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EFFECTS OF AUTONOMIC STIMULATION ON THE RELEASE OF VASOACTIVE INTESTINAL PEPTIDE FROM THE GASTROINTESTINAL TRACT IN THE CALF

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SUMMARY

1. The effects of autonomic stimulation on the release of vasoactive intestinal peptide (VIP) from the gastrointestinal tract have been investigated in adrenalectomized calves 2–5 weeks after birth.

2. Stimulation of the peripheral ends of the splanchnic nerves (10 Hz for 10 min) caused a small fall in the concentration of VIP in portal and arterial plasma, together with a rise in the concentration in intestinal lymph. None of these changes achieved statistical significance.

3. The effects of stimulation of the peripheral ends of the thoracic vagi, below the heart (10 Hz for 10 min), were found to depend in part upon the integrity of the splanchnic sympathetic innervation. A substantial rise in the concentration of VIP in intestinal lymph occurred whether or not the splanchnic nerves had been cut whereas an associated rise in arterial plasma VIP was only observed in calves in which the splanchnic nerves had been sectioned.

4. The rise in the concentration of VIP in intestinal lymph, in response to vagal stimulation, was unaffected by concomitant stimulation of the splanchnic nerves, although the associated rise in arterial plasma VIP concentrations was suppressed. The response was also found to be resistant to atropine.

5. The minimum estimated concentration of VIP in the extracellular fluid of the gastrointestinal tract was estimated to be about 60 p-mole/l. at rest and to rise by 70–120 p-mole/l. in response to vagal stimulation.

6. Intravenous infusions of VIP at a dose of 50 ng kg^{-1} min^{-1} (16 p-mole kg^{-1} min^{-1}), which raised the minimum estimated concentration of VIP in the gastrointestinal tract to the highest range encountered during stimulation, produced no significant changes in the concentrations of glucose, insulin, pancreatic glucagon or pancreatic polypeptide in the arterial plasma.

7. It is concluded that a small amount of VIP is released from the gastrointestinal tract in response to vagal stimulation. In contrast, release of VIP is unaffected by stimulation of the splanchnic nerves except in so far as the rate at which the peptide passes into the circulation is reduced by adrenergic vasoconstriction.

INTRODUCTION

The effects of stimulation of the peripheral ends of the vagi and of the splanchnic

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nerves on the release of vasoactive intestinal peptide (VIP) from the gastrointestinal tract have been investigated recently by Fahrenkrug and his colleagues in Copenhagen (Schaffalitzky de Muckadell, Fahrenkrug & Holst, 1977; Fahrenkrug, Galbo, Holst & Schaffalitzky de Muckadell, 1978*a*; Fahrenkrug, Haglund, Jodal, Lundgren, Olbe & Schaffalitzky de Muckadell, 1978*b*). These workers examined the changes in the concentration of the peptide in arterial and portal venous plasma in anaesthetized pigs and cats and concluded that VIP was released in response to vagal stimulation and that this effect was inhibited by stimulation of the splanchnic nerves in the pig.

The interpretation of variations in the concentration of the peptide in portal plasma is necessarily complicated by the likelihood of concomitant changes in portal blood flow. Furthermore the changes in mean VIP concentration in arterial plasma in response to stimulation of either the vagi or the splanchnic nerves are comparatively small, generally amounting to between 50 and 100 %. Although this peptide has been found to have a number of different biological actions (Makhlouf & Said, 1975; Said, 1975) it is not yet known whether any of these are of physiological significance or even if the peptide is capable of acting hormonally. The discovery by immunocytochemical and radioimmunochemical techniques that VIP is largely confined within the nerve terminals in the gastrointestinal tract (Bryant, Bloom, Polak, Albuquerque, Modlin & Pearse, 1976; Sundler, Alumets, Håkanson, Ingemansson, Fahrenkrug & Schaffalitzky de Muckadell, 1976; Larsson, Fahrenkrug, Schaffalitzky de Muckadell, Sundler, Håkanson & Rehfeld, 1976) suggests that it exerts actions locally within the tissue. If this is the case, changes in the concentration of the peptide in the circulating plasma may merely reflect the rate at which it is washed out of the tissue and have no functional significance *per se*. For these reasons the changes in the concentration of VIP in intestinal lymph have been measured in the present experiments in order to obtain a better estimate of the concentration of the peptide in the gastrointestinal extracellular fluid.

Certain of the findings have been published previously in a preliminary form (Edwards, Bircham, Mitchell & Bloom, 1978; Bloom, Edwards & Mitchell, 1979).

METHODS

Animals

The experiments were carried out on pedigree Jersey and Jersey × Charollais calves which were obtained from local farms shortly after birth and used at ages ranging between 15 and 36 days (24·7–39·4 kg body weight). The animals were kept in individual pens in the laboratory animal house and maintained on a diet of milk (6–7 pints/day). Food was withheld for at least 6 hr before surgery and for a period of between 12 and 14 h before each experiment.

Experimental procedures

Anaesthesia was induced and maintained by the administration of sodium pentobarbitone (Sagatal; May and Baker). Preparatory surgical and experimental procedures were generally similar to those described previously (Edwards, 1971; Bloom, Edwards & Vaughan, 1973, 1974). Briefly, separate fluid electrodes were attached to the peripheral ends of both splanchnic nerves, sectioned below the diaphragm, and of both vagus nerves, sectioned below the heart and approached by lateral thoracotomy. Thereafter the animals were maintained by intermittent positive pressure ventilation. The pylorus was ligated and both adrenal glands removed when required. The right kidney was generally removed to facilitate identification of the intestinal lymph duct which was cannulated just anterior to the renal vein along its course over the ventro-

medial aspect of the posterior vena cava towards the cysterna chylae. A standard 20 V square wave stimulus (pulse width 1 msec) was applied throughout, usually for a period of 10 min. Arterial blood pressure was monitored continuously and blood samples for analyses, assays and haematocrit estimations were withdrawn at intervals from an arterial catheter, the tip of which was placed in the abdominal aorta. In some experiments atropine (atropine sulphate; B.D.H.) was given by I.V. injection (0·9 % (w/v) NaCl, 0·1 g/100 ml.) at a dose of 0·2 mg/kg 10 min before stimulation of the vagus nerves. Only one experiment was carried out on any individual animal.

Estimations

Samples of arterial blood were collected into heparinized tubes containing aprotinin (Trasylol, Bayer; 1000 K.I.U./ml. blood) for peptide assays. These tubes were centrifuged at +4 °C immediately and the plasma then stored at −20 °C.

Pancreatic glucagon was measured by a radioimmunoassay using an intiserum relatively specific for pancreatic glucagon which was C terminal reacting (Assan & Slusher, 1972); this assay reacted less than 5 % with glucagon-like immunoreactivity of ileal origin (enteroglucagon). Insulin, VIP and pancreatic polypeptide were also measured radioimmunoassay (Albano, Ekins & Turner, 1972; Mitchell & Bloom, 1978; Adrian, Bloom, Bryant, Polak, Heitz & Barnes, 1977). Plasma glucose concentration was estimated with glucose oxidase by means of a Beckman Glucose Analyser and intestinal lymph flow was measured gravimetrically. In several experiments the dead space of the intestinal lymphatic system was estimated from the change in radioactivity of the lymph following a single rapid injection of 0·1 mc tritiated water (THO; Radiochemical Centre, Amersham) into the thoracic aorta. Lymph samples were deproteinized with trichloracetic acid (TCA) (0·8 ml. lymph: 0·08 ml. 55% TCA) and centrifuged. The supernatant (0·25 ml.) was added to 5 ml. liquid scintillator (Kl 354; Nuclear Enterprises Ltd, Edinburgh) and counted in a Nuclear Enterprises 8312 liquid scintillation counter. Correction was made for quenching by means of an internal standard.

Where the effects of I.V. infusions of VIP were investigated, pure natural porcine VIP (Professor V. Mutt) was dissolved in sterile physiological saline for infusion at an intended maximum dose of 100 ng kg^{-1} min^{-1}. In view of the avidity with which the peptide is known to bind to polyethylene tubing and to glass a small volume was collected from the infusion system directly into calf plasma and Trasylol immediately before and after each infusion. These samples were subsequently assayed for VIP in the normal way and the actual rate of infusion calculated from these results.

Statistical analyses have been made according to the methods of Snedecor & Cochran (1967).

RESULTS

Responses to stimulation of the splanchnic nerves

Bilateral stimulation of the peripheral ends of the splanchnic nerves at 10 Hz for 10 min in adrenalectomized calves produced a small but sustained fall in arterial plasma VIP concentration. The resting VIP concentration in portal plasma was higher than that in arterial plasma and the fall in response to splanchnic nerve stimulation was more pronounced (Fig. 1*A*) although neither change was statistically significant. These changes were accompanied by a substantial increase in the flow of intestinal lymph which rose from a resting value of 45 ± 4 μl. kg^{-1} min^{-1}, immediately before stimulation to a peak at 12·5 min (103 ± 8 μl. kg^{-1} min^{-1}; $P < 0{\cdot}01$) and subsided slowly thereafter (Fig. 1*B*). In contrast to the falls in the concentration of VIP in arterial and portal plasma, the concentration of VIP in the intestinal lymph rose steadily, after a delay of several minutes, from an initial value of 31 ± 11 p-mole/l. to a peak of 56 ± 20 p-mole/l. 5 min after stimulation had been discontinued and then fell steadily back towards the initial level (Fig. 1*B*).

As both the flow of intestinal lymph and the concentration of VIP therein rose

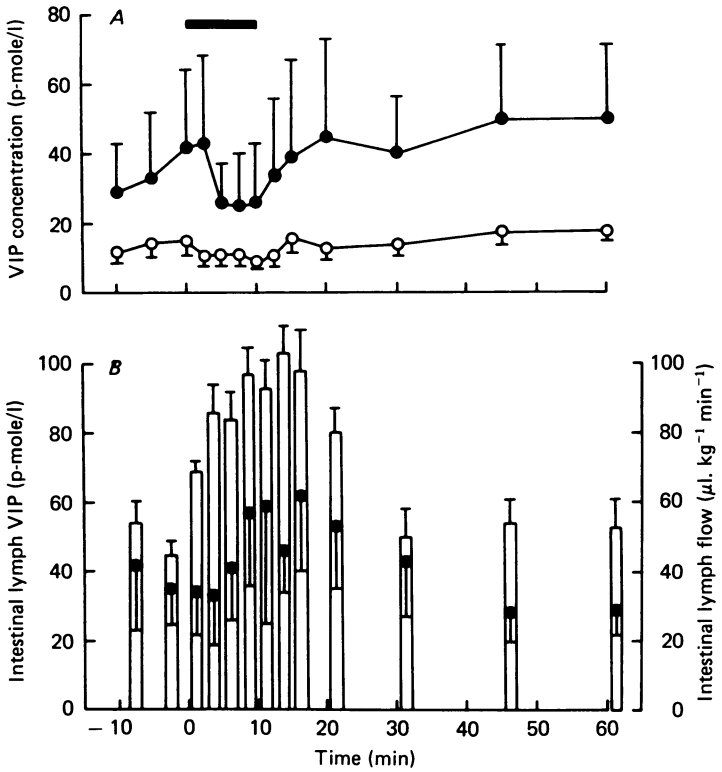

Fig. 1. Changes in mean arterial and portal plasma VIP concentration and in mean intestinal lymph flow and VIP concentration in response to stimulation of the peripheral ends of both splanchnic nerves (10 Hz, 10 min) in 2- to 5-week-old adrenalectomized calves. Horizontal bar: duration of stimulus. Vertical bars: S.E. of each mean value. *A*: ○, arterial plasma VIP ($n = 12$); ●, portal plasma VIP ($n = 5$). *B*: Histograms, lymph flow, ($n = 5$); ●, lymph VIP ($n = 5$).

during stimulation, the total output of the peptide from the gastrointestinal tract rose proportionately more than the concentration (from $1{\cdot}40 \pm 0{\cdot}36$ to $5{\cdot}68 \pm 2{\cdot}39$ f-mole kg^{-1} min^{-1} at 12·5 min.) It is important to note that the absolute amounts of VIP that are cleared from the gastrointestinal tract via the lymph (f-mole kg^{-1} min^{-1}) are far too small to have a significant effect on the tissue concentration. The pattern must be determined almost exclusively by the rate of release and inactivation of the peptide within the tissue and loss via the circulation. Thus, although the output of VIP in the intestinal lymph can easily be computed (simply by multiplying the concentration by the flow rate) it is highly flow-dependent, and changes in lymphatic output therefore provide a far less reliable index of the concentration of VIP in gastrointestinal extracellular fluid than the changes in lymphatic VIP concentration. For this reason the changes in VIP output in the intestinal lymph, which in each series of experiments exceeded the changes in the concentration of VIP in the lymph, are not presented. It should also be noted that the opposite argument applies to studies based on blood rather than lymph flow, because the amount of the peptide that is cleared from the tissue by this route is sufficiently large to affect the available

pool. It follows that changes in the concentration of VIP in portal plasma, for instance, are uninterpretable in the absence of any information about the changes in blood flow.

Responses to stimulation of the thoracic vagi

Bilateral stimulation of the peripheral ends of the vagus nerves, in the thorax below the heart, under the same conditions (10 Hz for 10 min) produced no significant rise in the arterial plasma VIP concentration of adrenalectomized calves with intact splanchnic nerves. In contrast there was a steady rise in the concentration of the peptide in the lymph, from 35 ± 8 p-mole/l. initially to 61 ± 3 p-mole/l. at the end of the period of stimulation (10 min; $P < 0{\cdot}02$) and a peak value of 69 ± 13 p-mole/l. 5 min later. Vagal stimulation also caused a steady increase in the flow of intestinal lymph which rose from 39 ± 8 μl. kg^{-1} min^{-1} initially to 68 ± 16 μl. kg^{-1} min^{-1} at 10 min. Mean aortic blood pressure fell abruptly by about 25 mmHg in response to vagal stimulation reflecting the intensity of the splanchnic vasodilation produced under these conditions, which presumably contributed to the increase in lymph production.

In view of the hypotensive effect of vagal stimulation it was thought that there might be some reflex increase in sympathetic efferent activity. As stimulation of the splanchnic nerves causes a fall in arterial plasma VIP concentration in these animals (Fig. 1*A*) such a reflex effect could be expected to antagonize any rise which might occur in response to the primary stimulus. This possibility was investigated by examining the responses of a further group of six adrenalectomized calves which were

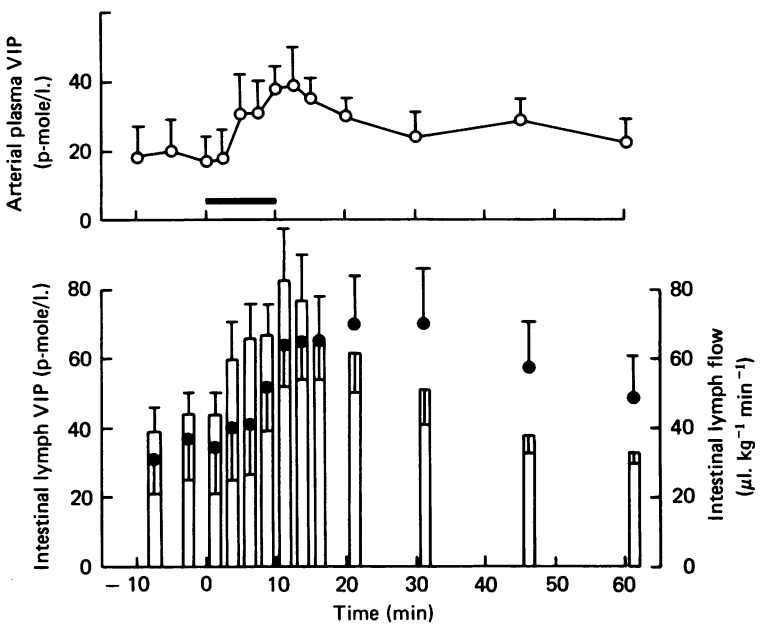

Fig. 2. Changes in mean arterial plasma and intestinal lymph VIP concentration and lymph flow, in response to stimulation of the peripheral ends of both vagus nerves (10 Hz, 10 min), in 2- to 5-week-old adrenalectomized calves with cut splanchnic nerves ($n = 6$). Horizontal bar: duration of stimulus. Vertical bars: S.E. of each mean value. ○, arterial plasma VIP; ●, lymph VIP. Histograms: lymph flow.

tested in precisely the same way after both splanchnic nerves had been cut below the diaphragm. The changes in lymph flow and lymphatic VIP concentration that occurred in response to vagal stimulation in this group of animals were both closely similar to those observed in the group with intact splanchnic nerves but there was a much more convincing rise in the concentration of the peptide in the arterial plasma which more than doubled, increasing from an initial value of 17 ± 7 p-mole/l. to 39 ± 11 at 12·5 min (Fig. 2).

The effects of vagal stimulation were also assessed in adrenalectomized calves with cut splanchnic nerves given atropine (0·2 mg/kg) 10 min prior to stimulation. The fall in aortic blood pressure in response to vagal stimulation was reduced following the administration of atropine (*ca.* 10 mmHg at 2·5 min), indicating that the vasodilator effect was partially suppressed. Likewise the rise in lymphatic flow was reduced by comparison with the control group, substantiating the supposition that this response is a consequence of splanchnic vascular changes. In contrast the rise in the concentration of VIP in the intestinal lymph was closely similar, both in rate and extent, to that observed in the absence of atropine; there was also a detectable rise in the concentration of the peptide in the arterial plasma.

Effects of combined vagal and splanchnic nerve stimulation

The fall in the concentration of VIP in arterial plasma which occurred in response to splanchnic nerve stimulation could be due either to direct inhibition of release within the tissue or to a reduction in the rate at which the peptide subsequently

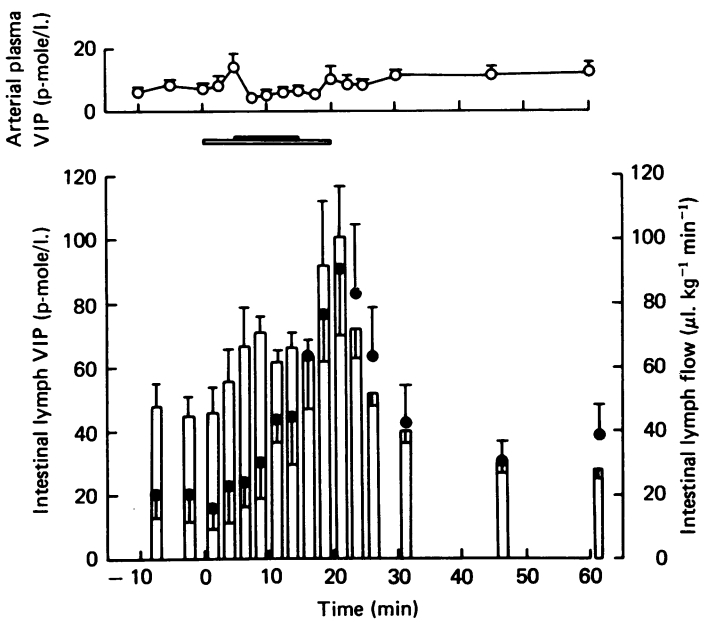

Fig. 3. Changes in arterial plasma and intestinal lymph VIP concentration and lymph flow in response to combined vagal and splanchnic nerve stimulation (10 Hz; 20 or 10 min) in 2- to 5-week-old adrenalectomized calves ($n = 5$). Horizontal bars: duration of vagal (open) and splanchnic nerve (filled) stimulation respectively. Vertical bars: S.E. of each mean value. ○, arterial plasma VIP; ●, lymph VIP. Histograms: lymph flow.

escapes into the circulation. This question was investigated in a further group of adrenalectomized calves in which both sets of nerves were stimulated simultaneously. In these experiments the vagi were stimulated at 10 Hz continuously for 20 min and the splanchnic nerves at the same frequency for 10 min from +5 to +15 min. This protocol produced a steady rise in the concentration of VIP in the intestinal lymph from an initial value of 20 ± 7 p-mole/l. before stimulation to a peak of 91 ± 21 p-mole/l. at 20 min ($P < 0{\cdot}02$). The extent of this rise (71 ± 16 p-mole/l.) corresponded to approximately double that obtained in response to vagal stimulation for 10 min in calves with intact splanchnic nerves (34 ± 19 p-mole/l.), cut splanchnic nerves (34 ± 7 p-mole/l.; Fig. 2) or those given atropine (32 ± 14 p-mole/l.). Furthermore there was no discernible change in the rate at which the concentration of the peptide in the lymph rose, in response to vagal stimulation, during the period of splanchnic stimulation (Fig. 3). In contrast the normal rise in the concentration of VIP in the arterial plasma which occurs in response to vagal stimulation in calves with cut splanchnic nerves such as these (Fig. 3) was completely suppressed by concomitant stimulation of the splanchnic sympathetic innervation.

The changes in lymphatic VIP concentration during these experiments show that there is no inhibition of VIP release within the gastrointestinal tract in response to splanchnic nerve stimulation. However, the fact that splanchnic nerve stimulation effectively abolished the associated rise in arterial plasma VIP concentration strongly suggests that loss to the circulation was reduced under these conditions, presumably due to adrenergic vasoconstriction.

Minimum estimated concentration of VIP in the gastrointestinal extracellular fluid

The changes in the concentration of VIP in the intestinal lymph reflect those in the mean concentration of the peptide in the gastrointestinal tract but are distorted by several different factors. These include the unavoidable delay between the formation of the lymph and collection, as it traverses the dead space which is represented by the intestinal lymphatics. This delay must clearly vary with flow rate. A significant proportion of the peptide may well be destroyed during its passage through the lymphatic system. In addition lymph from the gastrointestinal tract, in which VIP has been shown to be present in roughly similar amounts throughout, will be diluted with lymph from the liver, pancreas and spleen which are virtually devoid of VIP in the calf (Edwards *et al.* 1978). The combined weight of these viscera in a group of five 2- to 5-week-old calves was $28{\cdot}0 \pm 1{\cdot}9$ g/kg body weight while that of the gastrointestinal tract was $56{\cdot}4 \pm 2{\cdot}3$ g/kg. In occasional animals in which it has proved possible to cannulate the hepatic and intestinal tributaries separately we have found that lymph formation occurs at about the same rate in these different tissues, when expressed as flow/unit weight. A better estimate of the mean VIP concentration in the gastrointestinal extracellular fluid can therefore be obtained by correcting for the estimated dilution with hepatic, splenic and pancreatic lymph.

The dead space afforded by the lymphatic system will vary from region to region but the mean volume must correspond to the total volume collected between the administration of a freely diffusible marker intra-arterially and the subsequent peak in concentration in the lymph. In the present experiments the volume of the lymphatic dead space was estimated in six calves following a single rapid injection of tritiated

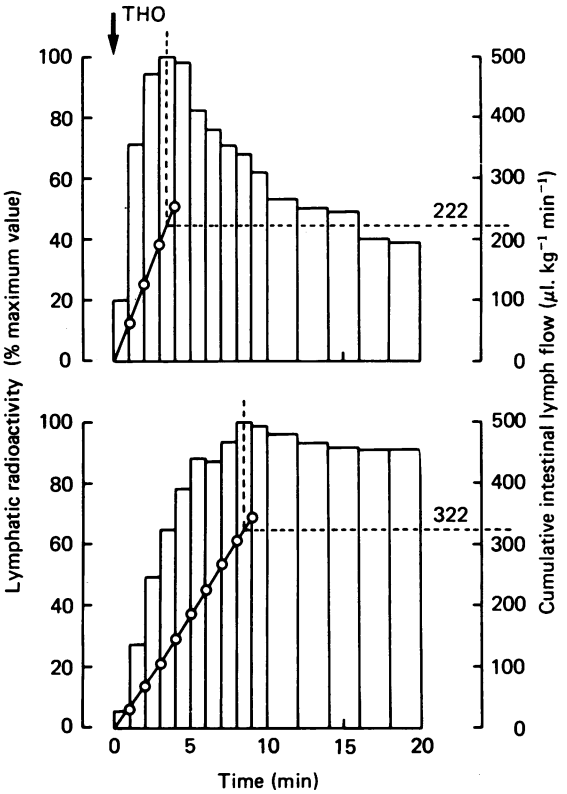

Fig. 4. Changes in the cumulative flow of intestinal lymph (○) and of lymphatic radioactivity (histogram) following a single intra-aortic injection of tritiated water (THO) (0·1 mC) in two individual calves 2–5 weeks after birth.

water (0·1 mc) into the thoracic aorta. The results of two typical experiments are shown in Fig. 4; mean lymphatic dead space was estimated to be 238 ± 24 μl./kg. With mean flow rates varying between about 40 and 100 μl. kg^{-1} min^{-1} during these experiments a change in the concentration of VIP in the tissue fluid would be reflected in that of the lymph collected between 2·4 and 6·0 min later. With this consideration in mind samples of intestinal lymph were incubated at 39 °C for 4 min in the absence of Trasylol in order to determine the rate at which the peptide is inactivated in lymph. Loss over 4 min was found to be $25 \pm 9\%$ in six samples collected from each of four 2- to 5-week-old calves.

Fig. 5 illustrates the changes in mean arterial plasma concentration and the minimum estimated concentration of VIP in the gastrointestinal extracellular fluid, in response to autonomic stimulation, having corrected the values obtained for lymph as indicated above. The resting levels range between about 35 and 70 p-mole/l. and rose by more than 130 p-mole/l. in response to combined autonomic stimulation. These estimated values are only approximate but they nevertheless provide the most reliable indication of the mean levels of VIP that occur within the gastrointestinal tract under physiological conditions and reveal responses to autonomic stimulation that cannot be detected by measuring changes in arterial plasma VIP concentration.

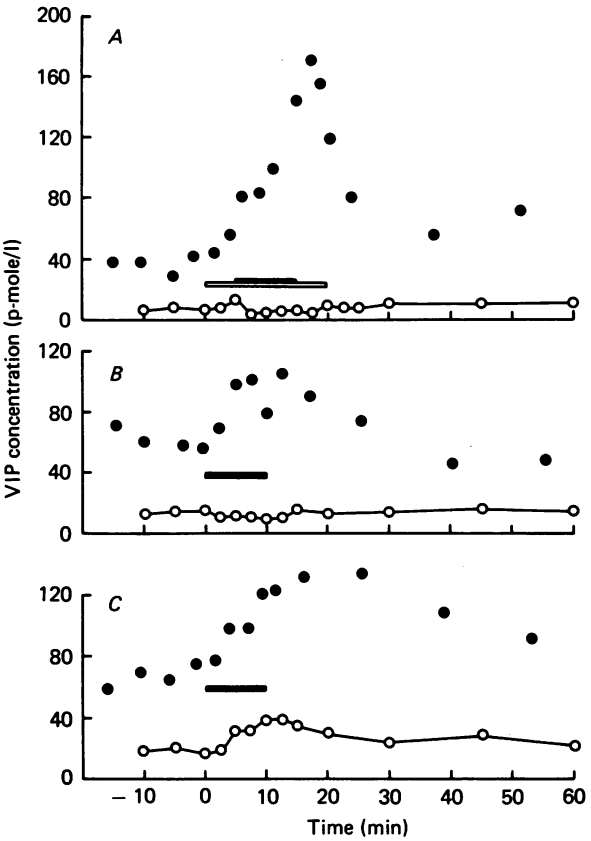

Fig. 5. Comparison of the changes in mean arterial plasma VIP concentration (○) and the estimated minimum VIP concentration in gastrointestinal extracellular fluid (●) in groups of adrenalectomized calves 2–5 weeks after birth. *A*: vagal stimulation, 10 Hz, 0–20 min (open bar) with splanchnic nerve stimulation, 10 Hz, 5–15 min (filled bar); $n = 5$. *B*: splanchnic nerve stimulation, 10 Hz, 0–10 Hz, 0–10 min, (filled bar); $n = 5$. *C*: vagal stimulation, 10 Hz, 0–10 min (filled bar); splanchnic nerves cut, $n = 6$.

Effects of intravenous infusions of exogenous VIP

Exogenous VIP was infused at a dose of either 50 or 10 ng kg^{-1} min^{-1} (15·6 or 3·1 p-mole kg^{-1} min^{-1}) for 15 min. The higher of these two doses raised the concentration of the peptide in the intestinal lymph to just above 100 p-mole/l. and the minimum estimated concentration of the gastrointestinal extracellular fluid to 175 p-mole/l. (In calculating these values the lymphatic concentration prior to infusion was corrected for dilution by lymph from sources other than the gastrointestinal tract as previously described. Thereafter the *increments* were uncorrected for dilution, on the assumption that the peptide passes from blood to lymph with equal facility in all the tissues that contribute to the flow of lymph through the intestinal duct.) The estimated mean value indicates that these intravenous infusions produced a concentration of VIP in the extracellular fluid corresponding to the highest that have been estimated to occur in the gastrointestinal tract in response to autonomic stimulation

TABLE 1. The changes in mean aortic blood pressure and heart rate in 2- to 5-week-old calves with cut splanchnic nerves, given atropine (0·2 mg/kg), in response to intravenous infusions of VIP between 0 and 15 min (50 ng kg^{-1} min^{-1}; $n = 4$)

Time (min)	Aortic blood pressure (mmHg)		Heart rate (beats/min)	
	Mean value	*D*	Mean value	*D*
−10	130 ± 12	1	163 ± 11	−3
− 2½	125 ± 13	0	162 ± 11	−4
0	125 ± 13	0	166 ± 13	0
2½	103 ± 13	−22	167 ± 11	1
5	102 ± 17	−23	169 ± 10	3
10	100 ± 18	−25	169 ± 9	3
12½	99 ± 18	−26	174 ± 11	8
15	99 ± 19	−26	174 ± 11	8
20	113 ± 16	−12	169 ± 11	3
30	117 ± 12	−8	167 ± 12	1

D, difference from value at time = 0.

in the intact animal. It follows that these infusions should therefore mimic any genuinely physiological paracrine responses to VIP in the peripheral tissue.

Aortic blood pressure fell in each of the four animals infused with VIP at the higher dose (50 ng kg^{-1} min^{-1}). Mean aortic blood pressure had fallen by about 20 %

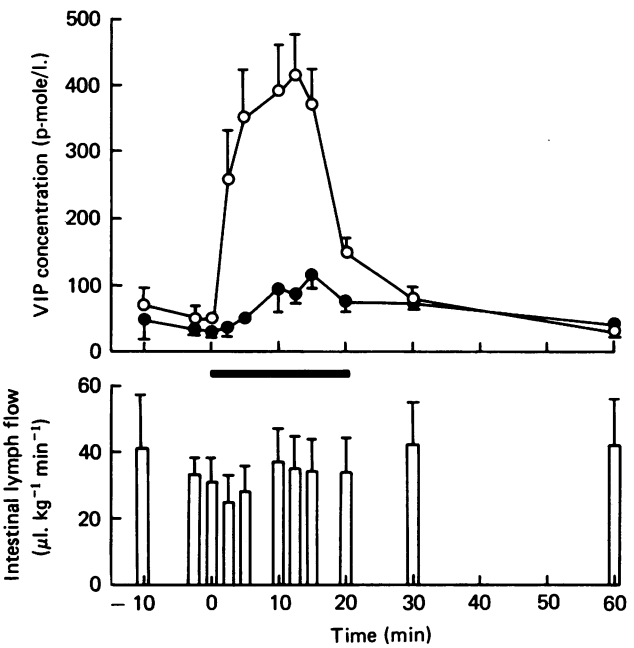

Fig. 6. Changes in mean VIP concentration in arterial plasma (○) and intestinal lymph (●) together with mean intestinal lymph flow in response to intravenous infusions of VIP (50 ng kg^{-1} min^{-1} for 15 min) in four 2- to 5-week-old calves with cut splanchnic nerves that were given atropine (0·2 mg/kg). Horizontal bar: duration of infusion. Vertical bars: S.E. of each mean value.

when the infusion was discontinued at 15 min (Table 1) and was associated with a comparatively small rise in heart rate (*ca.* 5 %), indicating that the observed hypotension was due to a fall in peripheral resistance, presumably attributable to the potent vasodilator effect of the peptide. The rate of formation of intestinal lymph remained relatively constant during these experiments (Fig. 6). It is therefore concluded that the vasodilatory response of the splanchnic vasculature was comparable to that which occurred in other tissues and sufficed to compensate for the fall in perfusion pressure. The constancy of lymph flow in these experiments provides further evidence to support the view that VIP exerts no specific effect on the rate of lymph formation and that any changes in the flow of lymph, which occur when the concentration of the peptide rises, are attributable to its vasodilator effects and the haemodynamic consequences.

Mean arterial plasma glucose, insulin, pancreatic glucagon and pancreatic polypeptide concentrations were found to remain relatively constant during intravenous infusions of VIP, at either dose employed, in calves with cut splanchnic nerves which had also been atropinized. The absence of any glycaemic or pancreatic endocrine response to VIP at the higher dose (50 ng kg^{-1} min^{-1}; Fig. 7) which raised the minimum estimated concentration of the peptide in gastrointestinal extracellular fluid to the highest range observed during the nerve stimulation experiments, provides strong evidence that the peptide does not produce these responses by diffusing generally throughout the target organ.

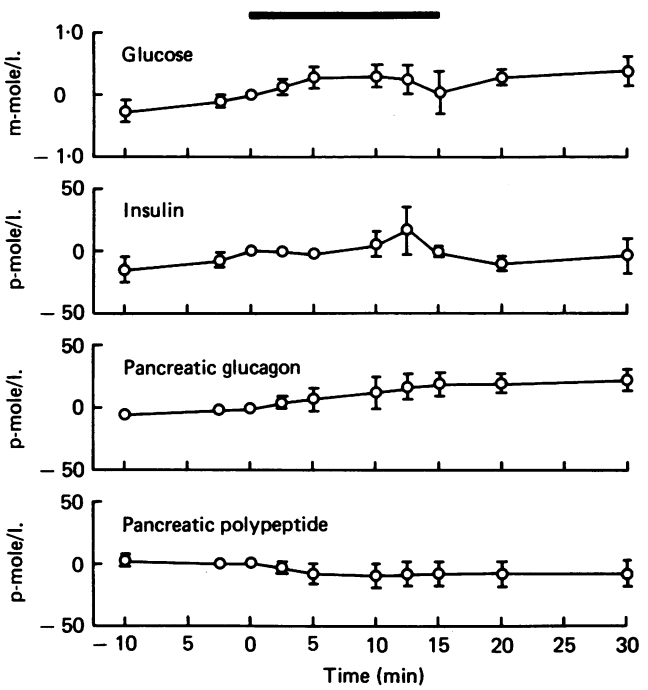

Fig. 7. Changes in the mean concentrations of glucose, insulin, pancreatic glucagon and pancreatic polypeptide in the arterial plasma in response to intravenous infusions of VIP (50 ng kg^{-1} min^{-1}) in four 2- to 5-week-old calves with cut splanchnic nerves that were given atropine (0·2 mg/kg). Horizontal bar: duration of infusion. Vertical bars: S.E. of each mean value.

DISCUSSION

The results of these experiments show that, in the case of a biologically active peptide, such as VIP, which has an extremely short half-life once it enters the circulation (Modlin, Mitchell & Bloom, 1978), changes in the concentration within the tissue of origin are not reliably reflected by those occurring in the blood. The concentration in the blood depends not only on the rate at which the peptide enters the vascular system but also on both the exit rate and the rate at which it is inactivated within the vascular compartment. The rapidity with which VIP is inactivated is reflected both by the low concentration of the peptide in arterial plasma and the restricted range within which it varies in response to nerve stimulation at high frequency, in both pigs and calves (Fahrenkrug *et al.* 1978*a*; present paper). At best therefore changes in the arterial plasma concentration of VIP can only provide a muted reflexion of changes in the entry rate of the peptide. However, as entry rate may be affected not only by the rate of release within the tissue, but also the adequacy of the blood flow through the tissue, the changes that occur in the blood may be positively misleading. A clear example of this is provided by the responses to stimulation of the peripheral ends of the splanchnic nerves. In these experiments stimulation caused a fall in the concentration of VIP in both the arterial and portal plasma, as found previously by Fahrenkrug and his colleagues in the pig, who concluded that release of the peptide was inhibited by adrenergic stimulation (Fahrenkrug *et al.* 1978 *a*). Our observation, that the concentration of VIP in the lymph rises steadily in response to splanchnic nerve stimulation, shows that release of the peptide within the gastrointestinal tract cannot be inhibited but rather that the rate at which it is lost into the circulation is reduced, presumably due to vasoconstriction of the splanchnic vascular bed. This conclusion is further supported by the finding that, in the pig, α-adrenoceptor blockade, which would abolish the splanchnic vasoconstrictor response to adrenergic stimulation, annulled the usual depression in portal plasma VIP concentration (Fahrenkrug *et al.* 1978*a*).

Whereas the changes in the concentration of VIP in the lymph seem to provide a more useful guide than those in either arterial or portal blood, to the changes which are occurring in the tissue of origin, they could be just as misleading in relation to the rate of release, in the absence of any other information. In the case of splanchnic nerve stimulation, for instance, the rise in the concentration of VIP in the intestinal lymph would suggest that it was being released in the gastrointestinal tract in response to adrenergic stimulation unless it were also known the concentration in portal and arterial blood had fallen. It follows that any assessment of changes in the rate at which the peptide is released necessitates a knowledge of the changes in both blood and lymph.

Stimulation of the peripheral ends of both vagi in the calf caused a rise in the concentration of VIP in the intestinal lymph which was associated with a rise in the arterial plasma. The latter response was enhanced by prior section of the splanchnic nerves, presumably because the rate at which the peptide was then 'washed out' into the circulation was increased in the absence of tonic sympathetic efferent activity. The conclusion from these results that VIP is released from the gastrointestinal tract in response to vagal stimulation is in complete agreement with findings in the pig, as is the observation that the response is resistant to atropine (Fahrenkrug *et al.* 1978*a*).

The finding that release of VIP from the gastrointestinal tract is unaffected by stimulation of the splanchnic sympathetic innervation, is relevant to the question as to how the elements, neural or otherwise, from which the peptide is released, are related to the classical sympathetic and parasympathetic systems. It is not consistent with the suggestion that neurones containing and releasing VIP receive a dual innervation, which exert opposing effects (Fahrenkrug *et al.* 1978*a*). However, it is entirely consistent with the idea that some vipergic elements in the gastrointestinal tract may be post-ganglionic parasympathetic neurones which, by releasing VIP in place of ACh, account for certain atropine-resistant responses to vagal stimulation. In the cat, atropine-resistant responses to either parasympathetic or mechanical stimulation, such as gastric relaxation and intestinal vasodilation, have been shown to be accompanied by an increase in the concentration of VIP in the effluent mesenteric venous blood (Fahrenkrug *et al.* 1978*b*). It has long been known that the vasodilator response of the cat submaxillary gland to stimulation of the chorda tymphani is resistant to atropine. The results of recent preliminary experiments have shown that the vasodilator effect of VIP in this tissue is at least twenty times that of ACh, on a molar basis, when the responses to intra-arterial infusions of the two agents are compared (S. R. Bloom and A. V. Edwards, unpublished observations). Neither release of VIP in response to vagal stimulation, nor the effects that it produces are affected by atropine, and the possibility that some of the vipergic neurones constitute a separate category of non-cholinergic post-ganglionic parasympathetic neurone is further supported by the finding that release of VIP in response to vagal stimulation is abolished by hexamethonium (Fahrenkrug, *et al.* 1978*a*).

If VIP acts as a neurotransmitter in the classical sense it may be expected to exert its effects on receptors close to the site of release at which high concentrations can be achieved for brief intervals. Alternatively, it might diffuse widely throughout the tissue and produce a generalized effect at necessarily lower concentrations. This question was examined by infusing the peptide intravenously at a dose which raised the minimum estimated concentration of the peptide in the extracellular fluid to the highest levels achieved in response to autonomic stimulation. The experiments were carried out in atropinized calves with cut splanchnic nerves in order to eliminate any secondary reflex responses to hypotension. Although specific gastrointestinal effects of VIP were not monitored, the peptide produced no significant change in the concentration of glucose or of any of the pancreatic hormones in the arterial plasma, although it has been shown to have a potent glycogenolytic action both *in vitro* and *in vivo* (Kerins & Said, 1973), and has been found to release both glucagon and insulin from the perifused rat pancreas (Bataille, Jarrousse, Vauclin, Gespach & Rosselin, 1977) and the isolated perfused cat pancreas (Schebalin, Said & Makhlouf, 1977).

The doses at which Kerins & Said (1973) administered VIP to their dogs (either 3·6 μg/kg infused I.V. over an unstated period of time or 1·0 μg/kg by intra-arterial injection) are large by comparison with those we have used (50 ng kg^{-1} min^{-1}) and it seems likely that they produced a correspondingly greater fall in arterial blood pressure. Both the glycogenolytic mechanism in the hepatocyte and the glucagon release mechanism in the pancreatic α-cell are extremely sensitive to stimulation via the sympathetic innervation in the dog (Edwards, 1972; Bloom & Edwards, 1975) and both reflex effects are readily reproduced in response to hypotension (Järhult &

Holst, 1978). It is therefore impossible to determine whether the hyperglycaemic response to VIP which they observed was due to a direct action of the peptide or arose secondarily.

Any estimate of the concentration of VIP in tissue fluid, based on the lymphatic content can only be a very rough approximation to the mean concentration throughout the whole of the tissue from which the lymph is flowing. In the case of the liver, which extraction studies have shown to contain very little VIP (Edwards *et al.* 1978) and is relatively homogeneous, the minimum estimated concentration of VIP is likely to be higher than the levels which are ever achieved under physiological conditions. The absence of any glycogenolytic response during VIP infusions, which must have raised the concentration throughout the hepatic extracellular fluid to this level, strongly suggests that VIP exerts no paracrine effect on glycogenolysis in the liver. As vagal stimulation has no direct glycogenolytic action in the liver, VIP could only act on the hepatocyte as a transmitter if it were released from post-ganglionic *sympathetic* fibres. In this connexion it may be significant that the increase in activity of hepatic phosphorylase that occurs in response to splanchnic nerve stimulation is unaffected by the β-blocker dichloroisoprenaline, in doses that abolish the glycogenolytic effect of catecholamines (Shimazu & Amakawa, 1968). Furthermore, splanchnic nerve stimulation leads to a rise in phosphorylase activity by inhibition of phosphorylase phosphatase (Shimazu & Amakawa, 1975) rather than by activation of cyclic AMP, suggesting involvement of some non-adrenergic transmitter. However, release of VIP in response to sympathetic stimulation has yet to be demonstrated.

No evidence was obtained in these experiments to suggest that VIP acts as a paracrine agent to influence the release of insulin, glucagon or polypeptide from the pancreas. However, as the α- and β-cells which produce glucagon and insulin are restricted to the pancreatic islets it is conceivable that the levels of VIP that were achieved during these infusions were less than those which might occur in response to the appropriate physiological stimulus *in vivo*. Release of all three pancreatic hormones in response to vagal stimulation in the calf is blocked by atropine (Bloom, Edwards & Hardy, 1978) which would appear to preclude the possibility that VIP could be implicated as a transmitter. However the pancreatic α-cell resembles the hepatocyte in that its response to stimulation of the sympathetic innervation is extremely resistant to the classical α- and β-adrenoceptor blocking drugs (Bloom & Edwards, 1978; Edwards & Bloom, 1978). It is therefore possible that release of pancreatic glucagen under these conditions is mediated by release of VIP or some other putative non-adrenergic transmitter.

The evidence at present available indicates that, with the exception of its vasodilator effect, which is produced at low concentration, VIP exerts its actions close to the site of release. Further studies are now required to establish whether release of this peptide, from the nerve fibres in which it has been shown to be stored, can account for any of the responses to autonomic stimulation which are known to resist either cholinergic or adrenergic blockade.

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