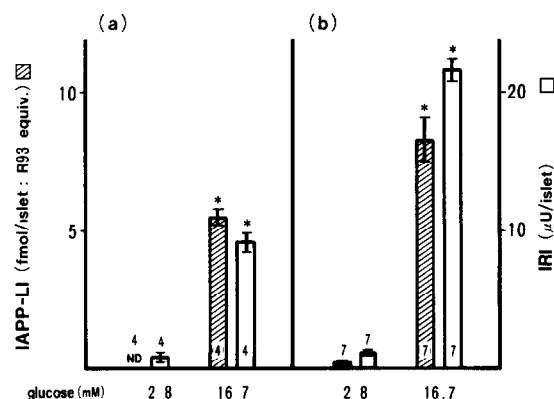
Fig.3. Secretion of IAPP-like substance and insulin from isolated rat pancreatic islets incubated for 30 min (a) or 60 min (b) in the presence of 2.8 or 16.7 mM glucose. Mean ± SEM for IAPP-like substance (▨) and insulin release (□) are expressed in fmol/islet and μU/islet, respectively. Numbers of observations are shown in parentheses. $*P<0.01$; compared with the release in the presence of 2.8 mM glucose.

present time we have no precise information regarding the molecular basis of the substances eluted in these fractions. As the substances reacted with a specific antiserum for IAPP, they are peptides related to IAPP, a precursor and a degraded peptide of IAPP. This hypothesis is supported by isolation of cDNAs encoding rat IAPP from an islet cDNA library and identification of a 0.9 kb mRNA encoding a 93 amino acid precursor that is proteolyzed to yield the final IAPP product [14]. Also, an immunohistochemical study indicated that IAPP immunoreactivity was found in the secretory granules of B-cells and in electron-dense regions of some lysosomal or lipofuscin bodies, suggesting partial degradation of IAPP in the cells [15].

As shown in fig.3, an IAPP-like substance was secreted from isolated rat pancreatic islets incubated in Krebs-Ringer bicarbonate buffer in the presence of 16.7 mM glucose. The secretion increased along with the duration of the incubation time. This is the first evidence that IAPP was co-secreted with insulin from the pancreatic islet cells and the secretion was regulated by glucose, the most important physiological stimulant of insulin secretion. IAPP immunoreactivity was shown to be localized in the outer lucent compartment of the B-cell secretory granule by an immunohistochemical study using an A-gold labeling technique [6]. A gene analysis using cDNA cloned from a human insulinoma cDNA library revealed that a cDNA encoding IAPP contained an open reading frame encoding a precursor having a typical signal peptide [8]. Also, sequence analysis of cDNA encoding rat IAPP showed that a 0.9 kb mRNA encoded a 93 amino acid precursor and the amino-terminal domain was hydrophobic and consistent with a signal sequence in a secreted protein [14]. The presence of a sequence with the properties of a signal peptide at the N-terminus of this precursor sug-

gests that IAPP may be secreted from pancreatic B-cells.

We recently found that synthetic IAPP inhibited insulin secretion from isolated rat pancreatic islets, thus, IAPP exerts an inhibitory effect on insulin secretion [16]. IAPP is also a potent inhibitor of basal and insulin-stimulated glycogen synthesis in stripped rat soleus muscle [17]. Since IAPP secretion is regulated by glucose, IAPP might be novel regulator for glucose homeostasis. Type 2 diabetes mellitus is characterized by impaired insulin secretion and insulin resistance in skeletal muscle, the chief site of insulin-mediated glucose disposal [18]. Changes in IAPP synthesis and secretion perhaps relate to both major traits of this form of diabetes mellitus.

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