Effects of vasoactive intestinal peptide and pancreatic polypeptide in rabbit intestine

M CAMILLERI, B T COOPER, T E ADRIAN, S R BLOOM, AND V S CHADWICK

From the Gastroenterology and Endocrinology Units, Department of Medicine, Hammersmith Hospital, Royal Postgraduate Medical School, London

SUMMARY The effects of porcine vasoactive intestinal peptide (VIP) and bovine pancreatic polypeptide (PP) on jejunal, ileal, and colonic fluid transport were studied in the rabbit. VIP produced secretion in the small intestine (jejunum > ileum) but did not affect absorption in the colon. PP had no secretory effects in jejunum, ileum, or colon. The small intestinal secretion induced by VIP was not associated with raised cAMP concentrations in the mucosa; this suggests that the secretory effects of VIP in vivo are mediated by a mechanism other than stimulation of adenylate cyclase.

Pancreatic tumours associated with the watery diarrhoea (WDS) or Verner Morrison syndrome may synthesise both vasoactive intestinal peptide (VIP) and pancreatic polypeptide (PP). VIP and PP have been proposed as the secretagogues responsible for the intestinal hypersecretory state in this syndrome. VIP infusions caused diarrhoea in pigs and intestinal secretion in dogs thus demonstrating its powerful secretory activity, while PP has recently been reported to have no secretory activity in the rat, in vivo. The mechanism of VIP-induced secretion is generally believed to involve activation of the adenylate cyclase cyclic AMP system. In vitro studies have shown that both the rabbit ileum and colon have a VIP-sensitive adenylate cyclase.

PP was patented by Eli Lilly as a veterinary laxative because it resulted in retching and decaecation in dogs. However, PP infusions, which produce plasma levels much greater than those found in patients with the watery diarrhoea syndrome, do not cause diarrhoea.

The aims of this study were to compare the effects of VIP and PP (at plasma concentrations seen in patients with VIPomas and PPomas) on fluid transport in vivo in the rabbit jejunum, ileum, and colon, and to monitor mucosal cAMP levels to assess the role of this system in the secretory response.

Methods

In vivo, steady-state, single-pass perfusions of the jejunum, ileum, and colon were performed in New Zealand white rabbits (weight range 2.5 to 3.5 kg) anaesthetised with intravenous pentobarbitone sodium (30 mg/kg body weight, Sagatal, May and Baker Limited, Dagenham, England). The perfusion solution was an isosmolar, plasma-like electrolyte solution consisting of NaCl 105 mM, KCl 4 mM, NaHCO3 30 mM, calcium gluconate 5 mM, and polyethylene glycol (PEG) 4000 5 g/l (BDH Chemicals Limited, Poole, England). A mixture of 95% oxygen and 5% carbon dioxide was bubbled through the perfusion fluid continuously. Alterations in fluid transport were determined from changes in the concentration of the non-absorbable marker PEG 4000 assayed by a turbidimetric method. A Schuco pump (multi-mini model) was used to drive the perfusion solution through the intestinal loops at a rate of 1.5 ml/min. A polyethylene catheter (external diameter 3 mm—McCarthy’s Surgical Limited, London, England) was inserted (via a cut-down on the right femoral artery) with its tip high up in the abdominal aorta. Throughout the experiments, pooled rabbit plasma, diluted 1 in 4 in isotonic saline, to which 5% (v/v) of the protease inhibitor aprotinin (Trasylol 20000 K IU/ml, Bayer, Germany) was added, was infused infra-aortically. This infusate also served as a vehicle for infusing porcine VIP (kindly donated by Professor V Mutt) at a rate of 0.1 µg/kg/min (30 pmol/kg/min) or bovine pancreatic polypeptide (kindly donated by Dr R E Chance) at a rate of 0.2 µg/kg/min (50 pmol/
kg/min). A similar cannula was inserted into the right femoral vein to obtain blood samples. Patency of the cannula was maintained with heparin saline (1000 IU lithium heparin per litre normal saline).

Jejunal and ileal loops (measuring approximately 30 cm in length) and a post-caecal total colonic loop were isolated by means of 16F Foley catheters at either end of the loop and were cleaned out by gentle flushing through with the perfusion solution, until the effluent was absolutely clean. At the end of the perfusion, the length of the perfused segments was measured after inserting a glass rod (external diameter 1 cm) through its lumen. Each level of intestine was studied in 10 rabbits (five each for the two test hormones). Bowel biopsies were taken by full-thickness clamping of the bowel wall with non-traumatic artery forceps and rapid excision with a sharp scalpel blade. This technique was shown not to result in blood seeping into the perfusion fluid or leakage of fluid from the perfused bowel loop. Mucosa was stripped from the full thickness biopsy and plunged into a mixture of ethanol and dry ice at −70°C, to ensure rapid inactivation of the enzymes that could affect the cAMP levels. The mucosa was homogenised. After centrifugation, the alcohol supernatant was dried under a stream of dry nitrogen at room temperature and reconstituted in 4 mM EDTA buffer pH 7.2. The protein content of the biopsies was measured by the colorimetric method of Lowry et al.10 cAMP was measured using a commercially available protein binding assay kit (Radiochemical Centre, Amersham, Buckinghamshire, England).

Plasma levels of VIP and PP were determined by specific radioimmunoassays. The VIP assay was specific and capable of detecting 1·5 pmol VIP/l plasma with 95% confidence.14 The PP assay detected changes of PP concentration between samples of 8 pmol/l with 95% confidence, on the sensitive part of the standard curve.17 18

Samples of loop effluent were collected every 10 minutes. There were six samples during two control periods and three samples during the test hormone infusion period, in each of the 10 rabbits studied (five for each hormone). Each plotted point refers to the mean ± SEM of all observations in each period—that is, mean ± SEM for 30 data points in control periods and 15 data points for test hormone periods.

**Experimental design**

An equilibration period of one hour to achieve a steady state was followed by a control period of one hour followed by an infusion of VIP or PP over 30 minutes, and finally a second control hour. Rabbit plasma-saline was infused intra-arterially at a constant rate of 2·0 ml/kg/h with or without the hormone. Throughout the experiment the bowel loop studied was perfused with the plasma-like electrolyte solution and 10-minute samples were collected for assay of PEG 4000. Two millilitres of blood samples were taken at the end of the first and second hours as well as after 15 and 30 minutes of hormone infusion. Samples were collected into lithium heparin bottles, containing 0·1 ml Aprotinin (Trasylol) and rapidly centrifuged at 8000 rpm for two minutes. The plasma was then separated and stored at −20°C until it was assayed. Bowel biopsies were taken from the perfused loops of bowel at the end of the two control hours and at the end of the VIP or PP infusions.

In two separate experiments, bowel biopsies were taken at frequent intervals (one, two, four, eight, 16 and 32 minutes) after starting VIP infusions to assess the time course of any cAMP response.

**Results**

**Effect of VIP intestinal fluid transport** (Fig. 1)

At all levels in the rabbit intestine, there was net fluid absorption in the first control hour before VIP infusion. During VIP infusion, there was a reversal from net absorption to secretion in both jejunum

![Fig. 1 Effect of intra-aortic vasoactive intestinal peptide on fluid transport (30 pmol/kg/min) in the rabbit intestine. Figures indicate mean ± SEM of all data points (30 and 15, respectively) during control and VIP periods.](image_url)
and ileum but no change in fluid transport by the colon.

During the second control hour after VIP infusion, ileal absorptive function was restored to normal; however, secretion persisted in the jejunum during the first 30 minutes of this second hour (24.8±0.6 μl/cm loop/10 min) though net absorption occurred in the subsequent 30 minutes (−3.6±4 μl/cm loop/10 min).

The Table shows the effect of VIP infusions on mucosal cAMP in the jejunum and ileum. There was no significant change in the mucosal cAMP concentration at either level of intestine.

In the two separate experiments in which mucosal cAMP concentrations in jejunum and ileum were measured one, two, four, eight, 16, and 32 minutes after starting VIP infusion, no significant changes in cAMP concentration were observed at any time, being 65±8 (mean ±SEM) pmol/mg protein in the jejunum and 56±5 pmol/mg protein in the ileum.

The mucosal cAMP concentration in the rabbit colon during intra-arterial VIP infusion and at all three levels studied during PP infusion showed no significant changes, and are not shown.

**Discussion**

Intra-arterial infusion of VIP achieving plasma levels between 75 and 115 pmol/l resulted in fluid secretion in the rabbit jejunum and ileum but not in the colon. These findings corroborate the results of Rambaud et al., who perfused the intestine of a patient with the watery diarrhoea syndrome and found small intestinal secretion but normal colonic absorptive function. Nevertheless, in the rat, VIP appears to inhibit colonic fluid absorption in vivo and in vitro so that it is not necessarily exclusively a small intestinal secretagogue. Dose response studies would be required to assess whether the colonic mucosa were less sensitive to VIP than the small intestine.

Bloom et al. were the first to propose that VIP was the mediator of the Verner-Morrison syndrome. Said and Faloon later supported this hypothesis by showing raised plasma VIP levels in all their 13 patients with pancreatic and adrenal diarrhoeagenic tumours. However, in several series of patients with diarrhoea and a tumour, there are patients who have normal plasma VIP levels. Several patients with diarrhoea have been reported with raised plasma and/or tumour levels of one or more hormones, with or without raised VIP levels. One of these hormones is pancreatic polypeptide. PP has been proposed as a mediator of the watery diarrhoea syndrome.

In this study the infusion of pancreatic polypep-
Effects of vasoactive intestinal peptide and pancreatic polypeptide in the rabbit intestine

tide did not significantly alter fluid transport in rabbit jejunum, ileum, or colon; This suggests that pancreatic polypeptide is not a secretagogue in the rabbit, and supports observations in the rat by other workers.\(^8\)\(^9\)

Several of our patients with plasma PP levels greater than 20 000 pmol/l have not had any diarrhoea; indeed, one patient with a pancreatic glucagonoma and a plasma PP level of 20 000 pmol/l required laxative treatment for constipation (TE Adrian and SR Bloom, unpublished observations). Furthermore, PP is not secretory in other organs—for example, when infused at rates to produce typical post-prandial levels in man, PP inhibits pancreatic and biliary secretion.\(^10\) Thus, overall, there is little evidence for PP being a secretagogue.

In vitro studies with small\(^10\) and large\(^11\)\(^12\)\(^13\) intestinal mucosa revealed the presence of a VIP-sensitive adenylate cyclase; however, we failed to observe any stimulation of the adenylate cyclase-cAMP system in our in vivo experiments by infusion of VIP to achieve plasma levels found in patients with the watery diarrhoea syndrome. A close examination of these in vitro studies shows that the dose of VIP required to inhibit fluid absorption was very much lower than that required to stimulate adenylate cyclase activity: a factor of 10\(^8\) lower in rat colon sacs\(^12\) and a 100-fold difference in ileal mucosa.\(^10\) Furthermore, Schwartz et al.\(^25\) have estimated the cAMP concentration in the jejunal mucosa biopsy of a patient with WDS and a pancreatic VIPoma and found it to be within normal limits. It is therefore possible that, even though VIP-sensitive adenylate cyclase receptors can be demonstrated in intestinal epithelium, the intestinal secretion induced by VIP is not mediated by the adenylate cyclase system. Indeed, Mailman et al.\(^26\) has suggested two alternative mechanisms, notably a vascular effect or a mechanism depending on the release of acetylcholine by the gut. Krejs et al.\(^7\) also detected reversible capillary dilatation in the dog jejunal mucosa during VIP infusion, as well as increased protein output into the lumen, indicating a vascular permeability effect.

As the turnover of cAMP in intestinal mucosa may be very rapid, the technique of biopsy and the rapid inactivation of the synthetic and degradative enzymes in in vivo experiments are crucial to subsequent interpretation of the role of cAMP in induced secretion. The time interval between biopsy and inactivation in our techniques is approximately five to 10 seconds, and, using this technique, changes in cAMP levels associated with theophylline-induced secretion are readily detected.\(^27\) It remains possible that the rise in cAMP in response to hormonal stimuli may be transient, but, in control studies, cAMP levels were not raised at any time between one and 32 minutes of starting VIP infusion. It has been suggested that a smaller or distinct intracellular pool of cAMP may be involved in hormone-mediated secretion but there is no evidence for this to date. The role of the VIP-sensitive cyclase in VIP-induced secretion thus remains obscure and other mechanisms mediating secretion in vivo seem likely.

We thank Miss Berny Murphy for expert secretarial help. Michael Camilleri is indebted to the Association of Commonwealth Universities for financial support.

References

Camilleri, Cooper, Adrian, Bloom, and Chadwick


26 Jaffe BM. 'To be or not to VIP'. *Gastroenterology* 1979; 76: 417–20.


