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European Journal of Pharmacology 365 (1999) 205–215

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Hemodynamic actions of systemically injected pituitary adenylate cyclase activating polypeptide-27 in the rat

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Received 9 November 1998; accepted 13 November 1998

Abstract

The aims of this study were (1) to characterize the hemodynamic mechanisms underlying the hypotensive effects of pituitary adenylate cyclase activating polypeptide-27 (PACAP-27 0.1–2.0 nmol/kg, i.v.) in pentobarbital-anesthetized rats, and (2) to determine the roles of the autonomic nervous system, adrenal catecholamines and endothelium-derived nitric oxide (NO) in the expression of PACAP-27-mediated effects on hemodynamic function. PACAP-27 produced dose-dependent decreases in mean arterial blood pressure and hindquarter and mesenteric vascular resistances in saline-treated rats. PACAP-27 also produced pronounced falls in mean arterial blood pressure in rats treated with the ganglion blocker, chlorisondamine (5 mg/kg, i.v.). The hypotensive and vasodilator actions of PACAP-27 were not attenuated by the β-adrenoceptor antagonist, propranolol (1 mg/kg, i.v.), or the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME 50 μmol/kg, i.v.). PACAP-27 produced dose-dependent increases in heart rate whereas the hypotensive response produced by the nitrovasodilator, sodium nitroprusside (10 μg/kg, i.v.), was associated with a minimal tachycardia. The PACAP-27-induced tachycardia was unaffected by chlorisondamine, but was virtually abolished by propranolol. These results suggest that the vasodilator effects of PACAP-27 are due to actions in the microcirculation rather than to the release of adrenal catecholamines and that this vasodilation may not involve the release of endothelium-derived NO. These results also suggest that PACAP-27 produces tachycardia by directly releasing norepinephrine from cardiac sympathetic nerve terminals rather than by direct or baroreceptor reflex-mediated increases in sympathetic nerve activity. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: PACAP-27 (pituitary adenylate cyclase activating polypeptide-27); Nitric oxide (NO); Hemodynamics; (Rat)

1. Introduction

Pituitary adenylate cyclase activating polypeptide exists as 27 amino acid (PACAP-27) and 38 amino acid (PACAP-38) forms (Miyata et al., 1989, 1990; Arimura, 1992). These peptides exert their effects via activation of G protein-coupled PACAP receptors which activate adenylate cyclase and also increase intracellular levels of calcium (Christophe, 1993). The type I PACAP receptor is specific for PACAP whereas the type II receptor binds PACAP and vasoactive intestinal polypeptide (VIP) (Shivers et al., 1991; Christophe, 1993). The presence of PACAP in nerve fibers innervating smaller arteries in the brain (Koves et al., 1990), respiratory tract (Uddman et al., 1991) and intestine (Arimura, 1992) suggest that PACAP may regulate vascular resistance in these beds. Warren et al. (1991) found that the relaxant effects of PACAP-27 and -38 were similar in endothelium intact and endothelium denuded rabbit thoracic aortic rings. They also found that the vasorelaxant responses to these peptides correlated with increases in cAMP levels in the vessels (Warren et al., 1991). These findings suggest that the PACAP polypeptides exert their vasorelaxant effects in vitro by direct activation of G_s protein-coupled PACAP sensitive receptors on vascular smooth muscle which activate adenylate cyclase.

Nandha et al. (1991) reported that systemic injections of PACAP-27 and -38 produce hypotension in anesthetized female Wistar rats and that binding sites for these peptides exist on plasma membranes of femoral and iliac arteries of these rats. In addition, Minkes et al. (1992a) provided evidence that the hemodynamic effects of PACAP-27 in

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PII: S0014-2999(98)00852-8

anesthetized cats may involve the release of epinephrine from the adrenal glands. Moreover, they found that the nitric oxide (NO) synthesis inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) did not attenuate the vasodilator actions of PACAP-27 in pentobarbital-anesthetized cats (Minkes et al., 1992a). This suggests that the vasodilator effects of PACAP-27 do not involve the release of endothelium-derived NO in vivo. Although it is likely that the hypotensive effects of systemically injected PACAP-27 in the rat involves direct endothelium independent actions on the microcirculation, the possibility that PACAP-27 may affect vascular resistance by direct changes in autonomic function or the release of endothelium-derived NO or adrenal catecholamines have not been addressed.

The systemic injection of PACAP-38 produces a tachycardia in conscious sheep (Sawangjaroen et al., 1992). The magnitude of the PACAP-38-induced tachycardia exceeded that expected from activation of the baroreceptor reflex (Sawangjaroen et al., 1992). Nandha et al. (1991) found that the systemic injection of PACAP-27 and -38 produced a tachycardia which may have been due to activation of the baroreceptor reflex since these peptides had no effect on beating rate of isolated atria. In contrast, PACAP-38 increased the beating rate of cardiac myocytes cultured from neonatal rat hearts and stimulated adenylate cyclase activity in crude membranes and homogenates of rat heart (Arimura, 1992). These findings suggest that PACAP-induced tachycardia may be due to direct actions on the heart or to baroreceptor-mediated changes in autonomic nerve activity. At present, the mechanisms by which systemically administered PACAP-27 affects heart rate in the rat have not been fully elucidated.

The aims of the present study were (i) to determine the hemodynamic mechanisms underlying the hypotensive effects of PACAP-27 in the pentobarbital anesthetized rat, (ii) the roles of the autonomic nervous system, adrenal catecholamines and endothelium-derived NO in the expression of PACAP-27-mediated changes in hemodynamic function, and (iii) to determine whether the tachycardia produced by PACAP-27 is due to direct or baroreceptor-mediated increases in sympathetic drive to the heart or whether PACAP-27 may directly release norepinephrine from cardiac sympathetic nerve terminals.

2. Materials and methods

2.1. Rats and surgical procedures

The protocols were approved by the University of Iowa Animal Care and Use Committee. Sprague–Dawley rats (250–350 g; $n = 40$) were anesthetized with pentobarbital (50 mg/kg, i.p.). Polyethylene catheters were inserted into the femoral vein to administer drugs and into the lower abdominal aorta for measurement of pulsatile and mean arterial pressure and the determination of heart rate. The rats received supplemental doses of pentobarbital (5 mg/kg, i.v.) as needed. In some rats, a midline laparotomy was performed and pulsed Doppler flow probes were placed around the superior mesenteric artery and lower abdominal aorta to measure blood flow velocities and to calculate vascular resistances (Davisson et al., 1996a,b; Kooy and Lewis, 1996). Details of the Doppler technique, including construction of the probes, the reliability of the method for the estimation of blood flow velocity, and the quantitative determination of percent changes in resistance, have been described previously (Haywood et al., 1981). During surgery and experimentation, the body temperature of the rats was maintained at 37°C by a thermostat controlled heating pad and the rats were allowed to breathe room air supplemented with 95% O_2–5% CO_2 via a face mask. The arterial catheter was connected to a Beckman

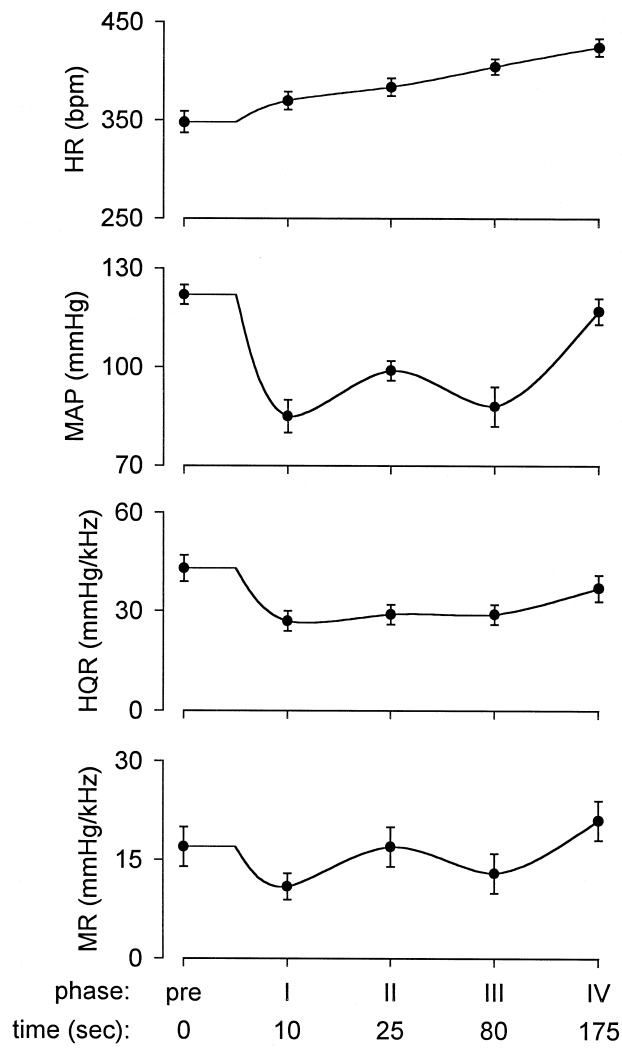

Fig. 1. A summary of the time dependent changes in heart rate (HR), mean arterial blood pressure (MAP) and hindquarter (HQR) and mesenteric (MR) vascular resistances produced by PACAP-27 (2 nmol/kg, i.v.) in pentobarbital-anesthetized rats ($n = 5$). Phase I represents the initial peak hypotensive and vasodilator effects of PACAP-27. Phase II represents a brief recovery phase. Phase III represents the secondary and more prolonged hypotensive phase. Phase IV represents the time of maximal PACAP-27-induced tachycardia.

Dynograph-coupled pressure transducer (Cobe Laboratories, Awada, CO) for the measurement of pulsatile and mean arterial blood pressure. Heart rate was determined from pulsatile arterial pressure by way of a Beckman Dynograph-coupled cardiotachometer. The wire leads from the flow probes were connected to a Beckman Dynograph-coupled pulsed-Doppler flowmeter (Department of Bioengineering, The University of Iowa, Iowa City, IA) to allow for the recording of blood flow velocities (Davisson et al., 1996a,b; Kooy and Lewis, 1996).

2.2. Experimental protocols

2.2.1. Protocol 1

One group of rats ($n = 5$) received L-NAME (50 μmol/kg, i.v.) and after 15–20 min, at which time the L-NAME-induced hemodynamic responses had reached their plateau levels, the rats received bolus injections of PACAP-27 (0.1–2.0 nmol/kg, i.v.). The dose of L-NAME was chosen on the basis that it produces substantial increases in mean arterial blood pressure and vascular resistances which are likely to involve the blockade of the de novo synthesis of NO and related nitrosyl factors (Davisson et al., 1996a,b). Another group of rats ($n = 5$) received an injection of saline (0.9% NaCl, i.v.) and after 15–20 min, they received the above doses of PACAP-27. In each case, the PACAP-27-induced responses were allowed to recover before the next injection of PACAP-27 was given.

2.2.2. Protocol 2

The changes in mean arterial blood pressure and heart rate produced by PACAP-27 (2 nmol/kg, i.v.), the β-adrenoceptor agonist, isoproterenol (10 μmol/kg, i.v.), and 5-hydroxytryptamine (5-HT; 30 μg/kg, i.v.) were determined in pentobarbital-anesthetized rats ($n = 5$) before and after injection of the ganglion blocker, chlorisondamine (5 mg/kg, i.v.). This dose of chlorisondamine was chosen because it blocks baroreceptor-mediated changes in heart rate in response to pressor and depressor agents (Lewis et al., 1989) and abolishes the vagal efferent-mediated bradycardia produced by systemic injection of 5-HT (see Section 3). The cardiovascular responses produced by each test agent were allowed to recover before the next test agent was given. The time course of the changes in mean arterial blood pressure following injection of chlorisondamine (5 mg/kg, i.v.) were examined in rats ($n = 5$) which received the angiotensin II receptor antagonist, losartan (1 mg/kg, i.v.), and the vasopressin V_1 receptor antagonist, [β-Mercapto-β,β-cyclopentamethylenepropionyl[1],*O*-Et-Tyr[2], Val[4], Arg[8]]vasopressin (10 μg/kg, i.v.), immediately after the hypotensive effects of chlorisondamine had developed. This dose of the vasopressin V_1 receptor antagonist was chosen because it abolishes the cardiovascular effects of high doses of arginine vasopressin (Ohta et al., 1991 and Section 3). The dose of losartan was chosen because it abolishes the pressor responses produced by systemically injected angiotensin II (10 ng/kg, i.v.) in ganglion blocked rats (see Section 3).

2.2.3. Protocol 3

The changes in mean arterial blood pressure and heart rate produced by PACAP-27 (2 nmol/kg, i.v.), epinephrine (10 nmol/kg, i.v.), and the NO-donor, sodium nitroprusside (10 μg/kg, i.v.), were determined in pentobarbital-anesthetized rats ($n = 5$) before and after administration of the β-adrenoceptor antagonist, propranolol (1 mg/kg, i.v.). In each case, the cardiovascular responses produced by each test agent were allowed to recover before the next test agent was given. This dose of propranolol was chosen because it produces a substantial reduction in heart rate and because it eliminates the pronounced hypotension, vasodilation and tachycardia produced by high concentrations of the β-adrenoceptor agonist, isoproterenol (10 μg/kg, i.v.; see Section 3).

2.3. Drugs

PACAP-27 was obtained from Bachem California (Torrance, CA). L-NAME, 5-HT, isoproterenol, propranolol,

Table 1
Effects of saline and L-NAME on resting hemodynamic parameters

Parameter	Treatment	Pre	Post	% Change
HR (bpm)	Saline	344 ± 8	353 ± 3	+3 ± 2
	L-NAME	317 ± 9	282 ± 16[b]	−11 ± 3[a]
MAP (mm Hg)	Saline	118 ± 4	119 ± 5	+1 ± 2
	L-NAME	119 ± 5	154 ± 2[b]	+30 ± 4[a]
HQR (mm Hg/kHz)	Saline	119 ± 11	121 ± 9	+4 ± 10
	L-NAME	100 ± 15	220 ± 26[b]	+125 ± 18[a]
MR (mm Hg/kHz)	Saline	37 ± 8	42 ± 9	+13 ± 9
	L-NAME	51 ± 5	146 ± 13[b]	+186 ± 13[a]

L-NAME is N^G-nitro-L-arginine methyl ester.
HR is heart rate, MAP is mean arterial blood pressure, and HQR and MR are hindquarter and mesenteric vascular resistances, respectively.
The dose of L-NAME was 50 μmol/kg, i.v.
Each value represents the mean ± S.E.M. of the resting values and the percent changes in these values.
The saline- and L-NAME-treated groups consisted of five rats each.
[a] $P < 0.05$, significant change from pre values.
[b] $P < 0.05$, post-L-NAME values vs. post-saline values.

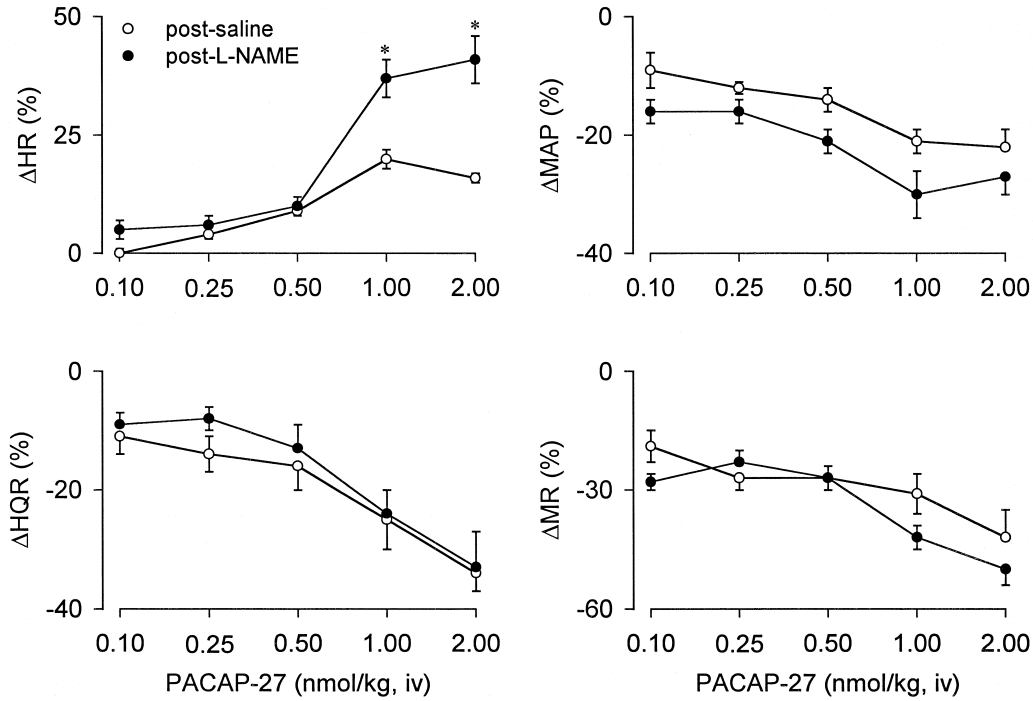

Fig. 2. A summary of the maximal initial (Phase I) changes in mean arterial blood pressure (ΔMAP) and hindquarter (ΔHQR) and mesenteric (ΔMR) vascular resistances produced by PACAP-27 (0.1–2.0 nmol/kg, i.v.) in saline-treated ($n = 5$) and L-NAME-treated (50 μmol/kg, i.v.; $n = 5$) rats. The changes in heart rate (ΔHR) are also shown. The data are expressed as mean ± S.E.M of the percent changes in these parameters. $* P < 0.05$, post-L-NAME vs. post-saline.

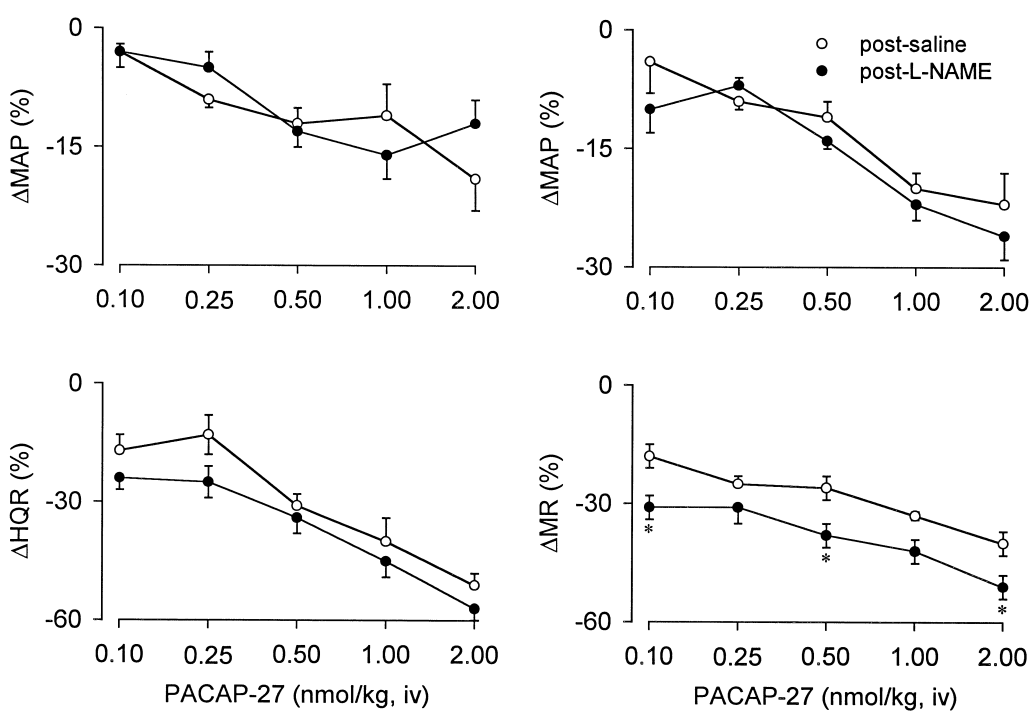

Fig. 3. A summary of the maximal secondary (Phase II) changes in hindquarter (ΔHQR) and mesenteric (ΔMR) vascular resistances produced by PACAP-27 (0.1–2.0 nmol/kg, i.v.) in saline-treated ($n = 5$) and L-NAME-treated (50 μmol/kg, i.v.; $n = 5$) rats. The changes in mean arterial blood pressure (ΔMAP) at the point of maximal vasodilation in these beds are also shown. The data are expressed as mean ± S.E.M of the percent changes in these parameters. $* P < 0.05$, post-L-NAME vs. post-saline.

Table 2
Effects of chlorisondamine on the cardiovascular actions of PACAP-27, isoproterenol and 5-HT

Agonist	Parameter	Treatment	Actual values		Δ Change	% Change
			Pre-CLX	Post-CLX		
PACAP-27	MAP (mm Hg)	pre-CLX	117 ± 2	76 ± 4	−40 ± 4[a]	−34 ± 3[a]
		post-CLX	87 ± 4[b]	56 ± 2	−32 ± 4[a]	−36 ± 4[a]
	HR (bpm)	pre-CLX	367 ± 15	449 ± 12	+81 ± 15[a]	+24 ± 6[a]
		post-CLX	309 ± 13[b]	409 ± 10	+100 ± 18[a]	+34 ± 7[a]
Isoproterenol	MAP (mm Hg)	pre-CLX	113 ± 4	69 ± 5	−44 ± 5[a]	−39 ± 4[a]
		post-CLX	88 ± 3[b]	50 ± 2	−38 ± 2[a]	−43 ± 2[a]
	HR (bpm)	pre-CLX	362 ± 14	514 ± 9	+152 ± 12[a]	+43 ± 6[a]
		post-CLX	317 ± 10[b]	517 ± 6	+200 ± 5[a,c]	+64 ± 4[a,c]
5-HT	MAP (mm Hg)	pre-CLX	109 ± 3	89 ± 4	−21 ± 2[a]	−19 ± 2[a]
		post-CLX	88 ± 2[b]	88 ± 2	0 ± 1	0 ± 1
	HR (bpm)	pre-CLX	366 ± 10	282 ± 17	−85 ± 9[a]	−23 ± 3[a]
		post-CLX	330 ± 13[b]	329 ± 12	−1 ± 1	0 ± 1

HR is heart rate and MAP is mean arterial blood pressure.
The dose of chlorisondamine (CLX) was 5 mg/kg, i.v.
The i.v. dose of PACAP-27 was 2 nmol/kg.
The i.v. dose of isoproterenol was 10 μg/kg.
The i.v. dose of 5-HT was 30 μg/kg.
Each value represents the mean ± S.E.M. of the actual values or the arithmetic or percent changes in these values.
There were five rats in the group.
[a] $P < 0.05$, significant change from pre-CLX values.
[b] $P < 0.05$, post-CLX resting values vs. pre-CLX resting values.
[c] $P < 0.05$, post-CLX responses vs. pre-CLX responses.

and [β-Mercapto-β,β-cyclopentamethylenepropionyl[1],*O*-Et-Tyr[2], Val[4], Arg[8]]vasopressin was obtained from Sigma (St. Louis, MO). Sterile saline, sodium pentobarbital and sodium nitroprusside were obtained from Abbott Laboratories (Chicago, IL). Chlorisondamine was obtained from Ciba-Geigy (Summit, NJ). Losartan was obtained from Merck (Rahway, NJ).

2.4. Statistical analyses

The data are presented as the mean ± S.E.M. of actual values or the arithmetic and percent changes in these values. Vascular resistances were determined by dividing mean arterial blood pressure by blood flow velocity. The data were analyzed by repeated measures analysis of variance (Winer, 1991) followed by Student's modified *t*-test with the Bonferroni correction for multiple comparisons between means (Wallenstein et al., 1980).

3. Results

3.1. Time course of effects of PACAP-27 on hemodynamic parameters

A summary of the time dependent changes in heart rate, mean arterial blood pressure and hindquarter and mesenteric vascular resistances produced by the i.v. injection of

Table 3
Effects of saline or propranolol on resting hemodynamic parameters

Parameter	Treatment	Pre	Post	% Change
HR (bpm)	Saline	337 ± 10	341 ± 9	0 ± 1
	Propranolol	348 ± 11	300 ± 9	−13 ± 3[a]
MAP (mm Hg)	Saline	117 ± 4	118 ± 5	+1 ± 2
	Propranolol	122 ± 3	116 ± 6	−4 ± 4
HQR (mm Hg/kHz)	Saline	53 ± 7	51 ± 6	−4 ± 5
	Propranolol	40 ± 4	43 ± 7	+4 ± 9
MR (mm Hg/kHz)	Saline	19 ± 4	21 ± 3	+12 ± 8
	Propranolol	17 ± 3	18 ± 3	+10 ± 10

HR is heart rate, MAP is mean arterial blood pressure, and HQR and MR are hindquarter and mesenteric vascular resistances, respectively.
The dose of propranolol was 1 mg/kg, i.v.
Each value represents the mean ± S.E.M. of the resting values and the percent changes in these values.
The saline- and propranolol-treated groups each consisted of five rats.
[a] $P < 0.05$, significant change from pre values.

PACAP-27 (2 nmol/kg) in pentobarbital anesthetized rats ($n = 5$) is shown in Fig. 1. As can be seen, the injection of PACAP-27 produced relatively rapid (within 5–10 s) falls in mean arterial blood pressure and vascular resistances and a rise in heart rate (Phase I). The initial hypotensive and mesenteric vasodilator effects of PACAP-27 then subsided after 20–30 s (Phase II) whereas the hindquarter vasodilation and tachycardia was sustained at this time. The mean arterial blood pressure and mesenteric resistance dropped again after 70–90 s (Phase III) whereas the hindquarter vasodilation remained constant and heart rate continued to increase. The mean arterial blood pressure and vascular resistances returned to baseline after approximately 175 s (Phase IV). There were no further changes in mean arterial blood pressure or resistances. In contrast, the PACAP-27-induced increases in heart rate reached maximal levels after approximately 175 s (Phase IV). This tachycardia subsided 300–340 s after the injection of PACAP-27.

3.2. Hemodynamic effects of PACAP-27 in saline-treated or in L-NAME-treated rats

The effects of systemic administration of saline or L-NAME (50 μmol/kg, i.v.) on resting hemodynamic parameters are summarized in Table 1. The post-saline- and post-L-NAME-treatment values are the averages of those recorded prior to each injection of PACAP-27 (see below). The post-saline injection values were not different than those observed prior to injection ($P > 0.05$, for all comparisons). The post-L-NAME heart rate values were lower than those prior to injection of the NO synthesis inhibitor ($P < 0.05$). The post-L-NAME-treatment mean arterial blood pressure and vascular resistances were higher than those before injection ($P < 0.05$, for all comparisons). These L-NAME-induced effects were sustained throughout the experiments.

The maximal initial (Phase I) changes in heart rate, mean arterial blood pressure and vascular resistances produced by PACAP-27 (0.1–2.0 nmol/kg, i.v.) in saline-treated ($n = 5$) or in L-NAME-treated rats (50 μmol/kg, i.v.; $n = 5$) are summarized in Fig. 2. PACAP-27 produced dose dependent falls in mean arterial blood pressure and vascular resistances and dose dependent increases in heart rate in the saline-treated rats. The PACAP-27-induced increases in heart rate were due to direct actions in the heart rather than to baroreceptor-mediated changes in sympathetic nerve activity (see below). The PACAP-27-induced falls in mean arterial blood pressure and vascular resistances were similar in the L-NAME-treated as compared to the saline-treated rats ($P > 0.05$, for all comparisons). However, the percent increases in heart rate produced by the two higher doses of PACAP-27 were greater in L-NAME than in saline-treated rats ($P < 0.05$, for both comparisons). The arithmetic increases in heart rate produced by these two doses of PACAP-27 were also larger in L-NAME-treated as compared to saline-treated rats. For example, the arithmetic increases in heart rate produced by the 2 nmol/kg dose of PACAP-27 in the saline and L-NAME-treated rats were 59 ± 4 vs. 115 ± 11 bpm, respectively ($P < 0.05$).

The secondary (Phase III) changes in hindquarter and mesenteric vascular resistances produced by the injection of PACAP-27 (0.1–2.0 nmol/kg, i.v.) in the saline-treated ($n = 5$) and L-NAME-treated rats (50 μmol/kg, i.v.; $n = 5$) are summarized in Fig. 3. The time of the maximal falls in hindquarter and mesenteric resistances did not exactly coincide. As such, the falls in mean arterial blood pressure shown in this figure are those which occurred at the times of maximal vasodilation in each bed. The injections of PACAP-27 produced dose dependent vasodilator responses

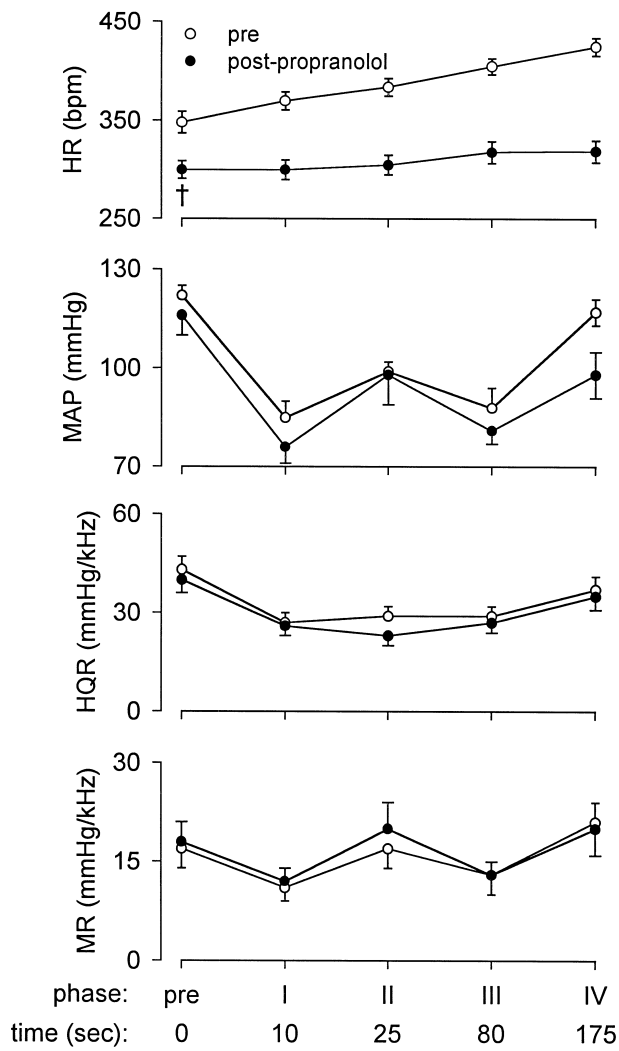

Fig. 4. A summary of the heart rate (HR), mean arterial blood pressure (MAP), and hindquarter (HQR) and mesenteric (MR) vascular resistance values of pentobarbital-anesthetized rats ($n = 5$) following the injection of PACAP-27 (2.0 nmol/kg, i.v.) both before and after the administration of propranolol (1 mg/kg, i.v.). The data are expressed as mean ± S.E.M. of the actual values. $^{\dagger}P < 0.05$, post-propranolol resting values vs. pre-propranolol values.

in the hindquarter bed which were unaffected by L-NAME ($P > 0.05$, for all comparisons). PACAP-27 produced a dose dependent vasodilation in the mesenteric bed of saline-treated rats. The mesenteric vasodilation produced by the 0.1, 0.5 and 2.0 nmol/kg doses of PACAP-27 were augmented in the L-NAME-treated rats ($P > 0.05$, for all comparisons).

3.3. Changes in mean arterial blood pressure and heart rate produced by PACAP-27, isoproterenol and 5-HT in ganglion blocked rats

The changes in mean arterial blood pressure and heart rate produced by PACAP-27 (2 nmol/kg, i.v.), the β-adrenoceptor agonist, isoproterenol (10 μmol/kg, i.v.), and 5-HT (30 μg/kg, i.v.) in pentobarbital-anesthetized rats ($n = 5$) before and after injection of the ganglion blocker, chlorisondamine (5 mg/kg, i.v.), are shown in Table 2. Chlorisondamine immediately reduced mean arterial blood pressure from 112 ± 3 to 66 ± 4 mm Hg (-46 ± 5 mm Hg, $P < 0.05$). However, the mean arterial blood pressure recovered to higher resting values (88 ± 4 mm Hg, $P < 0.05$ compared to initial hypotensive values) within 10–15 min. This increase in mean arterial blood pressure is due to the actions of circulating angiotensin II and arginine vasopressin. The mean arterial blood pressure values of another group of chlorisondamine treated rats ($n = 5$) fell immediately from 116 ± 3 to 73 ± 3 mm Hg (-43 ± 3 mm Hg, $P < 0.05$). The subsequent injection of the angiotensin II receptor antagonist, losartan (1 mg/kg, i.v.), and the vasopressin V_1 receptor antagonist, [β-Mercapto-β,β-cyclopentamethylenepropionyl^1,*O*-Et-Tyr^2, Val^4, Arg^8]vasopressin (10 μg/kg, i.v.), prevented the gradual rise in mean arterial blood pressure following injection of chlorisondamine. The mean arterial blood pressure values 15 and 30 min after injection of the ganglion blocker were 75 ± 3 and 76 ± 4 mm Hg, respectively ($P > 0.05$ compared to immediate hypotensive value). We established that this dose of losartan (1 mg/kg, i.v.) completely blocked the pronounced pressor response produced by angiotensin II (20 ng/kg, i.v.) in chlorisondamine-treated rats ($n = 5$). The angiotensin II-induced pressor responses before and after administration of losartan were $+47 \pm 5\%$ and $+1 \pm 1\%$, respectively ($P < 0.05$). We have established that this dose of the vasopressin V_1 receptor antagonist (10 μg/kg, i.v.) completely blocked the pronounced pressor response produced by arginine vasopressin (20 ng/kg, i.v.) in chlorisondamine-treated rats ($n = 5$). The arginine vasopressin-induced pressor response before and after administration of the vasopressin V_1 receptor antagonist were $+39 \pm 6\%$ and $+3 \pm 2\%$, respectively ($P < 0.05$). Chlorisondamine also produced a fall in heart rate which reached maximum after 10–15 min (see Table 2). The heart rate remained at these levels for the duration of the experiments.

PACAP-27 produced a substantial fall in mean arterial blood pressure and a pronounced increase in heart rate ($P < 0.05$ for both responses). These PACAP-27-induced responses were unaffected by chlorisondamine ($P > 0.05$ for both comparisons). Isoproterenol also produced a substantial fall in mean arterial blood pressure and a pronounced increase in heart rate ($P < 0.05$ for both comparisons). The isoproterenol-induced hypotension was unaffected whereas the tachycardia was augmented after administration of chlorisondamine ($P > 0.05$). 5-HT produced a substantial Bezold–Jarisch reflex-mediated fall in mean arterial blood pressure and heart rate ($P < 0.05$ for both comparisons). These 5-HT-induced responses were abolished by chlorisondamine ($P < 0.05$ for both comparisons).

3.4. Hemodynamic effects of PACAP-27 in propranolol-treated rats

A summary of the effects of saline (0.9% NaCl; $n = 5$) or propranolol (1 mg/kg, i.v.; $n = 5$) on resting hemody-

Table 4
Effects of propranolol on hemodynamic effects of PACAP-27

Parameter	Treatment	Response phase			
		I	II	III	IV
ΔHR (%)	Pre-propranolol	$+7 \pm 1^a$	$+11 \pm 1^a$	$+17 \pm 2^a$	$+23 \pm 2^a$
	Post-propranolol	0 ± 1^b	$+2 \pm 1^b$	$+6 \pm 1^{a,b}$	$+6 \pm 1^{a,b}$
ΔMAP (%)	Pre-propranolol	-32 ± 4^a	-18 ± 2^a	-27 ± 5^a	-4 ± 2
	Post-propranolol	-35 ± 2^a	-16 ± 4^a	-29 ± 4^a	-14 ± 6
ΔHQR (%)	Pre-propranolol	-33 ± 4^a	-41 ± 5^a	-31 ± 5^a	-12 ± 4^a
	Post-propranolol	-35 ± 5^a	-36 ± 4^a	-27 ± 3^a	-15 ± 3^a
ΔMR (%)	Pre-propranolol	-36 ± 4^a	-2 ± 6	-20 ± 5^a	$+26 \pm 7^a$
	Post-propranolol	-32 ± 6^a	$+5 \pm 4$	-28 ± 4^a	$+13 \pm 4^a$

HR is heart rate, MAP is mean arterial blood pressure, and HQR and MR are hindquarter and mesenteric vascular resistances, respectively.
The dose of propranolol was 1 mg/kg, i.v.
Each value represents the mean ± S.E.M. of the percent changes in the hemodynamic values from five rats.
[a] $P < 0.05$, significant change from pre-propranolol values.
[b] $P < 0.05$, post-propranolol vs. pre-propranolol.

Table 5
Effects of propranolol on the hemodynamic effects of sodium nitroprusside and epinephrine

Parameter	Sodium nitroprusside		Epinephrine	
	Pre-propranolol	Post-propranolol	Pre-propranolol	Post-propranolol
ΔHR (%)	$+5 \pm 1^{a}$	$1 \pm 1^{a,b}$	0 ± 2	-4 ± 2
ΔMAP (%)	-45 ± 5^{a}	-48 ± 3^{a}	$+20 \pm 3^{a}$	$+41 \pm 6^{a,b}$
ΔHQR (%)	-42 ± 6^{a}	-45 ± 6^{a}	-46 ± 4^{a}	$+32 \pm 9^{a,b}$
ΔMR (%)	-43 ± 2^{a}	-46 ± 3^{a}	$+210 \pm 20^{a}$	$+338 \pm 44^{a,b}$

HR is heart rate, MAP is mean arterial blood pressure, and HQR and MR are hindquarter and mesenteric vascular resistances, respectively.
The dose of sodium nitroprusside was 10 μg/kg, i.v.
The dose of epinephrine was 10 nmol/kg, i.v.
The dose of propranolol was 1 mg/kg, i.v.
Each value represents the mean ± S.E.M. of the percent changes in the hemodynamic values from five rats.
[a] $P < 0.05$, significant change from pre-propranolol values.
[b] $P < 0.05$, post-propranolol values vs. pre-propranolol.

namic parameters is shown in Table 3. Saline did not modify these resting hemodynamic parameters ($P > 0.05$ for all comparisons). Propranolol did not affect resting mean arterial blood pressure or hindquarter or mesenteric vascular resistances ($P > 0.05$ for all comparisons) but did lower heart rate ($P < 0.05$). The propranolol-induced bradycardia remained constant throughout the experiments. Summaries of the hemodynamic effects of PACAP-27 (2 nmol/kg, i.v.) before and after injection of propranolol (1 mg/kg, i.v.; $n = 5$) are shown in Fig. 4 and Table 4. As can be seen, the time dependent hypotensive and vasodilator effects of PACAP-27 were not affected by propranolol ($P > 0.05$ for all time point comparisons). However, propranolol markedly attenuated the tachycardia produced by PACAP-27 ($P < 0.05$ for all time point comparisons). The hemodynamic effects of PACAP-27 were similar before and after administration of saline ($P > 0.05$ for all time point comparisons; data not shown). In addition, this dose of propranolol (1 mg/kg, i.v.) eliminated the hypotension, regional vasodilation and the tachycardia produced by the β-adrenoceptor agonist, isoproterenol (10 μg/kg, i.v.; $n = 5$ rats). For example, the isoproterenol-induced falls in mean arterial blood pressure before and after administration of propranolol were $-37 \pm 5\%$ and $-2 \pm 2\%$, respectively ($P < 0.05$).

3.5. Hemodynamic effects of sodium nitroprusside and epinephrine in propranolol-treated rats

The hemodynamic effects of sodium nitroprusside (10 μg/kg; i.v.) and epinephrine (10 μmol/kg, i.v.) before and after injection of propranolol (1 mg/kg, i.v.; $n = 5$) are summarized in Table 5. Sodium nitroprusside produced pronounced falls in mean arterial blood pressure and vascular resistances ($P < 0.05$ for all responses) which were unaffected by propranolol ($P > 0.05$ for all comparisons). The hypotensive effects of sodium nitroprusside were accompanied by a minor increase in heart rate ($P < 0.05$) which was blocked by propranolol ($P < 0.05$). Epinephrine increased mean arterial blood pressure and mesenteric vascular resistance ($P < 0.05$ for both responses) but decreased hindquarter vascular resistance ($P < 0.05$). Epinephrine did not affect heart rate ($P > 0.05$). In the presence of propranolol, epinephrine produced an exaggerated increase in mean arterial blood pressure and mesenteric resistance and now an increase in hindquarter resistance ($P < 0.05$ for all comparisons). This increase in mean arterial blood pressure was associated with a minor decrease in heart rate ($P < 0.05$).

4. Discussion

The intravenous injection of PACAP-27 produced dose-dependent reductions in mean arterial blood pressure in pentobarbital-anesthetized male Sprague–Dawley rats that were allowed to breathe room air supplemented with 95% oxygen. These PACAP-27-induced falls in mean arterial blood pressure were considerably larger than that observed in female Wistar rats anesthetized with a mixture of fentanyl, fluanisone and midazolam (Nandha et al., 1991). The quantitative differences in the PACAP-27-induced responses in these two studies may therefore be due to the differences in the strain and sex of the rats, the differential effects of the anesthetics or the presence of oxygen-enriched room air. The greater effects of PACAP-27 in pentobarbital-anesthetized rats may be due to the inhibition of baroreceptor-mediated increases in sympathetic nerve activity since there were minimal changes in heart rate in response to the hypotensive effects of sodium nitroprusside. In addition, oxygen-enriched air was supplied to the rats to help maintain blood oxygen levels since these levels can fall over the course of an experiment in anesthetized rats (Lewis and Hoque, unpublished observations). It is therefore possible that the maintenance of blood oxygen levels by the administration of oxygen-enriched air allows for the full expression of the vasodilator effects of PACAP-27. The addition of PACAP polypeptides to isolated vessels produce a vasorelaxation which lasts for at least 60 min (Warren et al., 1991). In contrast,

we found that the vasodilator effects of PACAP-27 in pentobarbital-anesthetized rats lasted for approximately 3 min. To our knowledge, the reasons for the prolonged activity of PACAP polypeptides in vitro have not been determined. It is possible that the shorter duration of action of systemically injected PACAP polypeptides may be due to their degradation by blood-borne enzymes.

One principal finding was that the dose dependent reductions in mean arterial blood pressure produced by PACAP-27 were associated with dose dependent and pronounced decreases in mesenteric and hindquarter vascular resistances. From our studies, it appears that PACAP-27 is an equi-effective vasodilator agent in the mesenteric and hindquarter vascular beds. These findings would suggest that the hypotensive effects of PACAP-27 are mediated principally by vasodilation in the microcirculation of the peripheral vasculature. These results are consistent with previous evidence that PACAP polypeptides produce vasodilation in skin microvessels in humans and rabbits (Warren et al., 1992a,b, 1993; Wilson and Warren, 1993). The observation that the PACAP-induced fall in mean arterial blood pressure was substantially smaller than the falls in mesenteric or hindquarter vascular resistances suggests that an increase in cardiac output limited the expression of the hypotension. Although we have not directly examined this possibility, Minkes et al. (1992a) found that PACAP-27 produced substantial increases in cardiac output in pentobarbital-anesthetized cats.

Interestingly, the hypotensive and vasodilator effects of PACAP-27 consisted of two phases in these pentobarbital-anesthetized rats. The mean arterial blood pressure and vascular resistances fell immediately upon injection of PACAP-27, recovered toward baseline and then fell again. This contrasts to the monophasic falls in mean arterial blood pressure produced by PACAP-27 in female Wistar rats anesthetized with fentanyl, fluanisone and midazolam (Nandha et al., 1991). At present, we have not determined why PACAP-27 produces these biphasic changes in mean arterial blood pressure and vascular resistances. Nandha et al. (1991) suggested that the hypotensive effects of PACAP-27 may be due principally to the activation of type II PACAP sensitive receptors which also recognize VIP rather than PACAP specific type I receptors. In contrast, Minkes et al. (1992b) provided evidence that the vasodilator effects of PACAP-27 in pentobarbital-anesthetized cats may be due to activation of PACAP specific type I receptors. Taken together, it is possible that the initial effects of PACAP-27 in pentobarbital-anesthetized rats may be due to the activation of type I PACAP sensitive receptors and that the secondary effects are due to the activation of type II receptors, or vice versa. We are currently conducting studies with the specific VIP receptor antagonist, D-Phe^2-VIP (Minkes et al., 1992b) to test these possibilities.

On the basis of in vitro findings (Warren et al., 1991), it is likely that the vasodilation produced by the systemic injection of PACAP-27 is due to activation of PACAP sensitive receptors on vascular smooth muscle. Our finding that PACAP-27 and the β-adrenoceptor agonist, isoproterenol, produced robust falls in mean arterial blood pressure in rats treated with a 5 mg/kg dose of the long acting ganglion blocker, chlorisondamine, suggests that the vasodilator effects of these G_s protein-coupled receptor agonists are not primarily mediated by the withdrawal of sympathetic nerve activity. We have shown that a 2.5 mg/kg dose of chlorisondamine abolishes baroreceptor reflex-mediated changes in heart rate in conscious rats (Lewis et al., 1989). Since baroreceptor-mediated changes in heart rate are minimal in pentobarbital-anesthetized rats, we examined the effects of chlorisondamine on the Bezold–Jarisch reflex-mediated falls in mean arterial blood pressure and heart rate produced by 5-HT. These reflex-mediated falls in mean arterial blood pressure are due to the 5-HT-mediated activation of cardiopulmonary afferents which result in vagal efferent-mediated reductions in heart rate and cardiac output (Muntzel et al., 1996). The finding that chlorisondamine abolished the Bezold–Jarisch reflex suggests that this compound effectively blocked ganglionic transmission. Although the resting mean arterial blood pressure of the chlorisondamine-treated rats gradually increased over the course of the experiments, this was due to the exaggerated vasoconstrictor effects of angiotensin II and arginine vasopressin, rather than diminished ganglionic blockade. Taken together, these findings suggest that systemically injected PACAP-27 may exert its vasodilator effects by direct actions within the microvasculature.

PACAP receptors have been identified in the adrenal glands (Arimura et al., 1991) and the infusion of PACAP into rat adrenal gland increases catecholamine release (Wakade et al., 1992). In agreement with previous findings (Kooy and Lewis, 1996), we observed that epinephrine produced a pressor response and a vasoconstriction in the mesenteric bed and a pronounced vasodilation in the hindquarter bed. The hindquarter vasodilation was reversed to a vasoconstriction in the presence of the β-adrenoceptor antagonist, propranolol. However, it is unlikely that the hindquarter vasodilator effects of PACAP-27 involve adrenal-derived epinephrine since we found that the hypotensive and vasodilator effects of PACAP-27 were unaffected by propranolol. This would suggest that the 0.1–2.0 nmol/kg doses of PACAP-27 may not cause the release of physiologically relevant amounts of epinephrine in pentobarbital-anesthetized rats. Minkes et al. (1992a) found that the systemic injection of a 3 nmol/kg dose of PACAP-27 caused the release of adrenal catecholamines in the cat. However, these catecholamines produced an α-adrenoceptor-mediated vasoconstriction rather than a vasodilation in the hindlimb. More specifically, this dose of PACAP-27 produced vasoconstriction in the hindlimb that was abolished by adrenalectomy or the α-adrenoceptor antagonist, phentolamine (Minkes et al., 1992a).

The present study confirms that the NO synthase inhibitor, L-NAME, produces a substantial increase in mean arterial blood pressure in pentobarbital-anesthetized rats which is due to increases in peripheral vascular resistances (Davisson et al., 1996a, 1997). However, the hypotensive and vasodilator effects of PACAP-27 in the hindquarter bed were not diminished by L-NAME. Moreover, the maximal phase II vasodilation produced by PACAP-27 in the mesenteric bed was slightly augmented in L-NAME-treated rats. These results are consistent with evidence that PACAP-induced relaxation of isolated vessels is not attenuated by endothelium-denudation (Warren et al., 1991), and that the systemic administration of L-NAME did not attenuate the vasodilator effects of PACAP-27 in anesthetized cats (Minkes et al., 1992a). These findings suggest that the vasodilator effects of PACAP-27 do not involve the release of endothelium-derived NO. The observation that the maximal vasodilator actions of PACAP-27 in the hindquarter bed were unaffected by L-NAME also suggests that the mechanisms by which PACAP-27 relaxes vascular smooth muscle in this bed are not obviously regulated by tonically-released NO. In contrast, the augmented mesenteric vasodilation produced by PACAP-27 in L-NAME-treated rats suggests that the cAMP-dependent mechanisms by which PACAP produces vasodilation in this bed may be up-regulated following inhibition of NO release. However, the present study did not determine the degree of inhibition of NO synthesis produced by L-NAME. The observation that L-NAME produced pronounced increases in mean arterial blood pressure and vascular resistances suggests that L-NAME may have markedly diminished the de novo synthesis of endothelium-derived nitrosyl factors (Davisson et al., 1996a). Nonetheless, it is possible that NO synthesis was not diminished sufficiently enough to block PACAP-27-induced release of NO or related nitrosyl factors.

The systemic injection of PACAP-38 increases heart rate in conscious sheep (Sawangjaroen et al., 1992). This tachycardia was larger than that expected by baroreceptor-mediated activation of sympathetic drive and withdrawal of vagal drive as a result of the PACAP-induced fall in arterial pressure. Nandha et al. (1991) found that the systemic injection of PACAP-27 and -38 produced a tachycardia in anesthetized rats which they suggested was due to activation of the baroreceptor reflex since these peptides had no effect on the beating rate of isolated atrial preparations. In contrast, PACAP-27 and-38 increased the beating rate of cardiac myocytes cultured from neonatal rat hearts and also stimulated adenylate cyclase activity in crude membranes and homogenates of rat heart (Arimura, 1992). As such, these findings suggest that PACAP increases heart rate by direct actions on cardiac pacemaker cells or by baroreceptor mediated changes in sympathetic and vagal drive to the heart. We observed that PACAP-27 and the β-adrenoceptor agonist, isoproterenol, produced dose dependent increases in heart rate in pentobarbital-anesthetized rats. In contrast, the pronounced hypotension produced by the NO donor, sodium nitroprusside, was accompanied by a minor tachycardia. This suggests that pentobarbital anesthesia impairs baroreceptor reflex-mediated changes of autonomic input to the heart. These data and the observation that the ganglion blocker, chlorisondamine, did not affect PACAP-27- and isoproterenol-mediated tachycardia, suggest that these compounds increase heart rate by direct actions within the heart. The observation that PACAP-27-mediated tachycardia was markedly reduced by the β-adrenoceptor antagonist, propranolol, suggests that PACAP-27 increases heart rate by the direct release of norepinephrine from post-ganglionic sympathetic terminals in the heart. To our knowledge there is no direct evidence that PACAP receptors exist on cardiac sympathetic nerve terminals. However, the possibility that PACAP-27 causes the release of norepinephrine from sympathetic terminals is supported by the observation that PACAP-27 directly releases catecholamines from the adrenal gland (see above) and that the peripheral vasodilator effects of PACAP-27 in conscious rats are markedly exaggerated in the presence of the α_1-adrenoceptor antagonist, prazosin (Whalen et al., unpublished observations). The observation that the systemic injection of epinephrine did not increase heart rate in pentobarbital-anesthetized rats would argue that the release of adrenal epinephrine is not involved in the expression of PACAP-27-induced tachycardia in these rats. Interestingly, the tachycardia produced by PACAP-27 was augmented in the presence of the NO synthesis inhibitor, L-NAME. We have obtained preliminary evidence that the tachycardic effects of isoproterenol are also augmented in the presence of this NO synthesis inhibitor (Whalen et al., unpublished observations). Cardiac muscle contains the type III (endothelial) form of NO synthase and NO reduces the rate and force of contraction of cardiac muscle cells (Balligand et al., 1995). Balligand et al. (1995) reported that isoproterenol-induced increases in cardiac contractility are reduced by endogenous NO. Taken together, the exaggerated tachycardia produced by PACAP-27 in L-NAME-treated rats may involve the enhanced chronotropic effects of neurally released norepinephrine due to the loss of influence of cardiac-derived NO.

In summary, this study demonstrates that PACAP-27 produces dose dependent vasodilator responses in the mesenteric and hindquarter beds of anesthetized rats. The vasodilator effects of PACAP-27 may be due to direct actions on vascular smooth muscle rather than to changes in autonomic nerve activity, the release of adrenal catecholamines or the release of endothelium-derived NO. These findings support evidence that the effects of systemically injected PACAP may be due to the activation of PACAP sensitive receptors on vascular smooth muscle which activate adenylate cyclase (see above). This study also provides evidence that the tachycardia produced by PACAP-27 is due to the direct release of norepinephrine

from cardiac sympathetic nerve terminals and that the β-adrenoceptor-mediated actions of norepinephrine on cardiac cells are augmented by inhibition of NO synthesis. Determining the roles of PACAP type I and type II receptors in the hemodynamic actions of PACAP-27 will be an important step in understanding the mechanisms by which this peptide exerts its hemodynamic effects.

Acknowledgements

We wish to acknowledge the expert assistance of Mike Burcham in preparation of the figures. This study was supported in part by grants from the National Heart, Lung and Blood Institute HL-14388 and HL-57472, from NASA NAG5-6171, and from the Office of Naval Research N00014-97-1-0145.

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