Isolation of a brain peptide identical to the intestinal PHI (peptide HI)

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The isolation of a brain peptide identical to the intestinal peptide PHI (peptide HI) is described. The peptide was isolated from porcine brain extract using a chemical assay method based on its C-terminal isoleucine amide structure. The complete amino acid sequence of the peptide was found to be: His–Ala–Asp–Gly–Val–Phe–Thr–Ser–Asp–Phe–Ser–Arg–Leu–Leu–Gly–Gln–Leu–Ser–Ala–Lys–Lys–Tyr–Leu–Glu–Ser–Leu–Ile–NH_2. This sequence is identical to the intestinal peptide thus demonstrating PHI to be a brain–gut peptide. The role of PHI in the central nervous system as a neurotransmitter or neuromodulator is discussed.

Brain–gut peptide *Neuropeptide* *Glucagon–secretin family* *Amino acid sequence* *C-terminal amide* *Chemical assay*

1. INTRODUCTION

It has been well recognized that identical peptides may occur in brain and gut. To date, several such peptides have been isolated and shown to be identical in both tissues with respect to their amino acid sequences. These are: substance P [1], neurotensin [2], cholecystokinin octapeptide [3], somatostatin 28 [4–6], dynorphin [7–8] and the hydra head activator [9]. We have also shown that a brain peptide is identical to the vasoactive intestinal peptide (VIP) [10].

The intestinal heptacosapeptide PHI (peptide HI) was isolated in our laboratory based on its C-terminal isoleucine amide structure [11,12]. Subsequent investigation revealed that it is structurally similar to members of the secretin–glucagon family and exhibits biological activities similar to VIP [13–17]. We reported in [11] that porcine brain contains a peptide with C-terminal isoleucine amide and suggested the occurrence of PHI in both intestine and brain tissues. We report here the isolation of this peptide from porcine brain extracts. The amino acid sequence determination indicates that the brain peptide is indeed identical in respect to its amino acid sequence to the PHI of intestinal origin.

Abbreviations: PHI, peptide HI (the peptide having N-terminal histidine and C-terminal isoleucine amide); VIP, vasoactive intestinal peptide

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2. MATERIALS AND METHODS

Isoleucine amide was obtained from Vega Biochemicals and dansyl isoleucine amide was prepared from isoleucine amide by reaction with dansyl chloride [18]. Dansyl amino acids and phenylthiohydantoin amino acids were obtained from Calbiochem and Mann Research Laboratories, respectively. Dansyl chloride, phenylisothiocyanate, pyridine, butyl acetate, ethyl acetate, trifluoroacetic acid (TFA) were of se-

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quanal grade (Pierce Chemical Co.). Polyamide thin-layer plates were from Schleicher and Schüll. Acetic acid, ethanol and acetonitrile were of spectroscopic or HPLC grade and all other chemicals were of analytical grade and were used without further purification.

Peptide HI (PHI) was prepared from porcine intestine as in [12]. The chemical assay for PHI, amino acid analysis, N-terminal determination and TLC on silica gel layer were performed as in [12]. The methods of HPLC for separation of tryptic peptide fragments and subsequent microsequence analysis by an improved dansyl Edman technique were described in [19].

3. RESULTS

3.1. *Isolation procedure*

The details of the preparation of the methanol-soluble peptide concentrate (the starting material) from porcine brain extract were described in [10,20]. The methanol-soluble peptide concentrate (1.8 g) obtained from 400 kg porcine brain was subjected to gel filtration on Sephadex G-25 column. The PHI fractions, which also contained neuropeptide Y [20] and vasoactive intestinal peptide [10], were pooled and lyophilized. The lyophilized material (690 mg) was chromatographed on CM cellulose column (5 × 20 cm). The peptide was eluted with 0.01 M ammonium bicarbonate containing 0.025% mercaptoethanol. The fraction containing PHI was detected by the chemical assay and lyophilized. This procedure yielded 38 mg of the fraction containing PHI. The fraction was further purified by two successive HPLC on a reversed-phase silica gel column, μBondapak C-18 (Waters Ass.). The fraction was first eluted with 42% ethanol containing 0.2% acetic acid and 5 mM ammonium acetate under isocratic conditions (fig.1). This HPLC yielded a total of 0.8 mg of the lyophilized fractions containing PHI. The final purification was carried out in the second HPLC using a gradient elution system: 0.12% TFA/H_2O and 0.1% TFA/CH_3CN (fig.2). The peptide was found to be eluted in an identical position to the intestinal PHI. The peptide preparation (0.3 mg) was found to be homogeneous by amino acid analysis, N- and C-terminal determinations and by HPLC and TLC analysis.

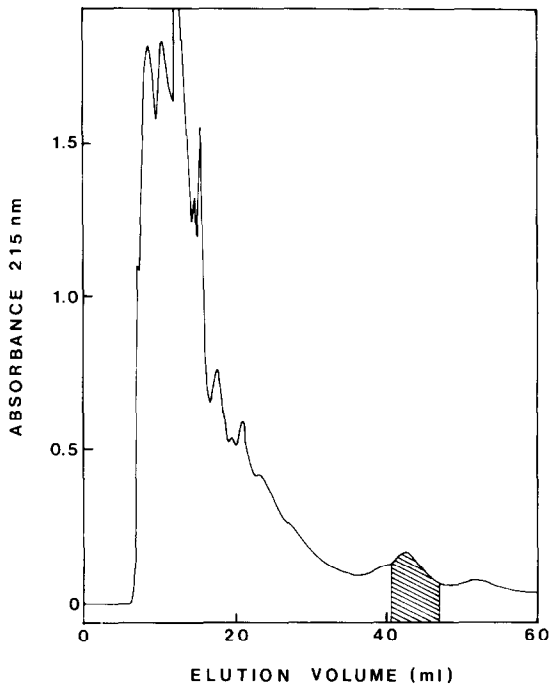

Fig.1. A typical HPLC elution profile of the PHI fractions (see text). The PHI fractions (4 mg for each run) were applied to a reversed-phase HPLC column (μBondapak C-18, 7.8 × 300 mm) and eluted with 42% ethanol/5 mM ammonium acetate/0.2% acetic acid at a flow rate of 2 ml/min under isocratic conditions. The peak fractions were collected and concentrated to 1/5th of the original volumes under reduced pressure. After the original volumes were restored by addition of water, the fractions were lyophilized and an aliquot of each was subjected to the chemical assay. The PHI peak is shown in oblique lines.

3.2. *Structural studies*

The results of amino acid analysis revealed that the brain peptide had an amino acid composition identical to that of the intestinal peptide (table 1). The N-terminal residue of the brain peptide was determined to be histidine, which is also identical to the intestinal PHI [12]. Further structural study, using a HPLC tryptic mapping technique, indicated that treatment of the brain peptide with trypsin yielded 5 fragments which were eluted in identical positions (fig.3) and had identical amino acid compositions (table 1) to the corresponding fragments of the intestinal peptide.

The amino acid sequences of tryptic fragments of the peptide were found to be:

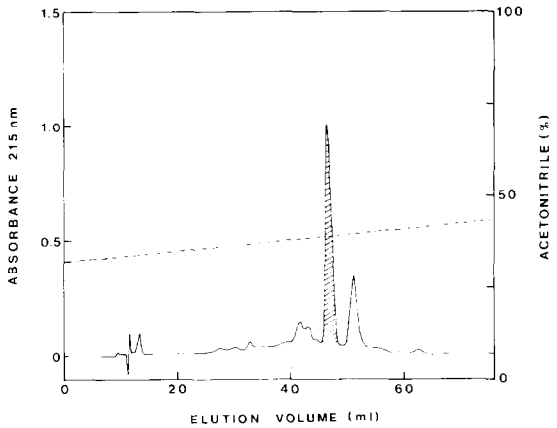

Fig.2. A HPLC elution profile of the PHI peak (fig.2). The PHI fraction (0.4 mg) was rechromatographed on a HPLC column (μBondapak C-18, 7.8 × 300 mm) using a linear gradient elution system (solvent A; 0.12% trifluoroacetic acid/water, solvent B; 0.1% trifluoroacetic acid/acetonitrile) at 2 ml/min. The PHI fraction (shown in oblique line) was lyophilized as in fig.2 and subsequently subjected to amino acid analysis and N- and C-terminal determinations.

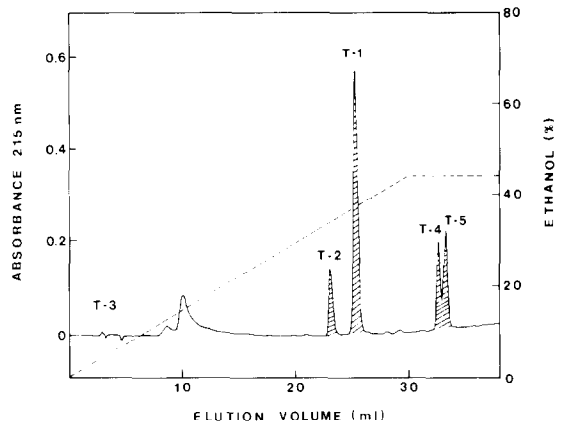

Fig.3. HPLC separation of tryptic fragments of brain PHI. Trypsin-treated PHI preparation (70 μg) was applied to a μBondapak C-18 column (3.9 × 300 mm). The tryptic peptides were separated at 1 ml/min using a linear gradient system of 0.05% acetic acid and 5 mM ammonium acetate (solvent A) and 80% ethanol containing 0.04% acetic acid and 5 mM ammonium acetate (solvent B, B; 0–55% for 30 min).

Table 1

Amino acid compositions and NH_2-termini of isolated tryptic fragments and whole molecule of brain PHI

Amino acid	T-1	T-2	T-3	T-4	T-5	Whole molecule
Ala	1.0 (1)	1.0 (1)				2.1 (2)
Arg	1.0 (1)					1.1 (1)
Asx	2.0 (2)					2.1 (2)
Glx		1.0 (1)		1.1 (1)	1.0 (1)	2.1 (2)
Gly	1.0 (1)	1.0 (1)				2.1 (2)
His	1.0 (1)					1.0 (1)
Ile				1.0 (1)	1.0 (1)	1.0 (1)
Leu		2.8 (3)		2.0 (2)	2.0 (2)	4.8 (5)
Lys		1.0 (1)	1.0 (1)	0.9 (1)		2.0 (2)
Phe	2.0 (2)					1.9 (2)
Ser	2.0 (2)	1.1 (1)		1.1 (1)	1.0 (1)	3.8 (4)
Thr	1.0 (1)					1.0 (1)
Tyr				1.0 (1)	1.0 (1)	1.0 (1)
Val	1.0 (1)					1.0 (1)
Total residues	12	8	1	7	6	27
NH_2-terminus	His	Leu	Lys	Lys	Tyr	His

T-1, His–Ala–Asp–Gly–Val–Phe–Thr–Ser–Asp–Phe–Ser–Arg;
T-2, Leu–Leu–Gly–Gln–Leu–Ser–Ala–Lys;
T-3, Lys;
T-4, Lys–Tyr–Leu–Glu–Ser–Leu–Ile–NH_2;
T-5, Tyr–Leu–Glu–Ser–Leu–Ile–NH_2.

Thus the complete amino acid sequence of the brain peptide should be:

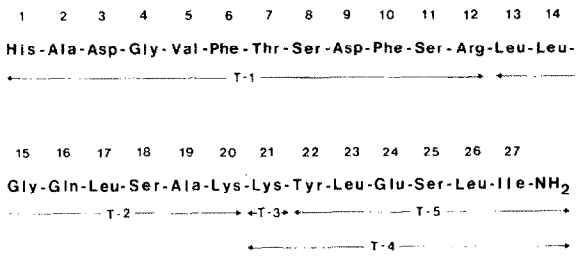

Fig.4. The amino acid sequence of brain PHI. The sequence of PHI was determined after separation of the tryptic fragments (T-1–T-5 in table 1) by HPLC using a modified dansyl-Edman technique [19].

His–Ala–Asp–Gly–Val–Phe–Thr–Ser–Asp–Phe–Ser–Arg–Leu–Leu–Gly–Gln–Leu–Ser–Ala–Lys–Lys–Tyr–Leu–Glu–Ser–Leu–Ile–NH_2 (fig.4).

This indicates that the peptide indeed has an identical amino acid sequence to intestinal PHI.

4. DISCUSSION

We reported the isolation and characterization of a novel intestinal peptide designated PHI (PHI-27) from porcine upper intestine in [12]. PHI is a linear chain peptide consisting of 27 amino acid residues and structurally similar to VIP, secretin, glucagon and the gastric inhibitory polypeptide. The peptide stimulates pancreatic endocrine secretion by releasing insulin and glucagon [14] and also stimulates pancreatic exocrine secretion [12,15,16]. PHI is capable of stimulating cyclic AMP production and inhibits the binding of VIP to its receptor [13]. PHI also inhibits fluid absorption and relaxes the wall of the gall bladder [17]. The physiological role of this peptide has not yet been established, but, it has been proposed to be a neurotransmitter or neuromodulator [21]. In this communication, we report that PHI is also present in the brain as well as in the intestine, demonstrating that it is a hitherto unknown gut–brain peptide. Immunoreactive PHI was reported in rat brain [22], the highest concentration being in the cortex (35 pmol/g) and hippocampus (35 pmol/g). Immunoreactive PHI was demonstrated in the external layer of the median eminence and PHI, not VIP, proposed to be responsible for the release of prolactin on the anterior pituitary [23]. Since PHI is present abundantly in the brain, this peptide may indeed have its role as a neurotransmitter or neuromodulator in the central nervous system.

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