Alimentary tract and pancreas

Effect of peptide histidine isoleucine on water and electrolyte transport in the human jejunum

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SUMMARY Peptide histidine isoleucine, a 27 amino acid peptide with close amino acid sequence homology to vasoactive intestinal peptide and secretin, is distributed throughout the mammalian intestinal tract, where it has been localised to intramural neurones. An intestinal perfusion technique has been used to study the effect of intravenous peptide histidine isoleucine (44.5 pmol/kg/min) on water and electrolyte transport from a plasma like electrolyte solution in human jejunum *in vivo*. Peptide histidine isoleucine infusion produced peak plasma peptide histidine isoleucine concentrations in the range 2000–3000 pmol/l, flushing, tachycardia and a reduction in diastolic blood pressure. Peptide histidine isoleucine caused a significant inhibition of net absorption of water, sodium, potassium and bicarbonate and induced a net secretion of chloride, these changes being completely reversed during the post-peptide histidine isoleucine period. These findings suggest that endogenous peptide histidine isoleucine may participate in the neurohumoral regulation of water and electrolyte transport in the human jejunum.

Peptide histidine isoleucine, isolated originally from mammalian small intestine, is a 27-amino acid peptide having close amino acid sequence homology to vasoactive intestinal peptide and secretin.^{1–3} Peptide histidine isoleucine-like immunoreactivity has recently been shown in intestinal intramural neurones of the dog, pig and mouse⁴ and also throughout the human gastrointestinal tract.⁵ The inhibitory effect of peptide histidine isoleucine on fluid absorption in pig⁶ and rat⁷ small intestine and in guinea pig gall bladder⁸ has led us to investigate its effect on water and electrolyte transport in the human jejunum.

Methods

SUBJECTS

Six healthy volunteers (three men, three women), each aged 21 years, gave written informed consent for the study which was approved by the Ethical Committee of King's College Hospital, London.

INTESTINAL PERFUSION

After an eight hour fast, each subject swallowed a double lumen intestinal perfusion tube, incorporating a proximal occluding balloon, a 30 cm test segment and a mercury bag.⁹ The tube was positioned under fluoroscopic control such that the balloon was situated at the ligament of Treitz with the infusion orifice located in the first 5 cm of jejunum. Using a peristaltic pump, a plasma like electrolyte solution at 37°C was perfused through the infusion orifice at a rate of 15 ml/min. The solution contained (mmol/l): Na, 135; K, 5; Cl, 110; HCO₃, 30; polyethylene glycol (PEG), 2.5 g/l and 1 µCi/l of [¹⁴C]PEG as a non-absorbable marker. The solution was continuously oxygenated throughout each experiment with 95% O₂–5% CO₂. After a 30 minute equilibration period, during which the aspirates were discarded, serial 10 minute aspirates were collected by siphonage. Aliquots were taken for immediate bicarbonate estimation and samples for determination of other electrolyte concentrations were stored at –20°C before analysis.

COURSE OF PERFUSION EXPERIMENTS

Natural porcine peptide histidine isoleucine

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Received for publication 27 September 1983

(Gastrointestinal Hormone Laboratory, Karolinska Institute, Stockholm)³ was dissolved in sterile isotonic sodium chloride containing 0.5% human serum albumin immediately before use in order to minimise peptide degradation and adherence to the glass and plastic used in its preparation and intravenous administration. The peptide used was highly purified, containing only trace amounts (<1%) of porcine secretin. Peptide histidine isoleucine was infused into a peripheral vein in the left arm of each subject at a rate of 44.5 pmol/kg/min during the second hour of the perfusion period. The peptide histidine isoleucine vehicle (sodium chloride containing human serum albumin) was infused intravenously for the first (pre-peptide histidine isoleucine) and third (post-peptide histidine isoleucine) hours. The infusion rate for each period was 50 ml/h. Pulse and blood pressure were recorded at 15 minute intervals throughout each experiment. Venous blood samples were collected at 15 minute intervals from an indwelling cannula into heparinised tubes containing 0.2 ml Trasylol/10 ml blood, centrifuged immediately, and the plasma stored at -20°C.

ANALYSIS OF SAMPLES AND CALCULATIONS

The concentration of sodium, potassium, chloride, bicarbonate, and [¹⁴C]PEG was determined in each aspirate. [¹⁴C]PEG was measured in an LKB 1210 Ultrabeta liquid scintillation counter.¹⁰ Sodium and potassium concentrations were measured using an EEL 227 flame photometer (Evans Electroselenium Ltd, Halstead, Essex) and chloride by an EEL chloridometer. Bicarbonate concentrations were derived from measurement of pCO₂ using an automated Corning 965 CO₂ analyser (Corning Ltd, Halstead, Essex). Absorption rates of water and solutes from the test segment were calculated from their measured concentrations in the perfusate and aspirates.¹¹ Net absorption (+) indicates a net transfer of water or solute from the lumen; net secretion (-) indicates net transfer of water or solute into the lumen.

PEPTIDE HISTIDINE ISOLEUCINE RADIOIMMUNOASSAY

Plasma samples for determination of peptide histidine isoleucine were stored at -20°C until assayed in one batch. The specific radioimmunoassay used showed no cross-reactivity with VIP, secretin, glucagon or gastric inhibitory polypeptide and had a lower limit of detection of approximately 10 pmol/l.⁵

STATISTICAL METHODS

Statistical analyses were performed using the paired Student's *t* test.¹²

Results

PLASMA PEPTIDE HISTIDINE ISOLEUCINE CONCENTRATIONS

Plasma peptide histidine isoleucine concentrations rose from 20–110 pmol/l in the pre-peptide histidine isoleucine period to 2000–3000 pmol/l during peptide histidine isoleucine infusion (Figure). Thereafter, plasma peptide histidine isoleucine concentrations decreased, returning to pre-peptide histidine isoleucine levels 60 minutes after discontinuation of the infusion.

EFFECT OF PEPTIDE HISTIDINE ISOLEUCINE ON WATER AND ELECTROLYTE TRANSPORT

Peptide histidine isoleucine caused a significant reduction in the net jejunal absorption of water, potassium and bicarbonate, virtually abolished net sodium absorption and reversed the direction of net chloride absorption to a net secretion (Table). These effects of peptide histidine isoleucine developed within 10 minutes of starting peptide histidine isoleucine infusion and reversed equally rapidly after its discontinuation. For each of the pre-peptide histidine isoleucine, peptide histidine isoleucine and post-peptide histidine isoleucine periods, net transport of water and ions achieved steady state values after an equilibration period of 30 minutes. Therefore, the results represent the mean net transport values calculated from analysis of three consecutive aspirates collected during 30–60 minutes of each of the three study periods.

Figure 1 Plasma peptide histidine isoleucine concentrations in pmol/l are represented on the vertical axis and time in minutes on the horizontal. Peptide histidine isoleucine was infused at a rate of 44.5 pmol/kg/min. Results are the means $\pm$ SEM for six subjects.

Table Effect of peptide histidine isoleucine on jejunal water and ion transport

	Pre-peptide histidine isoleucine	Peptide histidine isoleucine (44.5 pmol/kg/min)	Post-peptide histidine isoleucine
Net water transport (ml/30 cm/h)	192.3±29.4	48.7±13.7*	181.7±27.7
Net sodium transport (mmol/30 cm/h)	23.5±4.6	0.5±2.4*	20.5±4.3
Net potassium transport (mmol/30 cm/h)	1.12±0.15	0.51±0.10†	1.10±0.17
Net chloride transport (mmol/30 cm/h)	10.6±2.7	-6.3±2.3†	9.3±2.0
Net bicarbonate transport (mmol/30 cm/h)	12.3±2.3	6.3±2.0*	12.2±2.6

+ = absorption, - = secretion.

p values refer to the difference between the pre-peptide histidine isoleucine and peptide histidine isoleucine periods.

Numbers are mean ± SEM of six observations.

* p<0.01; † p<0.02.

SYSTEMIC EFFECTS OF PEPTIDE HISTIDINE ISOLEUCINE

Peptide histidine isoleucine caused facial flushing in all six subjects. Mean pulse rate increased from 73 ± 3 beats/min in the pre-peptide histidine isoleucine period to 98 ± 6 beats/min during peptide histidine isoleucine infusion ($p < 0.01$) and returned to baseline values (71 ± 4 beats/min) in the post-infusion period. Although there was no change in mean systolic blood pressure during the three hour study period (119 ± 3 , 119 ± 3 , 120 ± 4 mmHg respectively), mean diastolic blood pressure fell significantly from 77 ± 4 mmHg in the pre-peptide histidine isoleucine period to 68 ± 4 mmHg during peptide histidine isoleucine infusion ($p < 0.01$), returning to basal values (78 ± 4 mmHg) in the post-peptide histidine isoleucine period. All the systemic effects appeared and resolved within five minutes of starting or discontinuing peptide histidine isoleucine infusion. No subject developed diarrhoea during the study.

Discussion

Peptide histidine isoleucine, infused at a rate of 44.5 pmol/kg/min, produced plasma concentrations of 2000–3000 pmol/l, caused a marked inhibition of net jejunal water, sodium, bicarbonate and potassium absorption and converted chloride absorption to secretion. These changes in water and solute movement were rapidly reversible, absorption values returning to pre-peptide histidine isoleucine levels within 30 minutes of termination of the infusion.

The effects of peptide histidine isoleucine in human jejunum are similar to those previously reported in the small intestine of the pig⁶ and rat,⁷ and in guinea pig gall bladder.⁸ Similar changes in water and ion transport in human jejunum were produced by infusion of VIP.^{13,14} While peptide histidine isoleucine appears to be less potent than VIP in the human jejunum, studies in guinea pig gall bladder have shown that the two peptides are equipotent inhibitors of fluid absorption.^{8,15}

Secretagogues may influence jejunal water and electrolyte absorption by a variety of mechanisms. Vasoactive intestinal peptide has been reported to inhibit both active bicarbonate absorption and passive movement of sodium chloride and also to stimulate active chloride secretion.¹⁴ These changes in ionic movements result in the observed net reduction of salt and water absorption induced by VIP. Some of the effects of peptide histidine isoleucine, like those of VIP and secretin, may be mediated *via* increased intracellular cyclic AMP. Both peptide histidine isoleucine and VIP are thought to act *via* cyclic AMP to cause amylase secretion in guinea pig pancreas,¹⁶ while in rat intestinal epithelial cell membranes, peptide histidine isoleucine, in common with VIP and secretin, stimulates cyclic AMP production.¹⁷ It remains to be established whether peptide histidine isoleucine, as has been suggested for VIP,¹⁸ increases intracellular cyclic AMP, resulting in an inhibition of sodium chloride absorption¹⁹ and a stimulation of active chloride secretion^{20,21} across the mucosal membrane. Further studies are required to evaluate the effect of peptide histidine isoleucine on specific transport mechanisms. The tachycardia, hypotension and flushing observed in the subjects confirm that peptide histidine isoleucine, like VIP, is a vasodilator, although, in their study, Lundberg and Tatemoto found that peptide histidine isoleucine was about 1000-fold less potent in this respect than VIP.²² The rapid onset and offset of the haemodynamic changes is consistent with the short half-life of the peptide, which has been calculated to be about four minutes when administered intravenously to man.²³ The vasoactive properties of VIP and related peptides may also contribute to their effects on water and electrolyte transport, as ileal fluid secretion induced by VIP is associated with a reduction in mucosal blood flow.²⁴ The demonstration, however, that both peptide histidine isoleucine⁸ and VIP¹⁵ stimulate fluid secretion in the guinea pig gall bladder *in vitro*, where haemodynamic effects are eliminated, indicates that both peptides act to directly stimulate fluid secretion.

In view of the dual brain gut distribution of peptide histidine isoleucine²⁵ and its localisation in

neurones,⁴ it has been proposed that this peptide acts as a neurotransmitter or neuromodulator.^{5,25} In the present study, peptide histidine isoleucine has been administered intravenously in a sufficiently high dose to achieve plasma concentrations which influence jejunal water and electrolyte transport. It should be stressed that peptide histidine isoleucine is not a circulating hormone and although the findings of this study suggest that endogenous peptide histidine isoleucine may participate in the neurohumoral regulation of water and electrolyte transport in the human jejunum, a definitive role for the peptide in this regard remains to be established.

It is now generally accepted that the watery diarrhoea which is the predominant feature of the Verner-Morrison²⁶ or pancreatic cholera²⁷ syndrome is mediated mainly by VIP.²⁸⁻³¹ A recent study has shown, however, that some patients with this condition have raised plasma peptide histidine isoleucine concentrations, which suggests that peptide histidine isoleucine, in addition to VIP, may contribute to the diarrhoea.³² The findings of the present study showing that peptide histidine isoleucine inhibits water and electrolyte absorption in man lend some support to this suggestion. The plasma concentrations of peptide histidine isoleucine achieved in the present short term study, however, were 50-fold higher than those reported in patients with the watery diarrhoea syndrome.³² The absence of diarrhoea in the subjects in the present study may be attributed to the short duration of the peptide histidine isoleucine infusion, as a previous study in man using intravenous VIP, showed that the infusion had to be continued for four to five hours in order to produce watery diarrhoea.³³ The contribution of peptide histidine isoleucine to the pathogenesis of the diarrhoea in this syndrome is still to be determined.

KJM is in receipt of a Medical Research Council Training Fellowship.

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