Regional haemodynamic responses to pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal polypeptide in conscious rats

¹S.M. Gardiner, T. Rakhit, P.A. Kemp, J.E. March & T. Bennett

Department of Physiology & Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH

1 Regional haemodynamic responses to the homologous peptides, pituitary adenylate cyclase-activating peptide (1-27) (PACAP27) and vasoactive intestinal polypeptide (VIP) were assessed by giving 20 min infusions (1.5 – 15 nmol kg⁻¹ h⁻¹) in conscious, chronically-instrumented, Long Evans rats.

2 PACAP27 caused dose-dependent depressor and tachycardic effects associated with renal, mesenteric and hindquarters vasodilatations, although only in the latter vascular bed was there a sustained increase in flow.

3 VIP caused dose-dependent depressor and tachycardic effects that were not significantly different from those caused by equimolar doses of PACAP27. However, the hindquarters vasodilator effects of VIP (at 7.5 and 15 nmol kg⁻¹ h⁻¹) were greater than those of PACAP27 (at the same doses), and accompanied by reductions in renal and mesenteric flows and conductances.

4 In the presence of the nitric oxide (NO) synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME; 11 µmol kg⁻¹ h⁻¹), there was significant inhibition of the hindquarters vasodilator effects of PACAP27 and VIP (at 7.5 and 15 nmol kg⁻¹ h⁻¹). Under these circumstances the renal and mesenteric vasoconstrictor effects of VIP were abolished.

5 The β₂-adrenoceptor antagonist, ICI 118551 (670 nmol kg⁻¹ bolus, 335 nmol kg⁻¹ h⁻¹ infusion), reduced the matched hindquarters vasodilator responses to PACAP27 (15 nmol kg⁻¹ h⁻¹) and VIP (7.5 nmol kg⁻¹ h⁻¹), and also abolished the renal vasoconstrictor effects of VIP.

6 The AT₁-receptor antagonist, losartan potassium (20 µmol kg⁻¹), had no significant effect on the haemodynamic response to PACAP27 (15 nmol kg⁻¹ h⁻¹), but augmented the hypotensive action of VIP (7.5 nmol kg⁻¹ h⁻¹). This influence of losartan was associated with conversion of the renal and mesenteric vasoconstrictor effect of VIP to vasodilatation.

7 Our findings show that similar changes in mean systemic arterial blood pressure in response to PACAP27 and VIP conceal substantial differences in their regional haemodynamic actions. Although the hindquarters vasodilator effects of both peptides involve NO- and β₂-adrenoceptor-mediated mechanisms, it appears that activation of the renin-angiotensin system contributes significantly to the haemodynamic effects of VIP, but not to those of PACAP27.

Keywords: Pituitary adenylate cyclase-activating polypeptide (1-27) (PACAP27); vasoactive intestinal polypeptide (VIP); NO; N^G-nitro-L-arginine methyl ester (L-NAME); ICI 118551; losartan potassium; haemodynamics

Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) was originally isolated from the sheep hypothalamus as a 38 amino acid peptide (Miyata *et al.*, 1989). Subsequently, the existence of a peptide corresponding to the N terminal 27 residues of PACAP (PACAP27) was demonstrated (Miyata *et al.*, 1990); PACAP27 shows 68% sequence homology with vasoactive intestinal polypeptide (VIP; Miyata *et al.*, 1990). PACAP27, like VIP, causes hypotension in anaesthetized rats (Miyata *et al.*, 1990; Nandha *et al.*, 1991; Absood *et al.*, 1992) and cats (Minkes *et al.*, 1992). Nandha *et al.* (1991) concluded that the hypotensive effects of PACAP27 and VIP were through interaction with the same receptor type, whereas Minkes *et al.* (1992) provided evidence that distinct receptors were involved in the cardiovascular responses to PACAP27 and VIP. Since the study of Nandha *et al.* (1991) quantitated the cardiovascular effects of PACAP27 and VIP only on the basis of changes in mean arterial blood pressure, they would not have detected any putative differences in the regional haemodynamic effects of the peptides. Moreover, in the studies cited above, only responses to bolus injections of PACAP27 and VIP were assessed and hence effects under steady state conditions were not observed. Therefore, the first

objective of the present study was to determine regional haemodynamic changes during 20 min infusions of 3 doses (1.5 , 7.5 and 15 nmol kg⁻¹ h⁻¹) of PACAP27 or VIP, in conscious, unrestrained rats.

The *in vitro* vasorelaxant effects of PACAP27 and VIP on the rabbit aorta have been described by Warren *et al.* (1991) as endothelium-independent, although they did not formally compare responses to the peptides in the presence and absence of the endothelium. Nonetheless, the finding of Minkes *et al.* (1992), that responses to PACAP27 and VIP were unaffected by the nitric oxide (NO) synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), is consistent with the cardiovascular responses to the peptides *in vivo* being endothelium-independent. However, these observations were made in anaesthetized cats and there is evidence that the L-arginine/NO system is less important in the control of haemodynamics in cats than in rats (van Gelderen *et al.*, 1991). Moreover, prior to the publication of the paper by Minkes *et al.* (1992), we had preliminary unpublished data showing a marked effect of L-NAME on some haemodynamic responses to PACAP27 and VIP in conscious rats. Therefore, the second objective of the present work was to assess haemodynamic responses to infusions of PACAP27 or VIP, in the absence and presence of L-NAME, in the same conscious rats.

¹ Author for correspondence.

Since there is evidence that PACAP27 and VIP can stimulate release of catecholamines from the adrenal gland of the rat (Malhotra & Wakade, 1987; Wakade *et al.*, 1991a,b), and since adrenaline causes marked β_2 -adrenoceptor-mediated hindquarters vasodilatation in the conscious rat (Gardiner *et al.*, 1991a,b), our third objective was to determine the possible involvement of this mechanism in the hindquarters vasodilator effects of PACAP27 and VIP, by recording responses in the absence and in the presence of the β_2 -adrenoceptor antagonist, ICI 118551 (erythro-($\pm$)-1-[7-methylidano-4-yloxy]-3-isopropyl-aminobutan-2-ol)hydrochloride) (Bilski *et al.*, 1983).

In dogs (Porter *et al.*, 1982), and rabbits (Dimaline *et al.*, 1983), VIP stimulates renin release, possibly through direct and indirect mechanisms. However, there are no data available relating to the involvement of the renin-angiotensin system in the regional haemodynamic responses to VIP and so, our fourth objective was to determine the effects of the AT₁-receptor antagonist, losartan potassium (DuP 753; Wong *et al.*, 1990; Batin *et al.*, 1991) on regional haemodynamic responses to PACAP27 or VIP in conscious rats.

Methods

Male, Long Evans rats (350–450 g) bred in the Biomedical Services Unit in Nottingham were used for all experiments. Under sodium methohexitone (Brietal, Lilly) anaesthesia (40–60 mg kg⁻¹, i.p. supplemented as necessary), pulsed Doppler flow probes (Haywood *et al.*, 1981) were implanted around the left renal and superior mesenteric arteries, and the distal abdominal aorta, below the level of the ileocaecal artery (to monitor flow to the hindquarters). After surgery, animals were given ampicillin (Penbritin, Beechams; 7 mg kg⁻¹, i.m.) and returned to individual home cages with free access to food and water. At least 1 week later, animals were anaesthetized (sodium methohexitone, 40 mg kg⁻¹, i.p.) and had an intra-arterial (distal abdominal aorta via the ventral caudal artery) and 3 intravenous (right jugular vein) catheters inserted. The catheters were led subcutaneously to emerge at the back of the neck with the wires from the Doppler probes. The probe wires were soldered into a microconnector (Microtech Inc., Boothwyn, U.S.A.) that was clamped into a harness fitted to the rat. A flexible spring was connected to the harness, and the catheters ran through the spring to prevent the rat interfering with them. The spring was supported by a counterbalanced lever system that allowed the rat unrestrained movement. The animals were allowed at least 1 day to recover from anaesthesia and catheterisation before experiments were begun. The following experimental protocols were employed.

Regional haemodynamic effects of repeated infusions of PACAP27 or VIP

In order to ensure that responses to PACAP27 or VIP were reproducible, rats ($n = 2$) were given 20 min infusions of PACAP27 (15 nmol kg⁻¹ h⁻¹, see below) or VIP (7.5 nmol kg⁻¹ h⁻¹, see below) on separate experimental days. The renal and mesenteric vascular responses to both peptides were not consistently different on the separate experimental days. The tachycardia, hypotension and hindquarters vasodilatation were numerically less on the second day for both peptides in both animals, but in each case the reduction (area under or over curve for 0–20 min) was less than 10%.

Regional haemodynamic effects of PACAP27 in the absence and presence of L-NAME

One group of 9 rats was given 20 min infusions of PACAP27 at doses of 1.5, 7.5 and 15 nmol kg⁻¹ h⁻¹ (these doses were chosen on the basis of preliminary experiments). The infusions were all given on the same experimental day, in random order, but separated by at least 1 h to allow all variables to

return to baseline before the next infusion was started. On the first experimental day, an infusion of saline (154 mmol l⁻¹ NaCl, 0.3 ml h⁻¹) was started, 90 min before the first infusion of PACAP27 was begun. The saline infusion was continued throughout the experiment to control for L-NAME infusion (see below).

Animals were left undisturbed for the day after PACAP27 infusion, with the second arm of the experiment starting the day after. L-NAME (11 μ mol kg⁻¹ h⁻¹, 0.3 ml h⁻¹) was infused throughout this experiment with the first infusion of PACAP27 beginning 90 min after the L-NAME infusion was started, and the subsequent two infusions of PACAP27 being given at least 1 h apart. The order of administration of the 3 doses of PACAP27 was the same as in the absence of L-NAME.

Regional haemodynamic effects of VIP in the absence and presence of L-NAME

A separate group of 9 rats was used in this protocol, which was identical to that above except the animals received 20 min infusions of VIP (1.5, 7.5 and 15 nmol kg⁻¹ h⁻¹) in the presence of saline or L-NAME.

Regional haemodynamic effects of PACAP27 and VIP in the absence and presence of ICI 118551

Since the hindquarters vasodilator effects of PACAP27 at a dose of 15 nmol kg⁻¹ h⁻¹ and VIP at a dose of 7.5 nmol kg⁻¹ h⁻¹ were not significantly different (see Results), we assessed responses to randomized 20 min infusions of these doses of the peptides (separated by at least 24 h) before, and at least 60 min after the onset of a primed infusion of ICI 118551 (670 nmol kg⁻¹ bolus, 335 nmol kg⁻¹ h⁻¹ infusion) (Gardiner *et al.*, 1992) in 7 Long Evans rats.

Regional haemodynamic effects of PACAP27 and VIP in the absence and presence of losartan potassium

In 9 Long Evans rats, randomized 20 min infusions of PACAP27 (15 nmol kg⁻¹ h⁻¹) and VIP (7.5 nmol kg⁻¹ h⁻¹) were given before and 30 min after losartan potassium (20 μ mol kg⁻¹). Elsewhere (Batin *et al.*, 1991), we have shown this dose of losartan potassium blocks the haemodynamic effects of exogenous angiotensin II (AII, 125 pmol kg⁻¹) for at least 9 h.

Drugs and peptides

PACAP27 and VIP were obtained from Bachem (U.K.) and L-NAME hydrochloride from Sigma. ICI 118551 hydrochloride was a gift from ICI Pharmaceuticals plc, and losartan potassium was a gift from Dr R. Smith (DuPont, U.S.A.). The peptides were dissolved in sterile isotonic saline containing 1% bovine serum albumin (Sigma); L-NAME and losartan potassium were dissolved in saline, as was ICI 118551, but the latter required gentle warming.

Data analysis

Before and throughout the infusions of PACAP27 or VIP, continuous recordings were made of instantaneous heart rate, phasic and mean arterial blood pressures and phasic and mean Doppler shift signals from the renal, mesenteric and hindquarters probes. Values for heart rate, mean arterial blood pressure, and mean renal, mesenteric and hindquarters Doppler shift signals were averaged over 1 min epochs immediately before infusion of PACAP27 or VIP, and at 5, 10, 15 and 20 min during infusion of the peptides. Vascular conductances were calculated by dividing the mean Doppler shift signal by the corresponding mean arterial blood pressure signal (Gardiner *et al.*, 1991a,b; 1992). Responses to PACAP27 or VIP were assessed by Friedman's test; res-

ponses to each peptide in the absence and presence of L-NAME were compared by Wilcoxon's test applied to the integrated responses (i.e. areas under or over curves calculated from the values measured at the times above). Responses to PACAP27 and VIP in the absence or presence of L-NAME were compared by the Mann-Whitney U test applied to integrated responses. Effects of ICI 118551 or losartan on responses to PACAP27 or VIP were assessed by applying Wilcoxon's test to the integrated responses in the absence and presence of these drugs. A P value <0.05 was taken as significant.

Results

Regional haemodynamic responses to PACAP27 in the presence of saline

Infusion of PACAP27 at $1.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$ caused a slight hypotension and modest tachycardia but no significant

changes in renal, mesenteric or hindquarters flows, or vascular conductances (Figures 1 and 2, Table 1).

Infusion of PACAP27 at 7.5 and $15 \text{ nmol kg}^{-1} \text{ h}^{-1}$ caused marked, dose-dependent, depressor effects and tachycardia (Figures 1 and 3, Table 1). There were slight, and variable increases in renal and mesenteric flows, but substantial increases in hindquarters flow (Figures 1 and 3). There were increases in vascular conductances in renal, mesenteric and, particularly, hindquarter vascular beds (Figure 2, Table 1).

Regional haemodynamic responses to PACAP27 in the presence of L-NAME

In the presence of L-NAME, mean arterial blood pressure was increased significantly, in association with bradycardia and reductions in renal, mesenteric, and hindquarters flows and conductances (Figures 1 and 2).

Under these conditions, infusion of PACAP27 at a dose of $1.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$ caused a significantly greater hypotension than in the presence of saline, although the tachycardia was

Figure 1 Cardiovascular changes during 20 min infusions of PACAP27 at $1.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$ (●), $7.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$ (▲), or $15 \text{ nmol kg}^{-1} \text{ h}^{-1}$ (■) in the absence (left-hand panels) or presence (right-hand panels) of N^G -nitro-L-arginine methyl ester (L-NAME, $11 \mu\text{mol kg}^{-1} \text{ h}^{-1}$) in the same conscious, Long Evans rats ($n=9$). Values are mean with s.e.mean; * $P<0.05$ versus preinfusion baseline (Friedman's test).

Figure 2 Cardiovascular changes during 20 min infusions of PACAP27 at $1.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$ (●), $7.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$ (▲), or $15 \text{ nmol kg}^{-1} \text{ h}^{-1}$ (■) in the absence (left-hand panels) or presence (right-hand panels) of N^G -nitro-L-arginine methyl ester (L-NAME, $11 \mu\text{mol kg}^{-1} \text{ h}^{-1}$) in the same conscious, Long Evans rats ($n = 9$). Values are mean with s.e.mean; * $P < 0.05$ versus preinfusion baseline (Friedman's test).

Table 1 Integrated cardiovascular responses (areas under or over curves; AUC, AOC) during 20 min infusions of PACAP27 in the absence and presence of N^G -nitro-L-arginine methyl ester (L-NAME, $n = 9$), or vasoactive intestinal polypeptide (VIP) in the absence and presence of L-NAME ($n = 9$) in conscious, Long Evans rats

	PACAP27 ($1.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$) + L-NAME		PACAP27 ($7.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$) + L-NAME		PACAP27 ($15 \text{ nmol kg}^{-1} \text{ h}^{-1}$) + L-NAME	
Heart rate (AUC; beats)	917 ± 223	1078 ± 106	2122 ± 199	2640 ± 349	2957 ± 216	2543 ± 265
Mean arterial blood pressure (AOC; mmHg min)	-91 ± 19	$-371 \pm 66^*$	-270 ± 33	$-513 \pm 87^*$	-428 ± 34	-496 ± 96
Renal conductance (AUC; $[\text{kHz mmHg}^{-1}]10^3 \text{ min}$)	54 ± 19	$241 \pm 27^*$	343 ± 88	457 ± 56	414 ± 98	345 ± 60
Mesenteric conductance (AUC; $[\text{kHz mmHg}^{-1}]10^3 \text{ min}$)	89 ± 32	188 ± 60	322 ± 41	460 ± 73	276 ± 85	321 ± 68
Hindquarters conductance (AUC; $[\text{kHz mmHg}^{-1}]10^3 \text{ min}$)	36 ± 14	64 ± 12	363 ± 65	$223 \pm 46^*$	663 ± 41	$253 \pm 53^*$
	VIP ($1.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$) + L-NAME		VIP ($7.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$) + L-NAME		VIP ($15 \text{ nmol kg}^{-1} \text{ h}^{-1}$) + L-NAME	
Heart rate (AUC; beats)	432 ± 100	410 ± 104	2476 ± 166	2171 ± 282	3201 ± 136	$2228 \pm 342^*$
Mean arterial blood pressure (AOC; mmHg min)	-85 ± 24	$-134 \pm 21^\dagger$	-389 ± 59	-392 ± 36	-518 ± 31	$-653 \pm 53^*$
Renal conductance (AUC or AOC; $[\text{kHz mmHg}^{-1}]10^3 \text{ min}$)	67 ± 34	$69 \pm 16^\dagger$	$-105 \pm 29^\dagger$	$117 \pm 31^\dagger$	$-208 \pm 48^\dagger$	$60 \pm 25^\dagger$
Mesenteric conductance (AUC or AOC; $[\text{kHz mmHg}^{-1}]10^3 \text{ min}$)	82 ± 24	114 ± 27	$-240 \pm 60^\dagger$	$117 \pm 32^\dagger$	$-298 \pm 59^\dagger$	$-57 \pm 19^\dagger$
Hindquarters conductance (AUC; $[\text{kHz mmHg}^{-1}]10^3 \text{ min}$)	49 ± 13	26 ± 9	$822 \pm 135^\dagger$	$227 \pm 36^*$	$996 \pm 108^\dagger$	$426 \pm 73^*$

* $P < 0.05$ versus corresponding response in the absence of L-NAME (Wilcoxon's test);

† $P < 0.05$ versus corresponding response to PACAP27 (Mann-Whitney U test).

Figure 3 Contrasting cardiovascular changes at the onset of infusion of PACAP27 or vasoactive intestinal polypeptide (VIP) in different conscious, Long Evans rats. Note that although the fall in mean arterial blood pressure (BP) was very similar, VIP caused reductions in renal and mesenteric Doppler shift signals, whereas PACAP27 did not.

similar (Figure 1, Table 1). There were significant increases in renal and mesenteric flows but only the renal vasodilator response to PACAP27 was significantly greater than that seen during infusion of PACAP27 in the presence of saline (Figures 1 and 2, Table 1).

In L-NAME-treated animals, an infusion of PACAP27 at 7.5 and 15 nmol kg⁻¹ caused similar falls in blood pressure, and rises in heart rate and in renal, mesenteric and hindquarters flows and vascular conductances (Figures 1 and 2). Although the hypotensive responses to PACAP27 at a dose of 7.5 nmol kg⁻¹ h⁻¹ were enhanced relative to those in the presence of saline (Figure 1, Table 1), there was a significant reduction in its hindquarters hyperaemic vasodilator action, whereas its vasodilator effects in renal and mesenteric vascular beds were not changed significantly (Figures 1 and 2, Table 1). There was also a significant inhibition of the hindquarters haemodynamic effects of the highest dose of PACAP27 in the presence of L-NAME, but in this instance the hypotensive response was not different from that seen in the presence of saline (Figures 1 and 2, Table 1).

Regional haemodynamic responses to VIP in the presence of saline

VIP at a dose of 1.5 nmol kg⁻¹ h⁻¹ caused slight hypotension and tachycardia, but no significant regional haemodynamic changes (Figures 4 and 5, Table 1).

VIP at 7.5 and 15 nmol kg⁻¹ h⁻¹ caused dose-dependent hypotension and tachycardia accompanied by reductions in renal and mesenteric flows and vascular conductances, but increases in hindquarters flow and vascular conductance (Figures 3–5, Table 1).

Regional haemodynamic responses to VIP in the presence of L-NAME

As above, L-NAME caused hypertension, bradycardia and reductions in renal, mesenteric and hindquarters flows and vascular conductances (Figures 4 and 5). The cardiovascular effects of VIP at 1.5 nmol kg⁻¹ h⁻¹ in the presence of L-NAME were not significantly different from those in the

presence of saline (Figures 4 and 5, Table 1). The slight hypotensive response to VIP at 7.5 nmol kg⁻¹ h⁻¹ was not enhanced by L-NAME, relative to that in the presence of saline, but the renal and mesenteric vasoconstrictions were abolished and the hindquarters vasodilatation substantially reduced (Figures 4 and 5, Table 1).

A similar pattern of effects was seen with VIP at 15 nmol kg⁻¹ h⁻¹, but in this instance the hypotensive response was enhanced in the presence of L-NAME relative to that seen in the presence of saline (Figures 4 and 5, Table 1).

Comparison of the regional haemodynamic effects of PACAP27 and VIP

In the presence of saline, each of the equimolar doses of PACAP27 and VIP induced hypotensive effects which were not significantly different (Table 1). At the lowest dose (1.5 nmol kg⁻¹ h⁻¹) the hypotension caused by PACAP27 or VIP was accompanied by trivial regional haemodynamic effects that were not different between the two peptides (Table 1). However, at doses of 7.5 and 15 nmol kg⁻¹ h⁻¹, although the depressor effects of PACAP27 and VIP were similar, the former caused renal, mesenteric and hindquarters vasodilatation whereas VIP caused a significantly greater hindquarters vasodilatation, accompanied by renal and mesenteric vasoconstrictions (Table 1).

Effect of ICI 118551 on regional haemodynamic responses to PACAP27 and VIP

In the absence of ICI 118551, the patterns of haemodynamic response to PACAP27 (15 nmol kg⁻¹ h⁻¹; Table 2) and VIP (7.5 nmol kg⁻¹ h⁻¹; Table 2) were similar to those in the previous experiment (Table 1). In the presence of ICI 118551, the hindquarters vasodilator effect of PACAP27 was attenuated (Table 2), but there were no other significant changes (Table 2). ICI 118551 also diminished the hindquarters vasodilator response to VIP and abolished its renal vasoconstrictor action (Table 2).

Effect of losartan on regional haemodynamic responses to PACAP27 and VIP

In the absence of losartan, the patterns of haemodynamic response to PACAP27 (15 nmol kg⁻¹ h⁻¹; Table 3) and VIP (7.5 nmol kg⁻¹ h⁻¹; Table 3) were similar to those in the previous experiments (Tables 1 and 2).

In the presence of losartan, there were no significant changes in the haemodynamic effects of PACAP27 (Table 3). In contrast, losartan enhanced the hypotensive action of VIP, and this effect was associated with renal and mesenteric vasodilatation, rather than vasoconstriction.

Discussion

In the present work, 20 min infusions of equimolar doses of PACAP27 and VIP caused similar, dose-dependent, hypotensive responses. However, this similarity of effect on mean systemic arterial blood pressure was accompanied by different regional haemodynamic changes. Thus, at doses of 7.5 and 15 nmol kg⁻¹ h⁻¹, the hindquarters hyperaemic vasodilator effects of VIP were greater than those of PACAP27, and were accompanied by renal and mesenteric vasoconstrictions rather than vasodilatations, as seen with PACAP27. Previous work in anaesthetized dogs has shown that VIP can cause reductions in renal and superior mesenteric flows and vascular conductances (Blitz & Charbon, 1983; Naruse *et al.*, 1986). However, in those studies, VIP also caused no change, or a reduction, in femoral arterial flow. Our finding of a marked vasodilator effect of VIP in the hindquarters of the conscious rat is thus more in line with the recent observations of Minkes *et al.* (1992) in the anaesthetized cat. How-

Figure 4 Cardiovascular changes during 20 min infusions of vasoactive intestinal polypeptide (VIP) at $1.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$ (●), $7.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$ (▲), or $15 \text{ nmol kg}^{-1} \text{ h}^{-1}$ (■) in the absence (left-hand panels) or presence (right-hand panels) of N^{G} -nitro-L-arginine methyl ester (L-NAME, $11 \mu\text{mol kg}^{-1} \text{ h}^{-1}$) in the same conscious, Long Evans rats ($n = 9$). Values are mean with s.e.mean; * $P < 0.05$ versus preinfusion baseline (Friedman's test).

ever, the latter workers found that PACAP27 caused biphasic changes (increase followed by decrease) in hindquarters vascular conductance and systemic arterial blood pressure. The delayed pressor and vasoconstrictor effects of PACAP27 were abolished by phentolamine or adrenalectomy, but these interventions did not influence the hindquarters dilator or depressor responses to VIP. Moreover, L-NAME and propranolol were without effect on the haemodynamic actions of PACAP27 or VIP (Minkes *et al.*, 1992). It is clear, therefore, that there are fundamental differences between the haemodynamic effects of PACAP27 and of VIP, in conscious rats compared to anaesthetized cats.

Our results indicate that the hindquarters vasodilator effects of PACAP27 and VIP involved an L-NAME-sensitive

component, and that the latter was larger in the case of VIP. It is feasible that both endothelium-dependent and endothelium-independent mechanisms (Gaw *et al.*, 1991; Warren *et al.*, 1991) were involved, because in the presence of L-NAME there was a significant residual hindquarters vasodilator response to PACAP27 and VIP.

Since the β_2 -adrenoceptor antagonist, ICI 118551, caused significant inhibition of the hindquarters vasodilator effect of VIP and of PACAP27, and since both peptides have been shown to stimulate adrenal medullary catecholamine release in the rat (Malhotra & Wakade, 1987; Wakade *et al.*, 1991a,b), it is likely that a proportion of the hindquarters vasodilator action of PACAP27 and VIP involved adrenal medullary adrenaline release causing activation of hind-

Figure 5 Cardiovascular changes during 20 min infusions of vasoactive intestinal polypeptide (VIP) at $1.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$ (●), $7.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$ (▲), or $15 \text{ nmol kg}^{-1} \text{ h}^{-1}$ (■) in the absence (left-hand panels) or presence (right-hand panels) of N^{G} -nitro-L-arginine methyl ester (L-NAME, $11 \text{ } \mu\text{mol kg}^{-1} \text{ h}^{-1}$) in the same conscious, Long Evans rats ($n = 9$). Values are mean with s.e.mean; * $P < 0.05$ versus preinfusion baseline (Friedman's test).

Table 2 Integrated (area under or over curve, AUC or $\text{AOC}_{0-20 \text{ min}}$, respectively) cardiovascular responses during infusion of PACAP27 ($15 \text{ nmol kg}^{-1} \text{ h}^{-1}$) or vasoactive intestinal polypeptide (VIP) ($7.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$) in the same conscious Long Evans rats ($n = 7$) in the absence (control) or presence of ICI 118551 (670 nmol kg^{-1} bolus; $335 \text{ nmol kg}^{-1} \text{ h}^{-1}$ infusion)

		PACAP27	VIP
Heart rate	Control	2581 ± 222	2071 ± 89
(AUC; beats)	+ ICI 118551	2581 ± 249	1970 ± 238
Mean blood pressure	Control	-396 ± 27	-364 ± 32
(AOC; mmHg min)	+ ICI 118551	-312 ± 40	-319 ± 36
Renal conductance	Control	260 ± 50	-121 ± 48
(AUC or AOC; $[\text{kHz mmHg}^{-1}]10^3 \text{ min}$)	+ ICI 118551	248 ± 55	$63 \pm 21^\dagger$
Mesenteric conductance	Control	306 ± 36	-309 ± 42
(AUC or AOC; $[\text{kHz mmHg}^{-1}]10^3 \text{ min}$)	+ ICI 118551	262 ± 48	-235 ± 54
Hindquarters conductance	Control	638 ± 109	744 ± 108
(AUC; $[\text{kHz mmHg}^{-1}]10^3 \text{ min}$)	+ ICI 118551	$396 \pm 67^\dagger$	$540 \pm 107^\dagger$

Values are mean $\pm$ s.e.mean.

$^\dagger P < 0.05$ versus control (Wilcoxon's test).

quarters, β_2 -adrenoceptor-mediated vasodilatation (Gardiner *et al.*, 1991a,b). It is possible that a component of this response contributed to the L-NAME-sensitive hindquarters vasodilator effect of PACAP27 and VIP, since elsewhere (Gardiner *et al.*, 1991a,b) we have shown the hindquarters vasodilator effects of exogenous β_2 -adrenoceptor agonists are reduced in the presence of L-NAME.

Although the hypotensive effects of PACAP27 and VIP were matched, the former caused renal and mesenteric vasodilations whereas VIP caused vasoconstriction in these

vascular beds. Since there was little change in renal or mesenteric blood flows in the presence of PACAP27, it is possible that the increases in renal and mesenteric vascular conductances were a manifestation of autoregulation. However, it then remains to explain why such a phenomenon was not apparent during infusion of VIP. It could be that activation of indirect vasoconstrictor mechanisms during infusion of VIP had a predominant influence in the renal and mesenteric vascular beds. But, in the presence of L-NAME, the highest dose of VIP had an enhanced hypotensive effect,

Table 3 Integrated (area under or over curve, AUC or AOC_{0–20 min}, respectively) cardiovascular responses during infusion of PACAP27 (15 nmol kg⁻¹ h⁻¹) or vasoactive intestinal polypeptide (VIP) (7.5 nmol kg⁻¹ h⁻¹) in the same conscious Long Evans rats (*n* = 9) in the absence (control) or presence of losartan (20 µmol kg⁻¹)

		PACAP27	VIP
Heart rate	Control	3001 ± 159*	2229 ± 293*
(AUC; beats)	+losartan	2421 ± 90*	2163 ± 144*
Mean blood pressure	Control	-418 ± 32*	-406 ± 51*
(AOC; mmHg min)	+losartan	-428 ± 38*	-589 ± 43*†
Renal conductance	Control	406 ± 74*	-75 ± 32*
(AUC or AOC; [kHz mmHg ⁻¹]10 ³ min)	+losartan	340 ± 42*	184 ± 40*†
Mesenteric conductance	Control	389 ± 43*	-151 ± 33*
(AUC or AOC; [kHz mmHg ⁻¹]10 ³ min)	+losartan	543 ± 102*	138 ± 41*†
Hindquarters conductance	Control	613 ± 95*	582 ± 96*
(AUC; [kHz mmHg ⁻¹]10 ³ min)	+losartan	585 ± 80*	826 ± 81*

Values are mean ± s.e.mean.

**P* > 0.05 versus baseline;

†*P* < 0.05 versus control (Wilcoxon's test).

yet there was no renal or mesenteric vasoconstriction. Thus, it would have to be proposed that, in the presence of L-NAME-induced hypertension, activation of vasoconstrictor mechanisms, consequent upon VIP-induced hypotension, did not occur in the way it did in the absence of L-NAME because, in the presence of L-NAME, VIP did not cause blood pressure to fall below baseline levels. Since losartan augmented the hypotensive effects of VIP, and suppressed its renal and mesenteric vasoconstrictor actions it is likely that the latter effects were normally due to VIP-induced activation of the renin-angiotensin system. However, if this was a direct effect of VIP (Porter *et al.*, 1982) it should occur in the presence of L-NAME, unless VIP-induced stimulation of renin release involved NO.

An indirect action of VIP to stimulate renin release through β₂-adrenoceptors is consistent with the finding that ICI 118551 abolished the renal vasoconstrictor response to VIP. However, in conscious rabbits, the non-selective β-adrenoceptor antagonist, propranolol, had no effect on renin release in response to VIP (Calam *et al.*, 1982). Moreover, if VIP-induced renin release was secondary to its hypotensive effect, it is unclear why PACAP27 should not have a similar effect, but it did not appear to do so because losartan had no influence on the haemodynamic actions of PACAP27. It is feasible that the latter peptide interfered with renin release, or had such potent renal and mesenteric dilator actions that any constrictor effect of angiotensin II on the renal or mesenteric vascular bed was not expressed in its presence.

It is clear from the above that the regional haemodynamic effects of PACAP27 and VIP probably involved a number of different mechanisms, both direct and indirect, the contribu-

tions from which varied according to the peptide, and the dose. Thus, the differential effects of L-NAME on the hypotensive actions of PACAP27 and VIP probably reflected the changing relations between vasoconstriction and vasodilatation in the different vascular beds. For example, in one situation in the presence of L-NAME the hypotension was augmented in association with enhancement of renal vasodilatation (e.g. PACAP27 at 1.5 nmol kg⁻¹ h⁻¹), whereas in another situation there was no augmentation of the hypotension since, although in renal and mesenteric vascular beds, vasoconstrictions had been converted to vasodilatations, there was marked attenuation of the large hindquarters vasodilatation (e.g. VIP at 7.5 nmol kg⁻¹ h⁻¹). We have already discussed possible mechanisms underlying the inhibitory effects of L-NAME on vasodilator responses (see above). Since L-NAME inhibits NO production, and thereby decreases activation of cytosolic guanylate cyclase (see Ignarro, 1991, for review), then any augmented vasodilator responses to PACAP27 or VIP, in the presence of L-NAME, may have been due to enhanced effects of adenosine 3':5'-cyclic monophosphate (cyclic AMP), contingent upon decreased cyclic GMP-mediated stimulation of phosphodiesterase (see Lincoln & Cornwell, 1993, for review). However, additional experiments would be needed to demonstrate such putative interactions *in vivo*.

Whatever the details underlying the differences between the haemodynamic actions of PACAP27 and VIP, our findings are consistent with these peptides acting on different receptors (Minkes *et al.*, 1992) rather than on the same receptors (Nandha *et al.*, 1991) to exert their haemodynamic effects.

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