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A comparison of the differential effects of vasoactive intestinal peptide and peptide histidine isoleucine on the vascular and capsular smooth muscle of the dog spleen

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1 The actions of the two peptides, vasoactive intestinal peptide (VIP) and peptide histidine isoleucine (PHI) have been compared to that of isoprenaline on the smooth muscle systems of the isolated blood-perfused dog spleen.

2 Intra-arterial injections of VIP and PHI caused graded increases in splenic arterial blood flow at constant perfusion pressure indicative of splenic arterial vasodilatation.

3 VIP was significantly more potent than PHI, with their respective molar ED_{50} values being 9.9 ± 3.7 and 830 ± 141 pmol ($P < 0.002$). VIP was approximately 10 and 200 times more potent than isoprenaline and PHI respectively.

4 The maximum reduction in splenic arterial vascular resistance was the same ($P > 0.5$) in response to intra-arterial VIP and PHI, although both peptide maxima were significantly less ($P < 0.05$, 0.01 respectively) than that obtained with isoprenaline.

5 Small increases in spleen volume accompanied the splenic vasodilator responses to both peptides. They were probably passive in origin, secondary to splenic arterial vasodilatation.

6 The selective β_2-adrenoceptor antagonist, ICI 118,551, did not antagonize the splenic arterial vasodilator response to VIP or PHI but markedly attenuated the effect of isoprenaline.

7 These observations indicate that VIP and PHI, when either co-released locally or present together in the systemic circulation, may exert a differential action on different components of the circulation.

Introduction

Vasoactive intestinal peptide (VIP) was first isolated from extracts of porcine duodenum as a result of its vasodepressor effect and vasodilator action (Said & Mutt 1970a,b; Mutt & Said, 1974). Subsequently, a peptide having amino-terminal histidine and carboxy-terminal isoleucine, peptide histidine isoleucine (PHI), was also isolated from porcine intestinal extracts by a chemical assay that identified carboxy-terminal amidated amino acids which are frequently found in biologically active peptides (Tatemoto & Mutt, 1981). Structural comparison of these two peptides showed there to be substantial sequence homology between PHI and VIP. More recently a biosynthetic relationship has been established by investigations of the mRNA for the human VIP pro-hormone. This has shown that VIP and the human peptide analogue of PHI, peptide histidine methionine (PHM), are synthesized within a single precursor protein (Itoh *et al.*, 1983).

Previous studies of the immunocytochemical dis-

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tribution of VIP revealed a close association with the cardiovascular system; VIP being particularly abundant in perivascular nerves suggesting a role in modulating peripheral blood flow (Uddman *et al.*, 1981; Della *et al.*, 1983). Following the discovery of co-synthesis of VIP and PHI, the cellular localization of PHI in cat cerebral blood vessels has been examined and the co-existence of the two peptides in this vascular neuronal system demonstrated (Edvinsson & McCulloch, 1985). These findings, at least in the case of VIP, are concordant with observations that VIP is a potent vasodilator substance (Said & Mutt, 1970a,b). In spleen tissue VIP-like immunoreactivity is most closely associated with vascular smooth muscle and has been shown to produce splenic vasodilatation (Lundberg *et al.*, 1985), but neither the splenic distribution of PHI nor its actions have been described. We report here a comparison between the actions of VIP and PHI on the vascular and capsular smooth muscle of the isolated blood-perfused dog spleen. A preliminary account of these results has been published (Corder & Withrington, 1986).

Methods

The experiments were performed on 7 dogs (mean weight 26.2 ± 1.17 kg; range 22.0 ± 32.0 kg) anaesthetized with an intravenous mixture of chloralose and urethane (50 and 500 mg kg^{-1} respectively) after induction with methohexitone sodium (6 mg kg^{-1}). Systemic blood pressure was recorded from the cannulated left carotid artery with mean heart rate being derived electronically from this signal. The procedure for isolation and perfusion of the spleen and recording of splenic blood flow and spleen volume were as described recently (Corder *et al.*, 1987). Essentially, after vascular and nervous isolation from the donor, the spleen was placed in a plethysmograph and the splenic arterial system perfused with blood derived from the femoral artery; splenic venous blood drained into the femoral vein. An electromagnetic flow probe and strain gauge transducer were incorporated into the splenic arterial circuit to measure splenic arterial mean blood flow (SABF) and splenic arterial mean perfusion pressure (SAPP) respectively from which splenic arterial vascular resistance (SAVR) was derived. The plethysmograph was filled with liquid paraffin and connected to a weighed and calibrated reservoir. Displacement of liquid paraffin provided an indirect estimate of changes in spleen volume. A 'T' piece in the arterial inflow circuit allowed the close arterial administration of low doses of vasoactive substances. This procedure permitted establishment of the majority of the dose-response curves for the splenic smooth muscle systems without evoking general cardiovascular responses and consequently altering the conditions of the perfusion. The temperature of the spleen and of the donor dog was maintained at approx. 37°C whilst hourly arterial blood samples allowed the monitoring of arterial P_{CO_2}, P_{O_2} and pH and, if appropriate, correction to normal values was made by the intravenous infusion of $NaHCO_3$. The flow probe was calibrated with whole blood at the end of each experiment at which stage the spleen was also weighed after clamping of the artery and vein.

Drugs used and vehicles

Isoprenaline, VIP and PHI were administered as bolus injections directly into the splenic arterial line through a 'T' piece and washed in with saline (0.9% w/v NaCl solution) to give a total injectate of 2.0 ml. VIP and PHI were purchased from Bachem and made up in sterile saline which contained human serum albumin (10 mg ml^{-1}) and Polypep (2.5 mg ml^{-1}; low viscosity, Sigma) to reduce non-specific binding of the peptides to plastic surfaces. Intra-arterial injections of this vehicle produced no changes in splenic arterial blood flow or spleen volume. Isoprenaline hydrochloride (Pharmax) was diluted before injection with cold saline and maintained in ice. ICI-118,551 (erythro-DL-1-(7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol) was prepared in saline at a concentration of 1.0 mg ml^{-1}.

Statistics

Results are presented as means ± standard errors of mean (s.e.mean). Tests for significance refer to Student's *t* test.

Results

Control values

The mean spleen weight was 304 ± 50 g representing 1.14 ± 0.17% of body weight. The mean splenic arterial blood flow was 114 ± 15.5 ml min^{-1} or 43.3 ± 9.5 ml min^{-1} 100 g^{-1}. Since the mean splenic arterial perfusion pressure was 139 ± 7.7 mmHg then the mean calculated splenic arterial vascular resistance was 1.38 ± 0.21 mmHg ml^{-1} min or 3.86 ± 0.69 mmHg ml^{-1} min 100 g.

Splenic vascular responses to close arterial injections

Isoprenaline Isoprenaline, when injected close-arterially to the spleen in the dose range 0.01–10 μg, caused a characteristic vascular response observed in

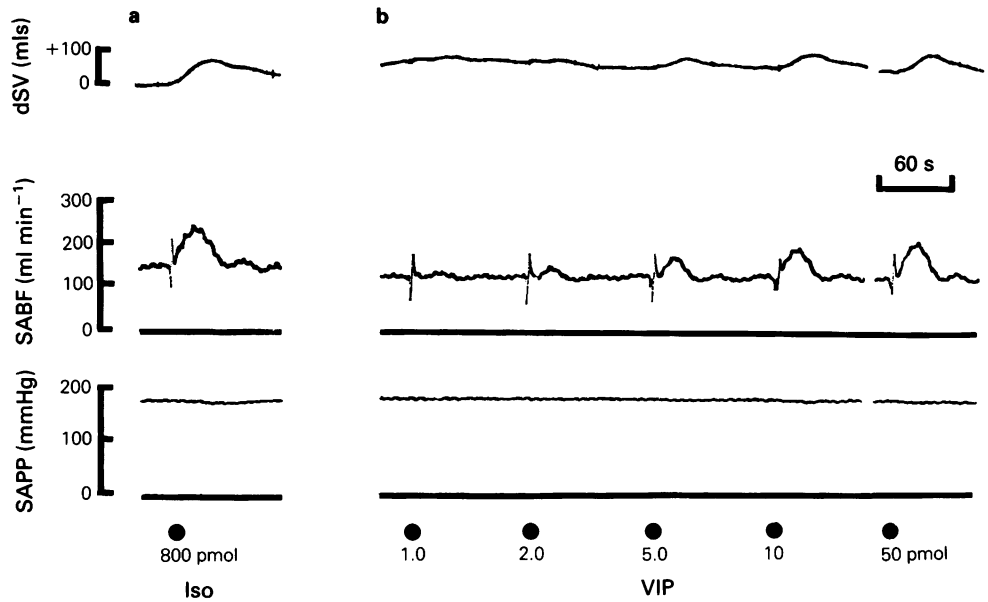

Figure 1 Spleen 152g. Records are from the top; dSV, change (increase) in spleen volume; SABF, splenic arterial mean blood flow; SAPP, splenic arterial mean perfusion pressure. The two panels, (a) and (b) illustrate the splenic vasodilator responses to the intra-arterial injection of isoprenaline (Iso, 800 pmol) and 5 graded doses of vasoactive intestinal peptide (VIP, 1.0, 2.0, 5.0, 10 and 50 pmol) respectively.

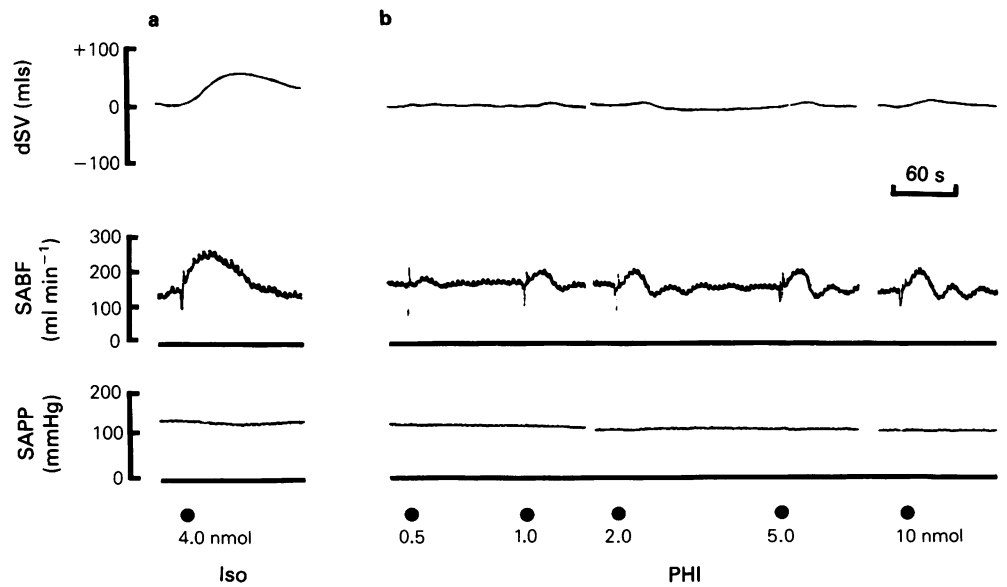

Figure 2 Spleen 371g. Records are the same as for Figure 1; dSV, changes in spleen volume; SABF, splenic arterial mean blood flow; SAPP, splenic arterial mean perfusion pressure. The two panels, (a) and (b) illustrate the splenic vasodilator responses to intra-arterial injection of isoprenaline (Iso, 4.0 nmol) and 5 increasing doses of peptide histidine isoleucine (PHI, 0.5, 1.0, 2.0, 5.0 and 10 nmol) respectively.

all preparations (Figures 1 and 2). This consisted of a rapid increase in splenic arterial inflow of relatively short duration. This response, observed at constant splenic arterial perfusion pressure, represents a fall in splenic arterial vascular resistance and splenic arterial vasodilatation. A splenic arterial vasoconstrictor phase was never observed with isoprenaline. In all the present splenic perfusion experiments isoprenaline was used as the standard vasodilator agonist for potency comparison; a complete dose-response curve for isoprenaline on the splenic vascular and extravascular (capsular) smooth muscle was established in each of the preparations.

Vasoactive intestinal peptide VIP was injected intra-arterially, over the dose range 1.0–500 pmol, in 7 separate spleen perfusions. The sole splenic vascular response to intra-arterial VIP was an increase in splenic arterial inflow representing splenic arterial vasodilatation. This vasodilatation was of short duration and graded with dose (Figure 1). Splenic arterial vasoconstriction was never observed.

In individual experiments the dose-response relationship between the intra-arterial molar dose of VIP and the percentage increase in splenic arterial blood flow always lay to the left of the comparable curve for intra-arterial injections of isoprenaline (Figure 3). That is, on a molar basis, VIP was the more potent splenic vasodilator. However, in each experiment, it was apparent that the maximum splenic vasodilator response to VIP was less than that to isoprenaline.

The mean results are illustrated in Figure 4, which compares the splenic vasodilator potency, assessed as the percentage fall in splenic arterial vascular resistance, to the intra-arterial molar dose. The mean molar ED_{50}, i.e. the mean molar dose to cause 50% of the maximum reduction in splenic arterial vascular resistance, was for VIP 9.9 ± 3.7 pmol a value significantly less ($P < 0.01$) than the ED_{50} for isoprenaline (79.3 ± 13.7 pmol) in the same experiments. In contrast the maximum vasodilator effect to VIP, achieved at intra-arterial doses of either 50 or 100 pmol was to reduce splenic arterial vascular resistance by $42.6 \pm 3.70\%$ of the control, significantly less than the mean maximum vasodilatation to intra-arterial isoprenaline ($51.3 \pm 1.96\%$; $P < 0.05$). The mean maximum splenic vasodilator response to VIP, expressed as a percentage of the maximum to isoprenaline in each experiment was $82.5 \pm 4.70\%$.

Peptide histidine isoleucine PHI was injected intra-arterially into 6 separate spleen perfusions over the dose range (0.1–10 nmol) which covered the entire dose-response curve from threshold to maximum. In all of these experiments the molar dose-response

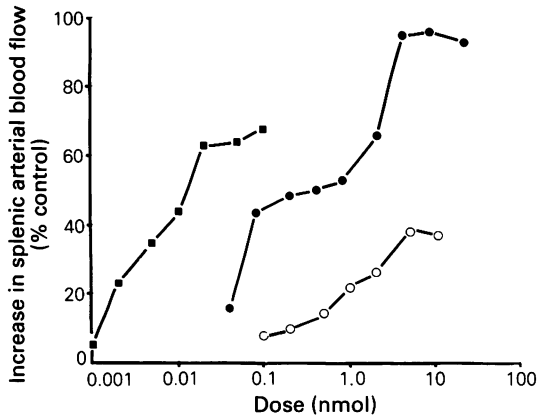

Figure 3 Spleen 152g. The dose-response curves relate the increase in splenic arterial blood flow, expressed as a percentage of the control flow just prior to injection, to the intra-arterial molar dose of isoprenaline (●), vasoactive intestinal peptide (■) and peptide histidine isoleucine (○). All the points were obtained in the same experiment under comparable conditions of splenic arterial flow (mean 118 ± 2.6 ml min^{-1}), and perfusion pressure (mean 172 ± 1.1 mmHg).

relationship to both VIP and isoprenaline was also established (Figure 4).

In each experiment PHI caused a graded, dose-dependent, increase in splenic arterial blood flow or splenic arterial vasodilatation of short duration (Figure 2). The time course of these splenic vascular

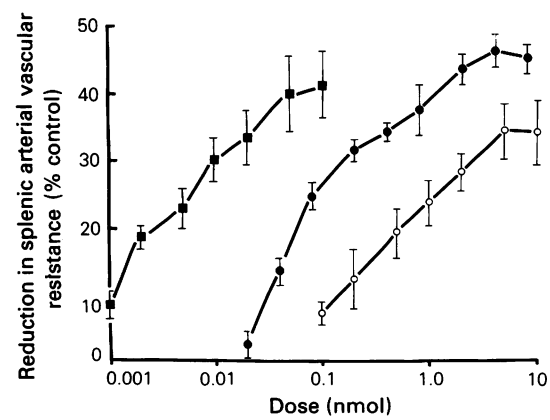

Figure 4 Results obtained in seven separate spleen perfusions, relating the molar dose of intra-arterial isoprenaline (●), vasoactive intestinal peptide (■) and peptide histidine isoleucine (○) to the reduction in splenic arterial vascular resistance (splenic vasodilatation), expressed as a percentage of the control value prior to the injection (mean splenic arterial vascular resistance = 1.38 ± 0.20 mmHg ml^{-1} min). Points represent the means and bars the s.e.means.

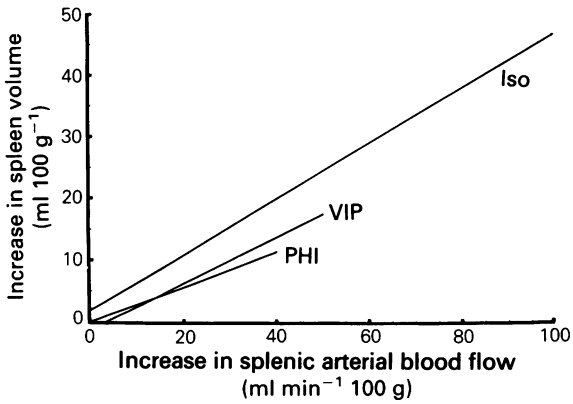

Figure 5 Computer plotted regression lines of the individual experimental observations (127) in 7 separate spleen perfusions. The abscissa scale is the increase in splenic arterial blood flow (ml min^{-1} 100 g) and the ordinate scale the increase in spleen volume (ml 100 g^{-1}) to intra-arterial bolus injections of isoprenaline (Iso, 43), vasoactive intestinal peptide (VIP, 47) and peptide histidine isoleucine (PHI, 37). The slope of each of the three regression lines is significantly greater than zero but they are not significantly different from each other. The intercepts for all three substances are not significantly different from zero. All three substances cause increases in spleen volume that are quantitatively closely related to the vasodilator effect of each substance irrespective of dose and intrinsic potency.

responses to PHI were not different from the splenic vascular responses to either isoprenaline or VIP. However in individual experiments (e.g. Figure 3) the dose-response curve to PHI always lay to the right of both VIP and isoprenaline, indicative of a lower molar potency. In addition, in most experiments the maximum vasodilator activity of PHI, achieved at intra-arterial doses of 5 or 10 nmol, was less than that of both VIP and isoprenaline.

Considering the 6 common perfusion experiments, the mean ED_{50} for PHI was 830 ± 141 pmol, significantly greater than the ED_{50} for either VIP or isoprenaline ($P < 0.0001$ and < 0.01 respectively). In addition the mean maximum vasodilatation to PHI i.e. decrease in splenic arterial vascular resistance, was 37.2 ± 3.40%, significantly less than the maximum to isoprenaline (51.3 ± 1.96%; $P < 0.001$) but not significantly different to the mean maximum splenic vasodilatation to VIP ($P > 0.50$). The mean maximum vasodilatation to PHI achieved at either 5.0 or 10 nmol, expressed as a percentage of the maximum response to isoprenaline, was 72.0 ± 4.10%.

Splenic capsular responses to isoprenaline, vasoactive intestinal peptide and peptide histidine isoleucine

In all the perfused spleen preparations, when isoprenaline, VIP and PHI were injected intra-arterially there were increases in spleen volume accompanying the increases in splenic arterial blood flow (Figures 1 and 2). However, increases in spleen volume were not observed with any of the substances in the absence of a splenic arterial vasodilator response, i.e. below the vascular threshold. A reduction in spleen volume was never observed with any injection of isoprenaline, VIP or PHI irrespective of whether a vascular response was achieved or not. To evaluate the vascular and capsular responses in all the experiments to all the close-arterial injections, a regression analysis (Figure 5) was made of the relationship between the increase in spleen volume (per 100 g spleen weight) and the increase in splenic arterial blood flow (per 100 g spleen weight). There was a positive correlation between these parameters for all three substances isoprenaline, VIP and PHI ($r = 0.80$; 0.81; 0.80 respectively). The slope of the regression lines for each substance was significantly greater than zero ($P < 0.05$) whilst the intercepts for each were not significant ($P > 0.5$). Such an analysis indicates that the increase in spleen volume and the increase in splenic arterial blood flow are closely related quantitatively. Further analysis revealed that the slope of the individual regression lines for VIP and PHI were not significantly different from each other ($P > 0.5$) and that neither was significantly different from the regression slope of the β-adrenoceptor agonist isoprenaline ($P > 0.5$ for both).

Splenic vascular and capsular responses to isoprenaline, VIP and PHI after β_2-adrenoceptor antagonism

In 3 experiments the splenic vascular and capsular responses to intra-arterial isoprenaline, VIP and PHI were examined before and after the i.v. administration of ICI 118,551, a selective β_2-adrenoceptor antagonist. The splenic vasodilator responses to isoprenaline were clearly antagonized in a competitive manner (Figure 6), without any concomitant alteration in the tachycardia induced by i.v. isoprenaline; the capsular responses, i.e. increases in spleen volume, were also reduced in a parallel manner. In contrast, the splenic vascular (Figure 6) and capsular responses to VIP and PHI were unchanged.

Discussion

In recent years a number of biologically active peptides with potent cardiovascular effects have been isolated and characterized. The subsequent tissue

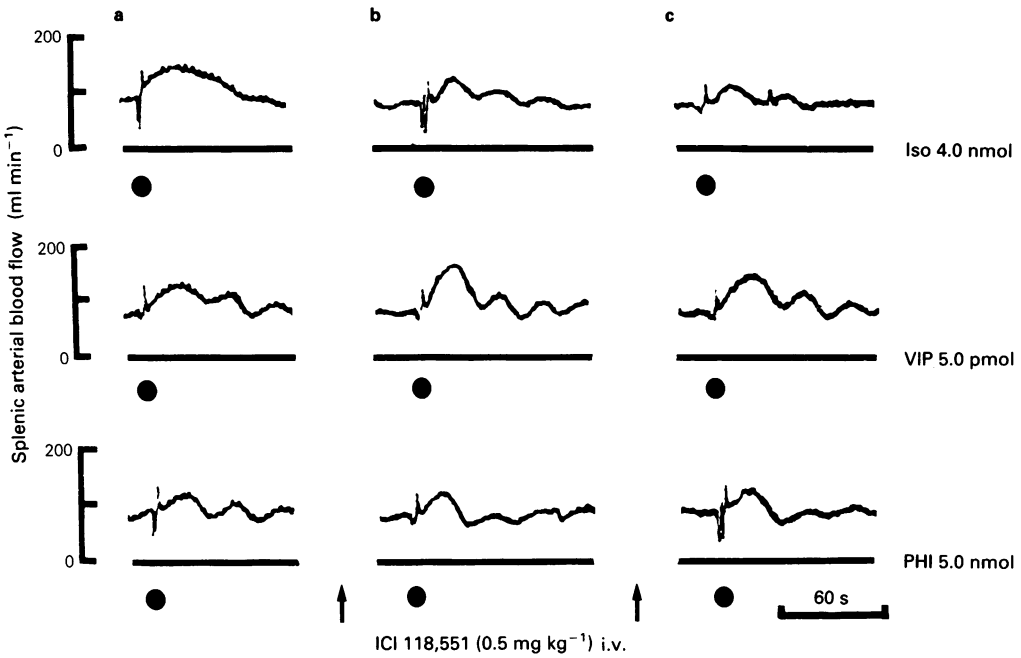

Figure 6 Spleen 371 g. The splenic arterial vasodilator response to intra-arterial bolus injections of upper trace, isoprenaline (Iso, 4.0 nmol) middle trace vasoactive intestinal peptide (VIP, 5.0 pmol) and lower trace, peptide histidine isoleucine (PHI, 5.0 nmol). The control responses are illustrated in (a) whilst (b) and (c) show the responses after two i.v. doses (indicated by the arrows) of the selective β_2-adrenoceptor antagonist ICI 118,551. The vasodilator response to isoprenaline is reduced in a graded manner by the β_2-adrenoceptor antagonist whilst the splenic arterial vasodilator response to the two peptides are not diminished. Clearly the two peptides do not act through activation of the β_2-adrenoceptor system. The mean perfusion pressure was unchanged by ICI 118,551 administration.

localization of these vasoactive agents has revealed a complex picture of neuronally located perivascular regulatory factors. Because of the comparable distribution, structural homology and biosynthetic relationship, the actions of VIP and PHI within the spleen have been considered in parallel. The results described here show that VIP and PHI, like isoprenaline, are both potent vasodilator substances in the vasculature of the dog spleen. However, the two peptides have only weak effects on the capsular smooth muscle. These findings are consistent with earlier results obtained from feline spleen, where VIP-like immunoreactivity was associated predominantly with blood vessels and intra-arterial infusion produced a vasodilatation ascertained by the fall in perfusion pressure (Lundberg *et al.*, 1985). The acute sensitivity of the dog spleen to VIP may indicate a physiological role for this peptide in altering splenic vascular tone and therefore modulating spleen function.

The profound difference in vasodilator potency between VIP and PHI in canine spleen (1:187) has also been observed previously in cat submandibular salivary gland (Lundberg & Tatemoto, 1982) and feline cerebral blood vessels (Edvinsson & McCulloch, 1985) where PHI was found to be 1000 and 25 times less potent respectively. Evidence has been put forward that PHI may act either through the VIP-receptor or a common second messenger system; cerebral vessels maximally released with PHI could not be relaxed to a greater extent by the addition of VIP (and *vice versa*), although further relaxation could be obtained with papaverine or nimodipine (Edvinsson & McCulloch, 1985). More recently human submandibular arteries have been shown to be equally sensitive to VIP and PHM (the human analogue of PHI), although VIP was approximately 5 times more potent in stimulating cyclic AMP production (Larsson *et al.*, 1986), thus raising questions of the nature of the PHI/PHM-receptor. The two amino acid residue sequence difference between PHI and PHM is unlikely to account for the greater potency of PHM if the action is mediated through the VIP-receptor as neither of these peptides have greater than 46% sequence homology with VIP (Itoh *et al.*, 1983).

Currently there is no information concerning the biosynthesis and post-translational processing of the

VIP precursor in the spleen, so that neither the importance of PHI in the canine spleen nor its vasodilator potency have been determined. VIP induces large changes in cyclic AMP in vascular smooth muscle cells (Ganz *et al.*, 1986), exploration of the inter-relationship between VIP and PHI at the second messenger level may provide a useful insight into these two peptides which appear to be co-synthesized, and presumably co-stored and co-released. In addition, as ICI 118,551 was unable to antagonize the effects of either peptide it would be interesting to determine if any link exists between β-receptor mediated and VIP- or PHI-induced vasodilatation, since β-stimulation also results in cyclic AMP production.

The potent vasodilator effects of VIP and, to a lesser extent, of PHI contrast with the relative inactivity of both peptides on capsular smooth muscle. Quantitative analysis indicates a passive relationship between the vasodilator action of these peptides (Figure 5) and the concomitant increase in spleen volume. The change in volume is unlikely to be a direct consequence of active relaxation of capsular smooth muscle due to activation of peptide receptors. This implies an absence of VIP and PHI receptors in the splenic capsule of the dog; functional evidence partially corroborated by histochemical studies (Lundberg *et al.*, 1985), which indicate a lack of VIP-receptor sites in the capsule of the spleen. This differential activity of the peptides VIP and PHI on the splenic vascular smooth muscle can be compared to recent similar observations (Corder *et al.*, 1987) on the actions of the neuropeptides NPY and PYY; these two substances are potent splenic arterial vasoconstrictor agents but lack any marked activity on the splenic capsule. The functional implications of these findings are important since they represent a further mechanism by which differential modification of one component of the circulation through an organ may be evoked without involvement of others. In the present context, changes in arterial resistance and flow can be brought about in the absence of any capacitative alterations and storage.

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