The Presence of Peptide Histidine-Isoleucine in Human, Dog, Guinea-Pig and Rat Pancreas

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Summary. Peptide histidine isoleucine-like immunoreactivity has been detected in the pancreas of man (10.3 ± 2.6 pmol/g), dog (6.5 ± 1.1 pmol/g), guinea-pig (5.8 ± 0.9 pmol/g) and rat (0.7 ± 0.1 pmol/g) using a specific radioimmunoassay. Chromatographic analysis of the pancreatic extracts by high performance liquid chromatography indicated that both

guinea-pig and rat have a molecular form of the immunoreactivity which is distinct from the porcine intestinal peptide histidine-isoleucine.

Key words: Peptide histidine-isoleucine, radioimmunoassay, high performance liquid chromatography, pancreas.

Introduction

Recently a polypeptide with N-terminal histidine and C-terminal isoleucine amide, and therefore called peptide histidine-isoleucine (PHI), has been isolated from porcine upper small intestine [1]. It has an amino acid sequence with a striking resemblance to vasoactive intestinal polypeptide (VIP) and has therefore been assigned to the glucagon-secretin family of peptides.

Subsequent studies have shown that PHI-like immunoreactivity, like that of VIP, is present throughout the gastrointestinal tract [2] and is also found in the brain [3, 4]. In the human intestine PHI-like immunoreactivity has been localised exclusively in the non-endocrine cell layer (i.e. lamina propria and muscularis externa). This work suggests that any physiological role of PHI would most probably be as a neurotransmitter or a neuromodulator.

It has been reported that PHI is a potent stimulant of pancreatic juice secretion [5] and also potentiates insulin and arginine stimulated glucagon release from the isolated perfused rat pancreas [6]. The aim of our study was to determine whether PHI-like immunoreactivity is present in the pancreas of various species, and to characterise this immunoreactivity chromatographically.

Materials and Methods

Extraction Procedure

The pancreas of the following species were used for this study: – dog ($n = 14$), guinea-pig ($n = 5$), rat ($n = 5$) and man ($n = 5$).

Rats and guinea-pigs were killed by cervical dislocation, dogs with an intraperitoneal injection of sodium pentobarbitone and the pancreas immediately dissected out. Specimens (approximately 1 g) of human pancreas were collected at post mortem examination from five individuals and each gland was subsequently shown to be histologically normal. Two specimens were from the head, two from the tail, and one from the body of the pancreas. All pancreatic tissue was weighed and then rapidly placed in boiling 0.5 mol/l acetic acid (10 ml/g) for 10 min. After cooling, 50 μ l aliquots of the extract were measured in duplicate for PHI-immunoreactivity.

Radioimmunoassay

The radioimmunoassay is described in full elsewhere [2]. The antibody was raised by immunising lop-eared rabbits with pure natural porcine PHI (Karolinska Institute, Sweden) conjugated to bovine serum albumin (Miles Biochemicals, Slough, UK) with glutaraldehyde and was used at a final titre of 1/10,000. The PHI tracer was prepared by the iodogen method [13] and purified by gel chromatography on a Sephadex G-50 superfine column. The assay was carried out in sodium acetate buffer (0.1 mol/l, pH 4.0), containing 0.5% bovine serum albumin. 'Antibody-bound label' and 'free label' were separated by dextran-coated charcoal adsorption after 5–7 days incubation at 4 °C. The inter-assay and intra-assay variations were 13% and 5% respectively. The assay showed no significant crossreactivity with other available peptides, in particular < 0.002% crossreactivity with VIP, glucagon, secretin, gastric inhibitory peptide (GIP), insulin, somatostatin, and pancreatic polypeptide. The assay could detect changes of 2 fmol/tube with 95% confidence.

High Performance Liquid Chromatography

A Waters Associates (Milford, Massachusetts) high pressure liquid chromatographic system was used which included: – (1) M6000A and M45 pumps, (2) USK septumless injector, (3) M660 solvent programmer. The Bondapak C₁₈ column (10 μ m, 3.6 $\times$ 30 mm) was obtained pre-packed from Waters Associates. Octadecylsilylsilica cartridges

Table 1. Peptide Histidine-Isoleucine (PHI) in the pancreas

Species	PHI immunoreactivity (pmol/g)
Man	10.3 ± 2.6
Dog	6.5 ± 1.1
Guinea pig	5.8 ± 0.9
Rat	0.7 ± 0.1

Results expressed as mean ± SEM

Fig. 1. Gel permeation chromatographic profiles of representative pancreatic extracts on a column (0.9 × 60 cm) of Sephadex G-50 superfine. V_0 – void volume; CC – horse heart cytochrome c; PHI – peptide histidine-isoleucine; V_t – bed volume. Concentrations are expressed as fmol/fraction. K_{av} is the elution coefficient [7]

(Sep-pak C_{18} , Waters Associates) were activated by washing with acetonitrile (British Drug Houses, Poole, UK) followed by water. Pancreatic extracts, three from each species, of volumes 500–2000 μ l were applied. Weakly bound components were eluted with 20 ml of water, and the 'hydrophobic' components were then eluted by 60% acetonitrile (1 ml). The eluate was diluted with an equal volume of 0.12% trifluoroacetic acid (British Drug Houses, Poole, UK) and then injected into the system.

A combination of a linear gradient and isocratic elution was used. The gradient from 30% to 40% B (solvent A – 0.12% trifluoroacetic acid/water: solvent B – 0.12% trifluoroacetic acid/acetonitrile) was run for 10 min and then kept at 40% B for a further 10 min. The flow rate was 1 ml/min with 1 ml fractions collected for subsequent radioimmunoassay.

Gel Permeation Chromatography

Tissue extracts were applied to a 0.9 × 60 cm column (Pharmacia, Uppsala, Sweden) containing a 30 ml bed volume of Sephadex G-50 superfine gel. Columns were run at a flow rate of 4 ml/h and were precalibrated with Dextran blue, horse heart cytochrome c, $Na^{125}I$

Fig. 2. Fractionation of pancreatic PHI-like immunoreactivity from four different species, on a reverse phase high performance liquid chromatography Bondapak C-18 column. Recoveries were 90 ± 5%. The bottom panel shows the acetonitrile gradient. Pure natural PHI standard, human and dog pancreatic PHI immunoreactivities were eluted at 28 ml, while those of rat and guinea-pig were eluted at 22 ml

and porcine PHI standard. Dextran blue, cytochrome c and a trace amount of ^{125}I were added to each sample as internal markers. The columns were eluted with 0.1 mol/l sodium acetate buffer (pH 4.0) containing 2% bovine serum albumin. Aliquots (50–200 μ l) of the eluted fractions (1 ml) were then assayed for their PHI content. An estimation of the column recovery was achieved by assaying the neat extract.

Calculations

Concentrations of PHI-like immunoreactivity are expressed in pmol/g wet weight of tissue and quoted as the mean ± SEM. For the direct comparison of the elution profile in each gel chromatographic run, the elution coefficient (K_{av}) was calculated according to Laurent and Killander [7].

Results

Table 1 shows the concentrations of immunoreactive PHI in the pancreas of the species studied. The concentrations were lowest in the rat and highest in the human pancreas. Representative gel chromatographic profiles for PHI immunoreactivity in three species are shown in Figure 1. In each case (except for the dog) in which the peak was narrower the PHI immunoreactivities emerged as broad peaks with recoveries ranging from 73% to 102%. Figure 2 shows the fractionation of tissue extracts by reverse phase high performance liquid chromatography, on which both the rat and guinea-pig immunoreactivities eluted earlier than that of the other species studied, which were co-eluted with the porcine PHI standard.

Discussion

The results presented here suggest that PHI, a recently isolated putative neurotransmitter, is present in the pancreas of several mammalian species. Chromatographic characterisation on Sephadex G-50 of the pancreatic immunoreactivity suggested that the material from all the species studied included material of the same molecular size as the porcine PHI standard. However, the use of reverse phase high performance liquid chromatography revealed significant species differences. Both guinea-pig and rat PHI immunoreactivities appeared to be less hydrophobic than the porcine PHI standard and were thus eluted significantly earlier. Human and dog PHI immunoreactivities were co-eluted with the porcine PHI standard. The finding of very low apparent concentrations of PHI immunoreactivity in the rat pancreas may thus be due to a lower cross-reactivity of rat PHI with the antibody used in this study.

Recently it has been found that PHI immunoreactivity is not confined to the upper small intestine but is distributed widely throughout the body. Highest levels of PHI immunoreactivity occur in the ileum and colon [9], but significant amounts (similar concentrations to those found in the pancreas) were also found in the brain and respiratory tract [8]. The concentration of PHI immunoreactivity in the pancreas is of a similar magnitude to those of the other putative neurotransmitters, bombesin [12] and VIP [11], but several orders of magnitude lower than the pancreatic hormones, glucagon, insulin and pancreatic polypeptide [10]. The concentration of peptides of widely different functions cannot, of course, be equated with their physiological importance.

Our finding that PHI immunoreactivity is found in the pancreas suggests that reports of the action of PHI on exocrine and endocrine pancreatic secretion may be physiologically relevant.

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