Release of vasoactive intestinal peptide by distention of the proximal stomach in dogs*

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SUMMARY The effect of transient proximal gastric distention was studied in six conscious dogs previously submitted to antrectomy and Billroth I anastomosis (and in which the intragastric pH was not altered), and in five anaesthetised dogs prepared with an innervated acute fundic pouch in which the pouch lumen was acidified with 0.15 M hydrochloric acid. Levels of vasoactive intestinal peptide (VIP) in portal and peripheral plasma were measured by radioimmunoassay. In conscious antrectomised dogs, distention of the proximal stomach to volumes of 250 and 500 ml evoked a significant rise of portal VIP concentration, with a maximal variation of 32.7 ± 4.4 to 67.7 ± 14.8 pg ml⁻¹, which was hardly reflected in systemic blood. In the anaesthetised dogs, transient distention of the acute fundic pouch with 0.15 M hydrochloric acid under a pressure of 40 cm of water elicited a significant rise of portal VIP from 36.2 ± 8.9 to 59.8 ± 12.4 pg ml⁻¹ ($P < 0.05$) without any significant variation of the peptide concentration in peripheral plasma. These results indicate that vasoactive intestinal peptide is released under transient proximal gastric distention in dogs, and that this response is not solely dependent upon the acid secretory effect of distention in this species.

Vasoactive intestinal peptide (VIP), an octacosapeptide characterised from porcine upper intestine,¹ is widely distributed in the central nervous system, digestive tract, and urogenital tract.^{2,3} Gastrointestinal VIP is detected at immunocytochemistry both in mucosa and muscular layers.^{2,4} Releasing factors so far identified include intraduodenal fatty acid⁵ or hyperosmolar solutions,⁵⁻⁷ intravenous calcium infusion,⁸ and vagal stimulation.⁹ Recently, mechanical stimulation of the oesophagus and colon was found to induce a significant release of VIP in anaesthetised (atropinised) cats.⁹

The present study was undertaken to assess the effect of proximal gastric distention on portal and peripheral plasma VIP concentrations in dogs.

Methods

Two experimental groups were studied. Under Nembutal anaesthesia, six dogs (22-28 kg at the

time of the test) were submitted to antrectomy and Billroth I anastomosis. After a recovery period of at least three weeks, a gastric fistula was constructed with a Thomas cannula, and an indwelling portal catheter was positioned *via* a splenic vein tributary. The tests were started at least eight days after this second operation. All animals showed normal gastric emptying, and could easily ingest 400 ml canned dog food plus water three times a day.

After an 18-hour fast without water restriction, the gastric fistula was opened and rinsed with distilled water. A rubber balloon, connected to a thin catheter for inflation, was positioned into the stomach. A second catheter was secured along this, so as to permit intragastric infusion of phenol red (8 mg l⁻¹, pH 7.0) at a rate of 180 ml h⁻¹. Care was taken to keep the gastric fistula patent. The gastric contents were continuously collected by gravity drainage. A catheter was positioned in a hindleg vein and kept patent with a slow infusion of isotonic saline. Saline was used as well for intermittent flushing of the portal catheter which was connected to a three-way stopcock. After 30 minutes of recovery, the balloon was intermittently inflated with 125, 250, and 500 ml of air in random order. No side-effect of distention was observed, except nausea in one dog for the 500 ml distention.

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Each inflation period, lasting for five minutes, was preceded by a 10 minute basal period and followed by 30 minutes of recovery. Blood samples (3 ml) were taken simultaneously *via* portal and peripheral catheters at regular intervals. The gastric effluent was collected as 10 minute fractions and the volume was measured to the nearest 0.1 ml. The concentration of phenol red was estimated on a Beckman spectrophotometer at 520, 560 and 600 nm for correction of non-specific absorbance in coloured samples,¹⁰ and was used to calculate the recovery of gastric contents. The acid concentration was measured by titrating 1 ml aliquots with 0.1M NaOH, using phenol red as indicator. The acid output was calculated from volume and acid concentration, and corrected from the percentage of marker recovered during the corresponding 10 minute period.

The effect of gastric distention against luminal acidification was studied in five dogs (24–31 kg) under Nembutal anaesthesia. Through a mid-line laparotomy, a fundic pouch was prepared with two long intestinal clamps. Care was taken to preserve the vascular and nervous connections of the pouch. Three Foley catheters were inserted, two for infusion into and drainage from the pouch and one for gravity drainage of the isolated fundus above the proximal clamp. Venous catheters were positioned respectively in the portal vein, in one of the fundic veins, and in a hindleg vein. The peripheral catheter was kept patent with a slow infusion of isotonic saline and the portal catheter was flushed at intervals with the same solution. In order to prevent venous stasis in the wall of the pouch, the catheter in the fundic vein was left open throughout the test, with a blood loss of 0.5–2 ml/min. The dogs were allowed to recover for one hour before starting the experiment. The pouch was continuously irrigated with 0.15 M hydrochloric acid at a rate of 180 ml h⁻¹. The acid solution was used for intermittent distention of the pouch to pressures of 10, 20, and 40 cm of water in sequential order. Each distention was applied for five minutes, followed by gravity drainage of the pouch, and the irrigation was resumed until the next distention period. The pouches accommodated about 200 ml liquid under the 40 cm of water pressure. Blood samples were collected simultaneously from portal, fundic, and peripheral catheters.

Blood samples were collected in Trasylol (800 µ/ml) and heparin (8 µ/ml) in ice-chilled glass tubes. The plasma VIP concentration was measured with a radioimmunoassay previously described.¹¹ Antiserum 76A, used at a 1:200 000 final dilution, recognises the 18–28 portion of porcine VIP (gift from Professor M Bodansky), but shows full

reactivity only with the entire molecule. Porcine VIP (gift from Professor V Mutt) was used as standard and for iodination with ¹²⁵I using lactoperoxidase (B grade, Calbiochem, San Diego, Ca). Samples from each dog were tested against a standard curve prepared in 20% charcoal-treated plasma. Unknown samples and peptide-free plasma were acidified to pH 5.5–6.0 with 0.4 M acetic acid before incubation. The within-assay and between-assay variations of the system are less than 10%. The sensitivity of the assay is 7.5 to 15 pg/ml of plasma. The recovery of 2–16 pg VIP per tube is 80–100%.¹¹ Results were expressed as pg equivalents of porcine VIP per ml plasma. Student's *t* test for paired values was used for statistical analysis; *p* values of less than 0.05 were considered to be significant.

Results

In conscious dogs, fundic distention with air induced significant rises in portal VIP; levels rose from a mean basal value of 31.5±4.5 to 47.0±9.3 pg ml⁻¹ with 250 ml distention and from 32.7±4.4 to 67.7±14.8 pg ml⁻¹ with 500 ml (Fig. 1). The variation induced by inflation with

Fig. 1 Effect of five minute gastric distention with a balloon inflated with 125, 250, and 500 ml of air on portal (open symbols) and peripheral (full symbols) plasma VIP concentrations in six antrectomized dogs. **p* < 0.05.

Fig. 2 Mean $\pm$ SE percent marker recovered (upper panel) and acid output (lower panel) in six antrectomised dogs submitted to gastric distension with a balloon inflated with 125, 250, and 500 ml of air. Each distention lasted for five minutes. The gastric effluent was collected as 10 minute fractions and analysed for volume, phenol red concentration, and acid concentration.

125 ml of air (39.3 ± 4.0 to 49.6 ± 7.6 pg ml⁻¹) was not significant. A limited, but significant rise of peripheral VIP was observed after the 500 ml distention, from 22.3 ± 4.3 to 28.0 ± 5.0 pg ml⁻¹ at five minutes ($P < 0.05$). Except for two 10 minute periods, respectively, before and after the 250 and 500 ml distentions (Fig. 2), the recovery of phenol red in the gastric effluent was in the range of 60–75% (Fig. 2). Under the conditions of this experiment, none of the distention volumes tested induced a significant variation of acid output (Fig. 2).

In the antrectomised dogs submitted to irrigation of the fundic pouch with 0.15 M HCl, the mean plasma VIP concentration in the portal vein during the 15 minute basal period was 36.2 ± 8.9 pg ml⁻¹. No significant variation was observed when distending the pouch with 10 or 20 cm pressure, but 40 cm of water pressure induced a significant rise to a peak of 59.8 ± 12.4 pg ml⁻¹ ($P < 0.05$, Fig. 3).

Fig. 3 Mean $\pm$ SE plasma VIP concentrations in portal vein (upper panel), fundic vein (middle panel), and femoral vein (lower panel) in five anaesthetised dogs submitted to sequential distention of an acute fundic pouch at 10, 20, and 40 cm of water pressure with 0.15 M hydrochloric acid. * $P < 0.05$.

The same pattern was observed in the plasma drawn from the fundic vein, but the rise induced by the 40 cm distention (from basal 29.2 ± 7.3 to peak 53.2 ± 22.4 pg ml⁻¹) did not attain statistical significance, probably because of the wide variations in VIP among dogs. The systemic concentration, measured before and after each distention period, was not affected by any of the pressures applied.

Discussion

The present results indicate that transient distention of the proximal stomach in dogs evokes a significant release of VIP in portal blood, and that this response is not suppressed by luminal acidification.

In conscious animals, VIP was released in significant quantities at distentions of 250 and 500 ml, but its release in response to 125 ml was not significant. The technical problems involved in handling, simultaneously, portal and peripheral catheters in conscious dogs made it impossible to test the variations of plasma VIP at intervals shorter than five minutes, so that increases greater than reported possibly went undetected in the present study. From the results obtained under these conditions, there was no clear correlation between distention volume and VIP response, as seen in Fig. 1, a finding that has been confirmed in subsequent unpublished experiments performed in dogs

with antral pouches. The VIP release induced by distending the proximal stomach may thus be an all-or-none response, possibly mediated by long vagovagal or short intramural reflexes, as for the acid response to distention in the same species.¹² The fact that the first significant response was observed with a moderate distention volume strongly suggests that distention of the proximal stomach is a physiological releasing factor for VIP in dogs. This is indirectly supported by the observation of a significant increase of portal VIP concentration (about 30 pg/ml) in the same antrectomised animals five minutes after ingestion of their regular 400 ml meal (unpublished studies).

The possible mechanisms of the distention-induced VIP release could not be fully elucidated in the present set of experiments. In the group of conscious dogs, the recovery of phenol red infused constantly into the stomach was essentially unaffected by the short distention periods, indicating that the VIP response was not simply reflecting an increased load of gastric contents to the duodenum. Whether the release was attributable to variations of intragastric pH due to the stimulatory effect of proximal gastric distention on acid secretion in dogs¹² was apparently ruled out by the fact that none of the present distention volumes, applied for five minutes in conscious animals, evoked any significant variation of acid output. As small changes of juxtamucosal pH could have been overlooked, however, the effect of distention of an acute fundic pouch was tested against luminal acidification in anaesthetised animals. Although both anaesthesia and surgical trauma probably altered the sensitivity of the VIP-producing sites in the digestive tract, the results then obtained indicate that the VIP-releasing effect of proximal gastric distention is retained when any variation of the juxtamucosal pH is obscured by luminal acidification. Whether the peptide release was triggered directly by mechanical receptors located in the gastric wall, or indirectly by the motor response to distention, remains to be investigated. As intestinal ischaemia was shown to evoke a sharp release of VIP into the portal stream,¹³ an additional mechanism to be tested would be the transient reduction of mucosal blood flow possibly resulting from the rise of intragastric pressure.

In the group of anaesthetised animals, the variations of plasma VIP concentration were tentatively studied both in the portal vein and in a vein draining the acute fundic pouch. Though the increase of plasma VIP at the later site after distention under 40 cm of water pressure did not reach significance, contrasting with the steady concentrations recorded both in portal and fundic

vein during the first part of the experiment, it provides circumstantial evidence that at least part of the VIP detected in the portal vein originated from the fundic pouch itself.

The physiological importance of the distention-induced VIP response remains speculative. When given exogenously, the peptide displays relaxing properties on the digestive smooth muscle,^{14,15} but the changes in plasma VIP levels induced by such infusions, appear to be supraphysiological.¹⁶ This, however, leaves open the possibility that VIP has a local role as neurotransmitter or as paracrine substance, so that the rise of portal VIP found in this study might reflect the simple overflow of the peptide released in the tissues. Additional studies with VIP in amounts comparable with those found in portal plasma are needed to more clearly assess the effects of the peptide on the motor, vasoactive, and secretory mechanisms in the digestive tract.

In all mammals studied, VIP is broadly distributed in the gastrointestinal tract.^{2,4} After the demonstration of a significant release of the peptide by mechanical stimulation of the oesophagus and of the intestinal mucosa in anaesthetised cats,⁹ the present results suggest that mechanical stimuli may release VIP from most parts of the gastrointestinal tract. In addition, because of the experimental conditions chosen here, they indicate that proximal gastric distention is a physiological VIP-releasing mechanism operating in dogs.

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