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Isolation and primary structure of human PHI (peptide HI)

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The isolation of the human form of PHI (peptide HI) is described. The peptide was purified from human colonic extracts by using a chemical method for the detection of its C-terminal amidated structure. Human PHI consists of 27 amino acid residues and the complete amino acid sequence is: His-Ala-Asp-Gly-Val-Phe-Thr-Ser-Asp-Phe-Ser-Lys-Leu-Leu-Gly-Gln-Leu-Ser-Ala-Lys-Lys-Tyr-Leu-Glu-Ser-Leu-Met-NH_2. The differences between the structures of porcine and human PHI are at position 12 (Arg/Lys replacement) and at position 27 (Ile/Met).

Human PHI *Porcine PHI* *Bovine PHI* *Brain peptide* *Gut peptide* *C-terminal amide* *VIP/PHI precursor*

1. INTRODUCTION

PHI is a heptacosapeptide originally isolated from porcine upper intestine [1,2] by using a chemical detection method [3] for its C-terminal amidated structure, which is a unique chemical feature shared with many hormonal and neural peptides. PHI was found to be structurally similar to members of the glucagon-secretin peptide family, especially to vasoactive intestinal peptide (VIP) [2]. Further, the peptide has been shown to exhibit a number of biological activities similar to VIP. For example, it stimulates insulin secretion [4,5] and pancreatic exocrine secretion [2,6]. PHI causes vasodilation [7], increases intestinal fluid transport [8,9] and relaxes tracheal [7,10] and gallbladder [11] smooth muscle. It is also a potent prolactin releasing factor [12]. Immunoreactive PHI is widely distributed in various nerve-containing tissues, including brain [13,14], intestine [15], respiratory tract [7,10] and pancreas [16]. Recently, a peptide was isolated from porcine brain and shown to be identical in primary structure to the PHI of intestinal origin [17].

We report here the isolation of the human form of PHI from human colonic extracts. The results of amino acid sequence analysis establish that the primary structure of human PHI differs from porcine PHI in two positions.

Abbreviations: PHI, peptide HI, peptide having N-terminal histidine and C-terminal isoleucine; HPLC, high-performance liquid chromatography

2. MATERIALS AND METHODS

Reagents for sequence analysis were of sequanal grade or were redistilled from analytical grade, and acetonitrile was of HPLC grade (Rathburn Chemical, Scotland). Polyamide thin-layer plates were obtained from Schleicher & Schüll and thermolysin (EC 3.4.23.4) from Daiwa Kasei K.K., Osaka.

Human PHI was assayed by the chemical method in [3]. Reversed-phase HPLC was performed in a Waters instrument using a μBondapak C18 or phenyl column under conditions described in the legends to figs.1–3. Amino acid compositions were determined with a Beckman 121 M amino acid

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analyzer after hydrolysis of samples in 5.7 M HCl/0.5% phenol at 110°C for 24 h. Stepwise Edman degradation of the intact peptide was performed, using a 0.1 M Quadrol program, in a Beckman 890 D liquid-phase sequencer in the presence of glycine precycled with polybrene [18]. Phenylthiohydantoin amino acids were determined by HPLC [19] and N-terminal amino acids were identified by the dansyl chloride method [20].

3. RESULTS

3.1. Isolation procedures

Human PHI was purified from a starting material obtained as a side fraction during isolation of human VIP [21] from colonic extracts. Briefly, human colon (2.5 kg), obtained from patients undergoing surgery for carcinoma of the colon, was boiled in water for 10 min, frozen, minced and extracted at 5°C with 0.5 M acetic acid. Peptides in the extracts were adsorbed onto alginic acid, eluted with 0.2 M HCl, and precipitated with NaCl at saturation. The precipitate was collected and dissolved in water, the pH adjusted to 4, and then the peptides were reprecipitated with NaCl. This second precipitate (5 g wet wt) was suspended in methanol containing 0.05% mercaptoethanol under constant stirring for 15 min and then filtered. The pH of the filtrate was adjusted to 7.5 (glass electrode) and the resulting precipitate was removed by filtration. The pH of the methanol extract was re-adjusted to 2.7, and the peptides in the extract were precipitated by addition of 4 vol ether. The ether precipitate was collected by centrifugation and dried under vacuum. The dried precipitate (0.94) was dissolved in 0.2 M acetic acid and chromatographed on a Sephadex G-25 (fine) column with 0.2 M acetic acid. The fractions containing the major peak were combined and lyophilized. An aliquot (35 mg) of the fraction (55 mg) was further purified by ion-exchange chromatography on a CM–cellulose column with step-wise elution (0.02, 0.06, 0.1 and 0.2 M ammonium bicarbonate). The fraction, eluting at 0.02 M ammonium bicarbonate, was found to contain a high concentration of a peptide with C-terminal methionine amide. This fraction (9.9 mg) was therefore subjected to further purification by reversed-phase HPLC. Fig.1 illustrates the HPLC elution profile on a μBondapak C18 column. This HPLC purification step yielded a total of 0.26 mg of the fraction containing PHI. This material was rechromatographed successively, first on a μBondapak phenyl column (fig.2), which yielded 0.05 mg of the PHI-containing fraction, and then on a μBondapak C-18 column (fig.3). The final product

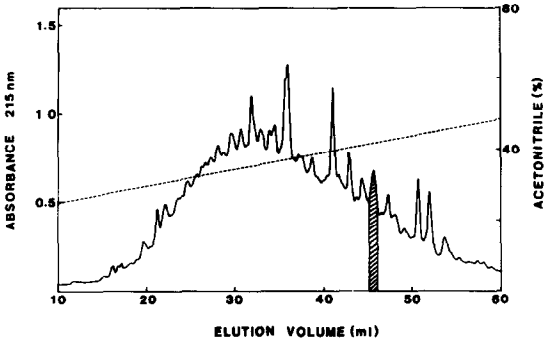

Fig.1. Reversed-phase HPLC profile of the PHI fraction from the CM-cellulose chromatography. An aliquot (3.5 mg) was applied to a μBondapak C18 column (7.8 × 300 mm) and eluted at a flow rate of 2 ml/min, using a linear gradient of 0.12% CF_3COOH/H_2O and 0.1% CF_3COOH/CH_3CN. The dashed line indicates the gradient profile. The HPLC fractions were lyophilized and aliquots were subjected to the chemical assay for PHI. The peak containing PHI is shown by the hatched area.

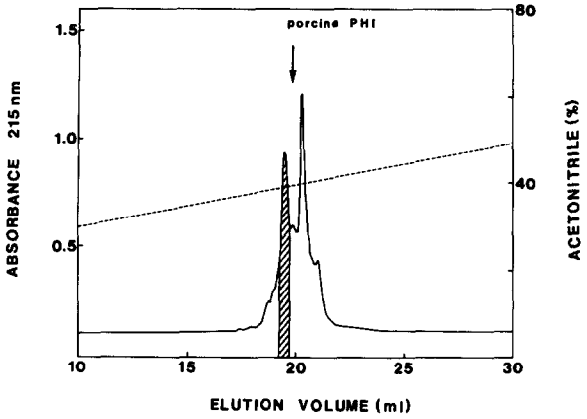

Fig.2. Reversed-phase HPLC profile of the PHI fraction (fig.1). The fraction (0.26 mg) was applied to a μBondapak phenyl column (3.9 × 300 mm) and eluted at a flow rate of 1 ml/min with the same solvent systems as described in fig.1. The peak (hatched area) contained human PHI. For comparison, the elution volume of porcine PHI is indicated by the arrow.

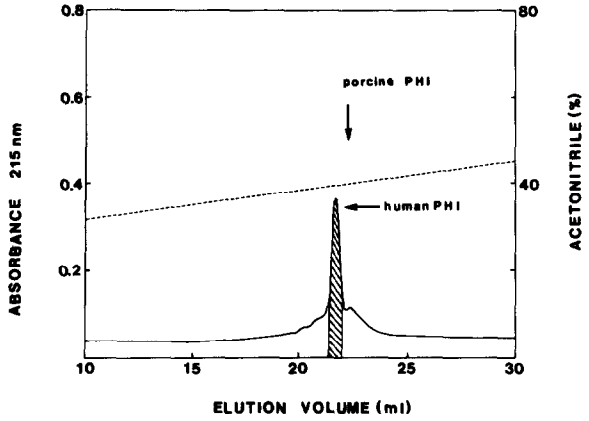

Fig.3. Final HPLC purification of human PHI. The PHI fraction (0.05 mg, Fig.2) was re-chromatographed on a μBondapak C18 column (3.9 × 300 mm) at a flow rate of 1 ml/min using the same solvent systems as in fig.1. The major peak (hatched area) contained pure human PHI and this fraction was subjected to structural analysis. The elution volume of porcine PHI is given by the arrow.

(8 nmol) was subjected to amino acid analysis, terminal determinations and sequence analysis.

3.2. *Structural analysis*

The results of amino acid analysis suggested that the peptide consisted of 27 amino acid residues: 2 Ala, 2 Asx, 2 Glx, 2 Gly, 1 His, 5 Leu, 3 Lys, 1 Met, 2 Phe, 4 Ser, 1 Thr, 1 Tyr, 1 Val. The N-terminal residue of the peptide was found to be histidine, which is identical to that of porcine PHI. Treatment of the peptide with thermolysin yielded both methionine amide and leucylmethionine amide as identified by the chemical method in [3]. Thus, the C-terminal structure of the human peptide should be -Leu-Met-NH_2, which is different from that of the porcine peptide, -Leu-Ile-NH_2. The intact peptide (5 nmol) was subjected to

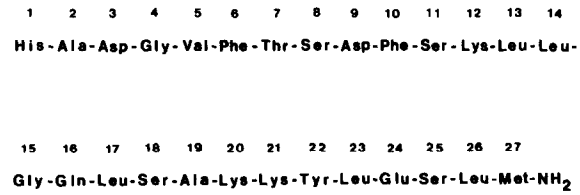

Fig.4. The complete amino acid sequence of human PHI.

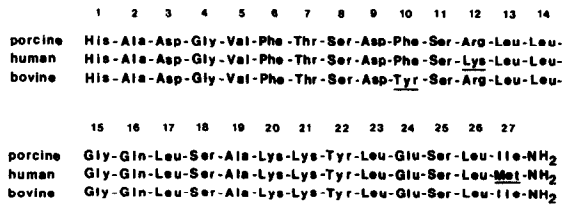

Fig.5. Comparison of the primary structures of porcine, human and bovine PHI. Positions with residue replacements in relation to porcine PHI are underlined.

Edman degradation in a Beckman 890 D liquid-phase sequencer which established the amino acid sequence up to residue 24 and traces of residue 26. The repetitive yield was Al_{2-19}: 89% and Leu_{13-23}: 97%. The complete amino acid sequence of the human peptide is deduced from the results combined with sequence analysis, terminal determinations and amino acid analysis, as in fig.4.

4. DISCUSSION

It has been demonstrated that high concentrations of immunoreactive PHI occur in human intestine, especially in colon [16]. We have therefore chosen the human colon as a starting material for the isolation of human PHI. The results of the chemical assay revealed that a material, obtained as a side fraction during the purification of human VIP [21], contained a high concentration of a peptide with C-terminal methionine amide. This side fraction was expected to contain PHI, since it was prepared by similar purification procedures as those used for the isolation of PHI from porcine intestine [2]. However, unlike porcine PHI, the human material contained no peptide with a C-terminal isoleucine amide, but one with C-terminal methionine amide. It was noted that this human peptide was eluted at slightly different volumes in HPLC from those of porcine PHI (fig.2,3). The structural studies show this peptide to be a human variant of PHI. The structural differences between the porcine and human peptides are found at two of the 27 residue positions, an Arg/Lys replacement at position 12 and an Ile/Met replacement at position 27. These replacements can be explained genetically by single nucleotide exchanges. The small structural differences suggest that the two peptides probably have similar biological properties.

Authors in [22] recently reported the primary structure of a human VIP precursor, deduced from the cDNA sequence of human neuroblastoma cells, contained the sequences of both VIP and a PHI-like peptide, PHM-27. The primary structure of human PHI, isolated from human colon in this study, is identical to that of PHM-27, indicating that PHM-27 in the VIP precursor can indeed become human PHI during the post-translational processing.

In addition to human PHI we recently isolated PHI from bovine intestine (submitted). The structural difference between porcine and bovine PHI is only one position in the 27 residues: Phe/Tyr replacement at position 10. All presently known PHI structures are compared in fig.5. It remains to be determined whether the differences in primary structures of the PHI molecules result in any significant difference in biological and immunological properties.

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