

Peptide histidine isoleucine: a secretagogue in human jejunum

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SUMMARY Peptide histidine isoleucine (PHI) is a newly discovered peptide from porcine intestine, which has sequence homologies with VIP, an established intestinal secretagogue. To study the effects of PHI in human jejunum, natural porcine PHI was infused intravenously at 10.7 ± 1.7 pmol/kg/min (mean \pm SEM) in normal volunteers during steady state perfusion of the jejunum with an isotonic bicarbonate-electrolyte solution. Plasma PHI concentrations rose to 279 ± 26 pmol/l (mean \pm SEM) in the first 20 minutes of the infusion reaching 417 ± 45 at 40 minutes. At these concentrations PHI induced a net secretion of chloride and sodium and either decreased net absorption or increased net secretion of fluid and potassium, while bicarbonate transport remained unaffected. Peptide histidine isoleucine is a potent jejunal secretagogue in man.

Peptide histidine isoleucine has recently been isolated as a heptacosapeptide from porcine small intestine.^{1,2} This peptide (P) has an N-terminal histidine (H) and C-terminal isoleucine (I) amide and therefore has been given the acronym PHI. Remarkable sequence homologies exist between PHI and VIP, secretin, glucagon, and GIP, indicating that PHI belongs to this family of peptides.^{1,2}

The effects of PHI have been studied in a variety of species. It has been shown to stimulate pancreatic juice³ and amylase secretion⁴, and to be potent stimulus for cyclic-AMP production in a variety of tissues – namely, fat cells, gastric and intestinal epithelial cells,⁵ and pancreatic acinar cells though to have little effect on blood flow.⁶ It is reported to interact with a class of receptors which have a high affinity for VIP and a low affinity for secretin.⁴

We have recently developed a sensitive radio-immunoassay for PHI which shows no cross-reactivity with the other members of the glucagon-secretin family. In animal and human intestine PHI is distributed along the entire length of the gastrointestinal tract with highest concentrations in the colon. It is found in the submucosa of the gut and is present in brain and neural tissues⁷ suggesting a

neurotransmitter or neuromodulator role. More recently we have shown that infusion of PHI in pigs and rats induced secretion of intestinal fluid.^{8,9} Its effect on the human jejunum was investigated as an essential preliminary step before studies of its possible role in human diarrhoeal disorders.

Methods

Studies were performed in six healthy volunteers (three men, three women), mean age 26.5 years (range 23–35 years). Informed written consent was obtained and the studies were approved by the Hammersmith Hospital and Royal Postgraduate Medical School Research Ethics Committee.

After an overnight fast a triple lumen perfusion tube with a mercury bag at the tip was passed into the jejunum under fluoroscopic control so that the infusion port was at the ligament of Treitz, the proximal sampling site at 10 cm distally and the distal sampling site at 40 cm (10 cm mixing segment and 30 cm test segment). The jejunum was perfused at 10 ml/min (by a peristaltic pump) with an isotonic bicarbonate-electrolyte solution (37°C) containing Na^+ 135, K^+ 5, Cl^- 110, HCO_3^- 30 (mmol/l) and polyethylene glycol (PEG) 4000 0.5 g/l with ¹⁴C-PEG 4000 5 $\mu\text{Ci/l}$, as non-absorbable marker. The perfusate was continually gassed with 5% CO_2 and 95% O_2 . After a 40 minute equilibration period and a 40 minute first control period, pure natural porcine

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PHI in saline with 1% human serum albumin, was infused intravenously at a nominal dose of 10 pmol/kg/min for either 40 (six subjects) or 60 minutes (three subjects), followed by a 40 minute final control period. Limited supplies of pure PHI for human studies precluded the use of 60 minute infusions in all subjects. Regular aspiration from the two sampling sites was performed by hand, using 20 ml plastic syringes to obtain 4 ml from each site in each 10 minute collection period. Aspirates were collected on ice and samples for bicarbonate assay immediately covered with paraffin oil. Blood samples were collected in heparinised trasylol tubes (400 KIUm) at 10 minute intervals, centrifuged immediately and plasma stored at -20°C for subsequent PHI assays.

LABORATORY MEASUREMENTS

Intestinal samples

These were assayed the same day for ^{14}C -PEG 4000 using a Packard Tricarb 2660 liquid scintillation counter with external standard quench correction. Sodium and potassium were measured using a Corning 40S flame photometer, chloride using an automatic Bucheler-Cotlove chloridometer, Bucheler Instruments Inc. Fort Lee, NJ, USA, and bicarbonate by a Technicon auto-analyser method.

Radioimmunoassay

The development and validation of the radioimmunoassay for PHI has previously been reported.⁷ The coefficient of variation between assays was 15% and within assays 9%. Changes of 10 pmol/l plasma could be detected with 95% confidence at the most sensitive point of the curve – that is, the assay could detect changes of 2 fmol per tube.

Calculation and statistics

Net absorption or secretion of fluid (μl) and electrolytes ($\mu\text{mol}/\text{cm}/10$ min) were calculated using standard equations. Mean \pm SEM values were then determined for sequential 20 minute experimental periods. The significance between periods was

assessed by the paired Student's *t* test.

Results

PLASMA PHI CONCENTRATIONS

Measured infusion rates of natural porcine PHI were 10.7 ± 1.7 pmol/kg/min (mean \pm SEM). Table 1 shows plasma PHI concentrations before, during and after PHI infusion. Peptide histidine isoleucine was undetectable in the plasma in the first control period (detection limit 10 pmol/l), rose to 279 ± 26 pmol/l in the first 20 minutes of infusion and reached 417 ± 45 pmol/l at 40 minutes and 500 ± 144 at 60 minutes ($n=3$). After discontinuation of infusion PHI concentrations returned to 105 ± 25 in subjects receiving PHI infusion for 40 minutes and 123 ± 35 in subjects receiving PHI infusion for 60 minutes.

SYSTEMIC EFFECTS OF PHI

No significant untoward effects were observed though transient facial flushing was observed in one subject towards the end of the infusion. No changes in blood pressure or pulse rate occurred.

EFFECT OF PHI ON FLUID AND ELECTROLYTE MOVEMENTS

Table 2 shows the effects of PHI infusion on fluid and electrolyte movement in the jejunal test segment. Net absorption of fluid and electrolytes was observed in all subjects in the first control period. Peptide histidine isoleucine infusion induced inhibition of fluid absorption in the first 40 minute period ($n=6$) and fluid secretion ($n=3$) between 40 and 60 minutes while net sodium and chloride secretion (Figure) was observed between 20 and 40 minutes (in five of six subjects). Potassium absorption was inhibited in all subjects, and no effect on bicarbonate transport were observed. ^{14}C -PEG concentrations in fluid entering the test segment were 1547 ± 187 (SEM) dpm/200 μl in the control period and 1625 ± 281 dpm/200 μl during PHI infusion (see discussion).

The composition of the fluid entering the test segment in control and PHI infusion periods is shown in Table 3.

Table 1 Peptide histidine isoleucine plasma concentrations before, during and after PHI infusion (pmol/l)

First control period		PHI infusion period			Final control period (time after cessation of PHI)	
-40' (n=6)	+20' (n=6)	+20' (n=6)	+40' (n=6)	+60' (n=3)	+20'	+40'
undetectable	undetectable	279 ± 26	417 ± 45	500 ± 144	A 105 ± 25	undetectable
					B 123 ± 45	undetectable

Values are mean \pm SEM. Detection limit 10 pmol/l.

A subjects receiving 40' PHI infusion ($n=3$).

B subjects receiving 60' PHI infusion ($n=3$).

Table 2 Effect of PHI infusion on jejunum

	First control period			PHI infusion period		Final control period (time after cessation of PHI)	
	-40	-20	+20	+40	+60 (n=3)	+20	+40
Water (ml/cm/10 min)	-2.0±0.6	-1.5±0.3	-0.41±0.2*	-0.09±0.2*	+0.39±0.3	-1.0±0.3	-1.3±0.3
Chloride (μmol/cm/10 min)	-103.0±15.0	-105.0±13.0	-0.8±12.3†	+27.5±2.04†	+101.0±18.0	-33.4±16.3	-105.0±30.8
Sodium (μmol/cm/10 min)	-117.0±23.0	-92.0±23.0	-28.3±25.8*	+18.3±25.8*	+72.0±46.0	-73.7±11.0	-94.0±21.0
Potassium (μmol/cm/10 min)	-12.0±2.5	-11.0±2.2	-5.7±0.9*	-4.4±1.3*	-0.9±2.0	-7.5±1.6	-12.2±3.26
Bicarbonate (μmol/cm/10 min)	-21.0±5.5	-21.0±5.3	-25.3±6.7	-49.6±21.5	+6.6±12.0	-49.5±10.0	-53.0±83.0

Negative signs indicate absorption and positive signs secretion.

* $p < 0.05$. † $p < 0.005$ (control periods vs PHI infusion period).

Numbers are mean ± SEM of six observations.

Discussion

Intravenous infusion of PHI at doses achieving plasma concentrations in the range 400–500 pmol/l induced net secretion of sodium and chloride and either secretion or a reduction in net absorption of fluid and potassium in human jejunum. These

observations confirm that PHI is a potent secretagogue in human small intestine. With triple lumen perfusion techniques, the possibility exists of contamination of the test segment with digestive secretions from above. In these studies the volume of fluid entering the test segment, calculated from ^{14}C -PEG concentrations, was marginally less during PHI infusion than during the control period. In separate studies (unpublished) we have shown that PHI has no effect on pancreatic, trypsin, and bicarbonate secretion in man in contrast with reports from animal studies,³ but reduces bile acid output into the duodenum. It is not likely therefore that entry of digestive secretions from above could be responsible even in part for the secretory effects and further studies in rat and porcine intestine^{8,9} using closed loops of small intestine confirm that PHI is a potent secretagogue.

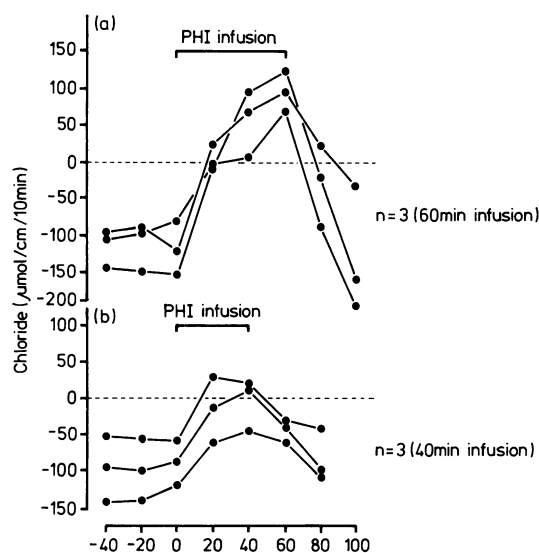


Figure Effects of PHI infusion on net chloride movements in six subjects. Upper panel shows results from three subjects receiving PHI over 60 minutes and lower panel shows results from three subjects receiving PHI over 40 minutes. Net chloride secretion was observed in five out of six subjects (negative values denote absorption, positive values denote secretion).

Table 3 Composition of the fluid entering the test segment in control and PHI infusion periods

	Initial control period	PHI infusion	Final control period
Fluid (ml/10 min)	25.7±7.6	27.3±7.2	27.2±11.0
^{14}C -PEG 4000 (dpm/200 μl)	1547.0±187.0	1625.0±281.0	1586.0±235.0
HCO_3^- (mmol/10 min)	2.3±0.5	2.5±0.6	3.0±0.6
Na^+ (mmol/10 min)	12.0±1.6	11.8±1.5	11.9±1.4
Bile acid (μmol/10 min)	299.0±90.0	191.0±33.0*	305.0±98.0

Numbers are mean ± SEM of six observations.

* $p < 0.01$ control vs PHI infusion periods.

Peptide histidine isoleucine shows considerable sequence homology with VIP and GIP both of which are established secretagogues in man,^{10 11} while it also has structural similarities to glucagon and secretin which are also intestinal secretagogues.¹²⁻¹⁵ In receptor studies with pancreatic acinar cells PHI appeared to interact with receptors having high affinity for VIP and low affinity for secretin⁴ so that its functional activity, which closely resembles that of VIP, might relate to that part of the molecule which binds to such receptors.

Like VIP, PHI activates adenylyl cyclase in a variety of tissues including intestinal epithelial cells,⁵ however, the potential for activating the adenylyl-cyclase-cAMP system does not necessarily imply a cyclic-AMP mediated secretory mechanism. For example, VIP may induce intestinal secretion without measureable changes in mucosal cAMP concentrations in animals¹⁶ or man,¹⁷ and plasma concentrations which produce intestinal fluid secretion are well below those capable of activating adenylyl cyclase in *in vitro* systems.¹⁸⁻²⁰ VIP may affect fluid transport *via* alterations in mucosal blood flow,^{21 22} a mechanism apparently not open to PHI.⁶ The as yet unresolved question about the mode of action of VIP may well apply to PHI *in vivo*.

Most patients with the Verner-Morrison syndrome who have pancreatic apudomas or ganglioneuromas have high circulating concentrations of VIP²³⁻²⁵ and it is generally believed that this peptide mediates the intestinal secretion of water and electrolytes which account for the secretory diarrhoea. Peptide histidine isoleucine appears to be elaborated by pancreatic or neuroendocrine tumours in those patients who have high circulating VIP concentrations.²⁶ The possibility that PHI may be raised in patients who are said to have the pseudo Verner-Morrison syndrome,²⁵ (with normal VIP concentrations), warrants investigation.

Our current studies do not provide information about the relative secretory bioactivity of PHI and VIP. In human studies Krejs showed intestinal secretion in response to VIP infusions with plasma concentrations of 400 pmol/l,¹⁰ comparable with the levels achieved in this study with PHI. The threshold molar concentrations for secretion of either peptide in man is not known though diarrhoea occurs with plasma concentrations in excess of 60 pmol/l in patients with vipomas.²⁵ Formal dose response studies must be performed to fully address these questions.

The anatomical location of PHI to nerves of the submucosal region in the gut²⁶ suggests that this peptide may subserve a neuroregulatory or neuro-modulatory function. Its role in normal physiology

and the possible relevance to human diarrhoeal disorders should now be investigated.

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