time twice that of the 2nd transit cell compartment. Thus, the 3rd peak would be expected to contain cells that in fact were undergoing their 2nd division (in which case their cell cycle time would be about 180 h) as well as some cells undergoing their 3rd division.

The model which best fitted all the data was one with about 12% of the basal cells being stem cells (with a cell cycle time of 180 h). The remainder of the basal layer consisted of transit proliferative cells and cells awaiting migration (post-mitotic?). The model, together with other considerations, suggested that there might be 3 transit divisions with the cell cycle time in the 1st transit population similar to that of the stem cell (i.e. 180 h) while for the following 2 transit divisions the cell cycle time would be about 90 h. The average basal layer residence time of the post-mitotic cells was concluded to be 45 h with a half-life of 30 h[16,17].

By about the 3rd, or 4th day after labeling some labeled cells in the granular layer would begin nuclear degradation which could release radioactive precursors that might be reutilized. This might result in the appearance of some new weakly labeled cells. This process has not been considered in the discussion above. The presence of clear labeled mitoses at the time of the peaks in LI, and the occurrence of labeled pairs, triplets, quads etc. at times that correspond to the peaks in LI suggests that reutilization alone is not likely to account for the appearance of the peaks in labeling.

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Infusion of a novel peptide, PHI, in man. Pharmacokinetics and effect on gastric secretion

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Summary. PHI, infused in man, achieved plateau plasma levels of 297 pmoles/l. The plasma half life was 3.1 min, metabolic clearance rate was 16.4 ml/kg/min and estimated volume of distribution was 73.2 ml/kg. No subjective side effects were noted during the infusion and there was no significant alteration in submaximal pentagastrin stimulated gastric acid or pepsin secretion.

Tatemoto and Mutt have recently isolated a 27 amino-acid peptide from porcine intestine[3,4]. This peptide has been given the abbreviated name PHI, referring to the peptide (P) having N terminal histidine (H) and C terminal isoleucine amide (I). PHI has sequence homologies with members of the glucagon-secretin family and has thus been included in this family of peptides[3,4].

There is only limited information regarding the biological activity of PHI and its physiological role in man has not yet been defined. Its in vitro actions so far are also shared by other members of the glucagon-secretin family, for example stimulation of pancreatic exocrine secretion[5], activation of isolated acinar cells[6] and release of insulin and glucagon from isolated rat pancreas[7]. Recently PHI has been shown to have powerful effects on the stimulation of intestinal secretion and thus mimics actions of vasoactive intestinal peptide (VIP)[8,9].

This study was designed to investigate the pharmacokinetics of infused natural PHI in man, and determine its effect on gastrointestinal hormones in vivo, and monitor its effects on gastric secretion of pepsin and acid.

Methods. Highly purified PHI was isolated at the Karolinska Institute. It was prepared immediately before use, being dissolved in 0.9% sterile saline containing 1% human serum albumin.

PHI was measured by a recently developed radioimmunoassay. Briefly, the antibody was raised to porcine PHI coupled to bovine serum albumin (BSA) with glutaraldehyde, and was used at a final dilution of 1/10,000. The iodinated (^{125}I) PHI was prepared using the Iodogen method. The assay did not significantly crossreact with other members of the glucagon-secretin family, and could detect changes of 10 pmoles/l plasma with 95% confidence[10].

Plasma taken before and during the infusion of PHI was also assessed for concentration of pancreatic polypeptide, motilin, insulin and pancreatic glucagon[11].

Five healthy male subjects (aged 24–31) were studied. Full

informed written consent was obtained for each subject and the experiment had prior approval from the Royal Postgraduate Medical School and Hammersmith Hospital Research Ethics Committee.

After an overnight fast a modified Anderson tube (AN 10. H.W. Anderson Inc. NY, USA) was positioned in the stomach. Throughout the procedure, phenol red (0.15%) a non absorbable marker was infused intragastrically at a rate of 12 ml/h via a separate polyvinyl catheter attached to the gastric tube.

Gastric juice was aspirated by continuous suction and pooled in 10 min collections. An indwelling catheter was inserted into the antecubital vein of one arm for blood sampling and 2 cannulae were inserted into the other arm for infusion of pentagastrin and PHI. After a 20-min period of basal gastric juice collection, pentagastrin was infused i.v. for 120 min at a dose of 0.1 µg/kg; this dose was chosen to achieve submaximal stimulation of acid and pepsin.

40 min after starting the pentagastrin infusion, PHI was infused in additon, at a rate of 4.7 pmoles/kg/min for a further 40 min. The pentagastrin infusion was then continued alone for a final 40 min.

Two blood samples were taken before starting the infusion, and further samples were taken 10, 30, 35 and 40 min after the PHI infusion began. On stopping the infusion, blood samples were taken at 1, 2, 3, 4, 5, 10 and 15 min. Blood was taken into chilled lithium heparin tubes containing 400 kallikrein inhibitory units of aprotinin (trasylol) per ml and immediately centrifuged. Plasma was stored at −20 °C before assay. A sample of the infusate was also collected into the subjects own basal plasma for assay.

Gastric aspirates were analyzed for volume, acidity (electrometric autotitration against 0.1 moles/l sodium hydroxide to end point pH 7.0) and pepsin ('Autoanalyzer' hemoglobin substrate method). Gastric aspirates were also analyzed for sodium content and phenol red to allow correction for duodenal reflux and pyloric loss. The outputs in the last 2 consecutive 10 min collections of each 40-min period were added and trebled to express acid secreted in mmoles/h and pepsin secretion in Ansons/h.

The results are expressed as mean and SEM. Analysis of the effect of PHI infusion was performed using a paired t-test for each individual, comparing the acid output during the pentagastrin infusion before and after cessation of the PHI with that during the PHI infusion.

The calculation of the metabolic clearance rate and the apparent distribution space were based on the plateau principle[12].

Results. 1. Vascular effects. The infusion of PHI caused no subjective effects. No change was noted in pulse rate or blood pressure during the infusion, and no cutaneous flushing was observed.

2. Infusion dosage and plasma characteristics. A calculated rate of infusion of 5 pmoles/kg/min was given i.v. Analysis of the infusate showed that the measured rate of infusion was 4.7±0.6 pmoles/kg/min showing minimal losses of PHI during preparation.

A plateau level of PHI in plasma of 297±46 pmoles/l was achieved. After stopping the infusion (fig. 1), the plasma levels of PHI rapidly fell with an apparently 1st order course of disappearance, the calculated half life of decay being 3.1±0.5 min (fig. 2). The metabolic clearance rate was 16.4±1.7 ml/kg/min and the apparent distribution space was thus 73.2±16 ml/kg.

3. Effect of PHI on gastric secretion of pepsin and acid. Submaximal stimulation of secretion using a low dose infusion of pentagastrin achieved a mean acid secretion of 30.6±1.4 mmoles/h in the 20 min prior to the PHI infusion. During the infusion of PHI there was an insignificant rise in acid secretion to a mean of 31.7±4.0 mmoles/h for the last 20 min and after stopping the PHI infusion, acid secretion remained at 30.3±4.8 mmoles/h (table).

Secretion of pepsin during pentagastrin stimulation was 3.37±1.6 Ansons/h. There was no significant change during the period of PHI infusion, the mean being 3.68±1.85 Ansons/h.

4. Effect of PHI infusion on plasma gut hormones. There was no significant alteration in plasma levels of insulin, pancreatic glucagon, PP or motilin during the infusion of PHI.

Discussion. PHI has remarkable amino acid sequence homologies with all members of the secretin family of peptides in particular with VIP, there being 13 identical positions between PHI and VIP in their 27 amino-acid residues. Human PHI appears to be immunochemically very similar but not identical to porcine PHI and they are indistinguishable by gel chromatography. It is noteworthy that the closely related peptide VIP does not vary in its human and porcine sequence. PHI has been demonstrated

Effect of PHI on pentagastrin stimulated gastric acid output

Patient No.	Acid output mmoles/h Pentagastrin alone	Pentagastrin and PHI	Pentagastrin alone after PHI
1	30.6	35.4	34.3
2	31.5	33.8	31.4
3	28.2	30.3	25.9
4	34.8	33.8	34.8
5	28.2	25.5	24.5

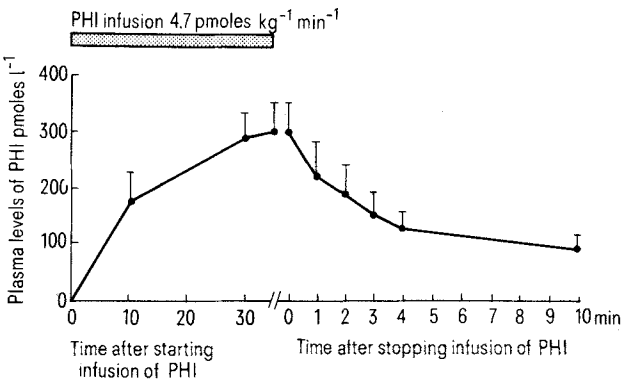

Figure 1. Plasma immunoreactive PHI in 5 volunteers during i.v. infusion of PHI at a rate of 4.7±0.6 pmoles/kg/min. Data points represent means and SEM of incremental post infusion values.

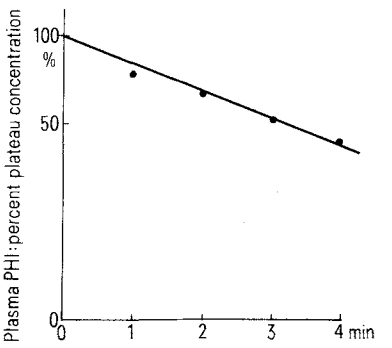

Figure 2. Rate of disappearance from the circulation of immunoreactive PHI after i.v. infusion of 4.7 pmoles/kg/min for 35 min in 5 subjects. The mean concentrations over 10 min before stopping the infusion were taken as 100% after subtracting basal PHI.

in human intestine by radioimmunoassay[10] where it is present in highest concentration in the lamina propria and muscularis externa. This peptide has also been demonstrated in brain[13] suggesting that PHI may act as a neurotransmitter or neuromodulator peptide. The plasma half life of PHI was calculated to be 3.1 min, similar to the reported plasma half life of VIP of 1.03 min[14]. The relatively short half life of PHI may possibly be more in favor of PHI being a neurotransmitter rather than a circulating hormone, being considerably shorter, for example, than the hormonal peptides, GIP and glucagon.

The plasma levels of PHI achieved during this study are comparable to the level at which the very similar peptide VIP results in facial flushing, hypotension and tachycardia[15]. No such effects were observed during the PHI infusion, suggesting that these 2 peptides, despite having similar sequences alter either in their biological activities or potency. At the plasma levels achieved during this study, no effect was noted on secretion of gastric acid and pepsin, and this contrasts with the findings on animal studies on the effects of the members of this family of peptides. In addition, infusion of PHI in man did not effect release of pancreatic glucagon or insulin in this study, and this contrasts with the stimulation observed in the isolated rat pancreas[7].

As this is the 1st study in which porcine PHI has been infused in man, there is no data on its possible other bioactivity. It is possible that the dose of PHI infused may have been too low to produce an effect. The dose however, probably produced pharmacological blood levels as normal basal levels of PHI in healthy controls are less than 20 pmoles/l, and the plateau levels achieved during the infusions reported here were more than 10 times this level. On the other hand, PHI may be a neurotransmitter or neuromodulator rather than a circulating hormone to be released by local nerves in the gastric mucosa. It is thus possible that the local cellular concentration of PHI may be much higher than that achieved after the exogenous PHI infusion in this study.

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Dopamine vasodilates human cerebral artery

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Summary. In human large pial arteries, dopamine-induced relaxations appear to be mediated via dopaminergic receptors, and predominate over contractions mediated via alpha-adrenoceptors.

Regional and species differences in the response of the vasculature to dopamine have been demonstrated in vivo and in vitro[1,2]. These differences are mainly associated with variable magnitudes of activation of postsynaptic dopamine receptors and alpha-adrenergic receptors in the vascular smooth muscle. Isolated mesenteric, renal, splenic and coronary arteries from dogs and rabbits[2-7], as well as isolated dog and cat cerebral arteries treated with high concentrations of alpha-adrenergic antagonists, respond to dopamine by relaxation, possibly mediated via dopamine receptors[8,9]. However, an analysis of dopamine action has not been made in primate cerebral arteries, despite evidence indicating the effectiveness of dopamine in treating acute brain ischemia[10]. The present study was undertaken to compare the effect of dopamine and norepinephrine in isolated human cerebral arteries and to clarify the mechanism of dopamine action.

Four basilar, 5 middle cerebral and 3 vertebral arteries were obtained during autopsy of 4 patients (13-, 49-, 55- and 71-year-old males). The arteries were helically cut into strips, approximately 20 mm long. The specimen was vertically fixed under a resting tension of 2 g in a muscle bath containing modified Ringer-Locke solution, which was maintained at 37 ± 0.3 °C and was aerated with a mixture of 95% O_2 and 5% CO_2. Detailed procedures have been described in an earlier report[11]. Preparations were allowed to equilibrate for 60 to 90 min, before the start of experiments. Cumulative dose-response curves for dopamine and norepinephrine were obtained.

Human cerebral arteries under resting conditions responded with a slight contraction to high concentrations of dopamine (2×10^{-5} to 10^{-4} M). However, when the arteries were contracted partially with prostaglandin (PG) $F_{2\alpha}$ or serotonin, dopamine (5×10^{-8} to 5×10^{-6} or 2×10^{-5} M)