ELSEVIER

European Journal of Pharmacology 275 (1995) 259–266

ejp

The effects of pituitary adenylate cyclase-activating polypeptide on cerebral arteries and vertebral artery blood flow in anesthetized dogs

Yukio Seki, Yoshio Suzuki *, Mustafa K. Baskaya [1], Takahisa Kano, Kiyoshi Saito, Masakazu Takayasu, Masato Shibuya, Kenichiro Sugita

Department of Neurosurgery, Nagoya University School of Medicine, 65-Tsurumai, Showa, Nagoya 466, Japan

Received 23 June 1994; revised 19 December 1994; accepted 30 December 1994

Abstract

We investigated and compared the effects of pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) on cerebral circulation in anesthetized dogs. The intracisternal administration of PACAP-27, PACAP-38, and VIP dilated canine cerebral arteries in a dose-dependent manner. A 10 nmol dose of PACAP-27, PACAP-38, and VIP dilated the basilar artery by 23 ± 3, 27 ± 3 and $30 \pm 3\%$, respectively. Rostrally located arteries tended to be more responsive to PACAP-27. Pretreatment with N^G-monomethyl-L-arginine did not affect PACAP-27-induced vasodilation. Vertebral artery blood flow was also affected by intra-arterial injection of these peptides in a dose-dependent manner. A 100 pmol dose of PACAP-27, PACAP-38, and VIP increased the vertebral artery blood flow by 42 ± 10, 29 ± 4, and $62 \pm 11\%$, respectively. The VIP receptor antagonist, [Lys[1],Pro[2,5],Arg[3,4],Tyr[6]]VIP, inhibited both the VIP- and PACAP-38-induced increase in vertebral artery blood flow. These findings suggest that PACAP plays a role in the regulation of cerebral circulation.

Keywords: PACAP (pituitary adenylate cyclase-activating polypeptide); Blood flow, vertebral artery; Cerebral artery, canine; VIP (vasoactive intestinal peptide); Vasodilation

1. Introduction

Immunohistochemical techniques have demonstrated that in addition to adrenergic and cholinergic nerves, cerebral blood vessels are richly innervated by peptide-containing nerve fibers. The major peptide-transmitter candidates are vasoactive intestinal peptide (VIP) (Larsson et al., 1976), neuropeptide Y (Lundberg et al., 1983), substance P (Chan-Palay, 1977), and calcitonin gene-related peptide (Hanko et al., 1985). Evidence of their role in the neurogenic regulation of the cerebral circulation is increasing (Allen et al., 1984; Edvinsson et al., 1985; Lee et al., 1984; McCulloch and Edvinsson, 1980; Suzuki et al., 1988).

Pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide recently isolated from ovine hypothalamic tissue, can stimulate adenylate cyclase in cultured rat pituitary cells (Miyata et al., 1989). This peptide is present in two different forms in the hypothalamus, one with 38 amino acid residues (PACAP-38), and the other with 27 amino acid residues (PACAP-27). PACAP-27 has the same 27 N-terminal amino acids as PACAP-38 with an amidated C-terminus. The two forms of PACAP have equipotent biological activity on adenylate cyclase stimulation (Miyata et al., 1990). The N-terminal portion (amino acids 1–28) of PACAP-38 has 68% homology with ovine VIP, but its adenylate cyclase-stimulating activity in cultured rat pituitary cells is at least 1000 times greater than that of VIP (Miyata et al., 1989).

Immunohistochemical studies in several species, including humans, have demonstrated that PACAP is present in neurons and is distributed predominantly in the hypothalamus (Arimura et al., 1991; Kivipelto et al., 1992; Köves et al., 1990; Vigh et al., 1991). This suggests that it plays a role as a hypophysiotropic hormone, neurotransmitter and/or neuromodulator. However, a subpopulation of VIP-containing nerve fibers, present in the adventitia and adventitia-media

* Corresponding author. Tel. (052) 741-2111, fax (052) 731-0638.

[1] Present address: Department of Neurosurgery, Ankara University School of Medicine, Ibni Sina Hospital, Ankara, Turkey.

SSDI 0014-2999(95)00011-9

border of cat cerebral arteries, displays PACAP immunoreactivity (Uddman et al., 1993). These fibers can also be found in close proximity to small vessels in the hypothalamus (Vigh et al., 1991; Köves et al., 1990). These findings suggest that PACAP may act as a cerebral vasoregulator.

The purpose of this study was to determine the role of PACAP on canine cerebral circulation and to compare it with that of VIP.

2. Materials and methods

2.1. Animal preparation

Our protocol followed the guidelines for the care and use of animals in the physiological sciences as approved by the Physiological Society of Japan. Mature mongrel dogs of either sex, weighing 9–14 kg, were anesthetized with ketamine hydrochloride (10 mg/kg i.m.) and pentobarbital (20 mg/kg i.v. followed by 5 mg/kg/h). A slow intravenous infusion of saline was maintained during the procedure to prevent dehydration. The animals were paralyzed with pancuronium bromide (0.08 mg/kg) and respiration was controlled through tracheal tubes with room air delivered by a respirator (model B2; Igarashi Ika Kogyo Co., Tokyo, Japan). The respiration rate (approximately 12 cycles/min) and tidal volume (20 ml/kg) were adjusted to maintain arterial blood gas levels and pH within physiological limits. In this series, the mean pO_2 was 104.3 ± 2.5 mm Hg, pCO_2 was 33.2 ± 0.8 mm Hg, and the pH was 7.369 ± 0.005. The body temperature was maintained between 37 and 38°C with a heating pad. The systemic blood pressure and heart rate were monitored with a pressure transducer catheter (model AP-601G; Nihon Kohden Co., Tokyo, Japan) placed in the right femoral artery, in conjunction with a heart rate coupler (model AT-600G; Nihon Kohden Co., Tokyo, Japan).

2.2. Angiographic measurement of vascular diameter

The vasodilator effects of PACAP-27, PACAP-38, and VIP on cerebral arteries were determined by means of angiography. A catheter (5 French) was inserted under fluoroscopic control into the vertebral artery via the left femoral artery. Angiography was performed at a fixed magnification with 3 ml of 65% iothalamate meglumine. Angiograms were obtained before and at 5, 10, 15, 20, 30, 45, 60, 90, and 120 min after the injection of 1 ml control or test solution into the cisterna magna. Physiological saline was used as a control, and all test substances were dissolved in physiological saline just before use. The pH of all test solutions was almost the same as that of the control solution. Solutions were injected gently (1 ml/min) through a 22-gauge spinal needle after the same amount of cerebrospinal fluid (CSF) was withdrawn. This kept the intracranial pressure as constant as possible. The middle third of the basilar artery was chosen as the site for the quantitative evaluation of cerebrovascular reactivity to these peptides. The internal diameters of the arteries were measured with a computerized image analysis system (Macintosh IIcx computer manufactured by Apple Computer, Cupertino, CA, USA; Image 1.47 software supplied by National Technical Information Service, Springfield, VA, USA).

The role of nitric oxide in PACAP-induced vasodilation was investigated with N^G-monomethyl-L-arginine, which inhibits the formation of nitric oxide from L-arginine. 10 μmol of N^G-monomethyl-L-arginine, in 1 ml of physiological saline, was injected intracisternally 30 min before the administration of 1 nmol PACAP-27. The dose of N^G-monomethyl-L-arginine was determined empirically from a previous study (Oyama et al., 1993).

A preliminary study demonstrated that the intracisternal administration of PACAPs at 10 nmol significantly increased the plasma concentration of arginine vasopressin (data not shown). Additional experiments therefore were performed to exclude any modulation by vasopressin of PACAP-induced vasodilation. The non-peptide vasopressin V_1 receptor antagonist, OPC-21268 (1-{1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl}-3,4-dihydro-2(1*H*)-quinolinone) (Yamamura et al., 1991), was infused intravenously (0.1 mg/kg/min) for 70 min starting 10 min prior to the administration of 10 nmol of PACAP-27. This dose of OPC-21268, given 30 min before the intravenous administration of 300 pmol of synthetic vasopressin, effectively suppressed an increase in blood pressure. The antagonist alone had no effect on the diameter of the basilar artery. OPC-21268 was dissolved in dimethyl formamide and diluted with distilled water just before use (15 mg/ml, dimethyl formamide 20%).

2.3. Assay of cyclic AMP in CSF

After the administration of PACAP-27 into the cisterna magna, 0.5 ml samples of CSF were collected periodically and stored at −80°C. A commercial radioimmunoassay kit was used to determine the levels of cyclic AMP.

2.4. Vertebral artery blood flow measurement

Vertebral artery blood flow was determined with a short extracorporeal loop placed between the femoral and vertebral arteries. An electromagnetic flow probe (Model MFV-1100, manufactured by Nihon Kohded Co., Tokyo, Japan) was used to measure the blood flow

in the extracorporeal fistula. Heparin sodium (1000 U/kg) was administered intravenously to prevent coagulation. After initial baseline values were obtained, the vertebral artery blood flow was measured continuously for 10 min after the injection of vehicle or test solutions into the femoral-vertebral artery shunt. The peptides in 0.1 ml of physiological saline were injected into the loop as a bolus. Data are expressed as the percentage change from the baseline vertebral artery blood flow. 10 nmol of the VIP receptor antagonist, [Lys^1,Pro^{2,5},Arg^{3,4},Tyr^6]VIP, was co-injected with 100 pmol of PACAP-38 or VIP to determine the PACAP receptor type in cerebrovascular beds.

2.5. Materials

Synthetic ovine PACAP-27, ovine PACAP-38, and human VIP were purchased from Peptide Institute (Osaka, Japan), [Lys^1,Pro^{2,5},Arg^{3,4},Tyr^6]VIP from Sigma Chemical Company (St. Louis, MO, USA) and N^G-monomethyl-L-arginine from Calbiochem (La Jolla, CA, USA). OPC-21268 was obtained from Otuka Pharmaceutical Co. (Tokushima, Japan). The cyclic AMP radioimmunoassay kit was obtained from Yamasa Shoyu (Choshi, Japan). All other chemicals were reagent grade or the best commercially available grade.

2.6. Statistical analysis

Data are expressed as means ± S.E. Differences were assessed by analysis of variance, Scheffe's F-test and Student's t-test. Values of P less than 0.05 were considered statistically significant.

3. Results

3.1. Angiography

There was a dose-dependent dilation of the major cerebral arteries, including the vertebral artery, the basilar artery, and the circle of Willis and its main branches, in response to the intracisternal administration of PACAP-27, PACAP-38, and VIP (Fig. 1). There

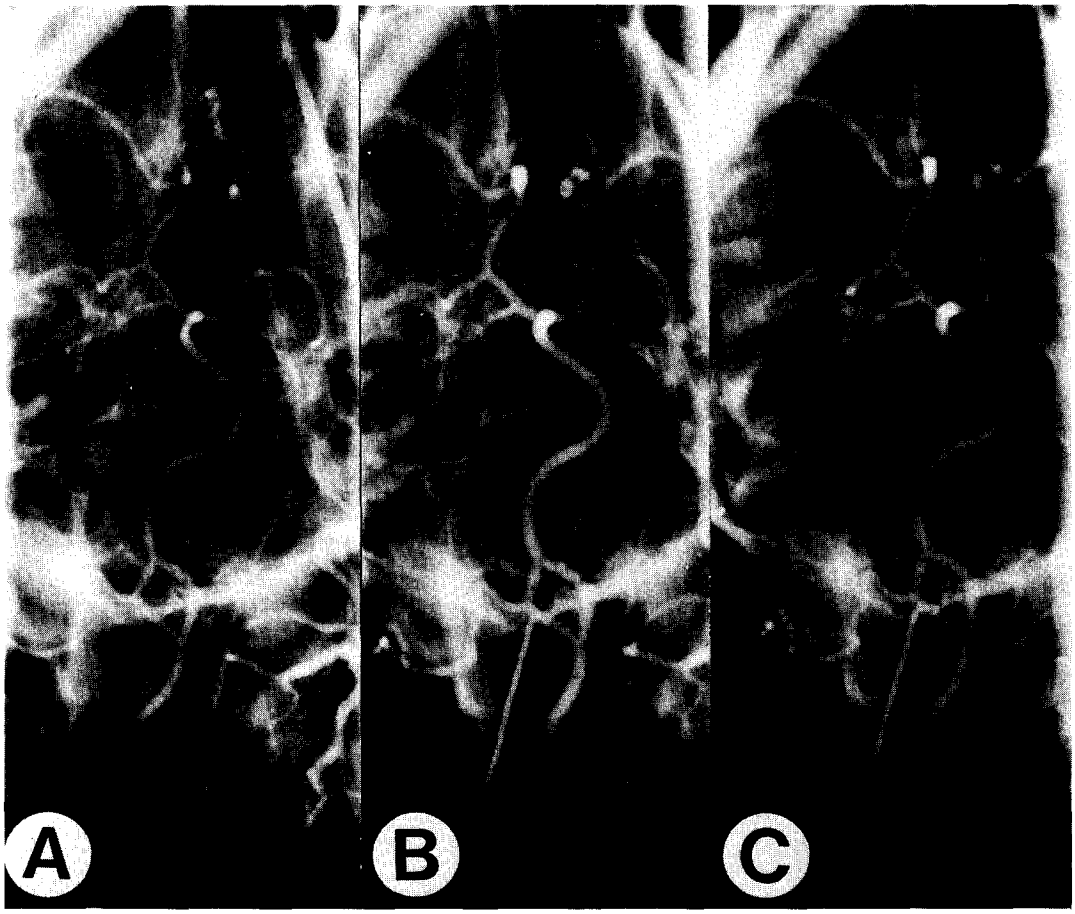

Fig. 1. Representative vertebral angiograms showing the vasodilator effect of pituitary adenylate cyclase-activating polypeptide (PACAP)-27 on the canine basilar artery. A: Control (before administration of PACAP-27). B: 15 min after intracisternal administration of 10 nmol PACAP-27. C: 90 min after intracisternal administration of 10 nmol PACAP-27.

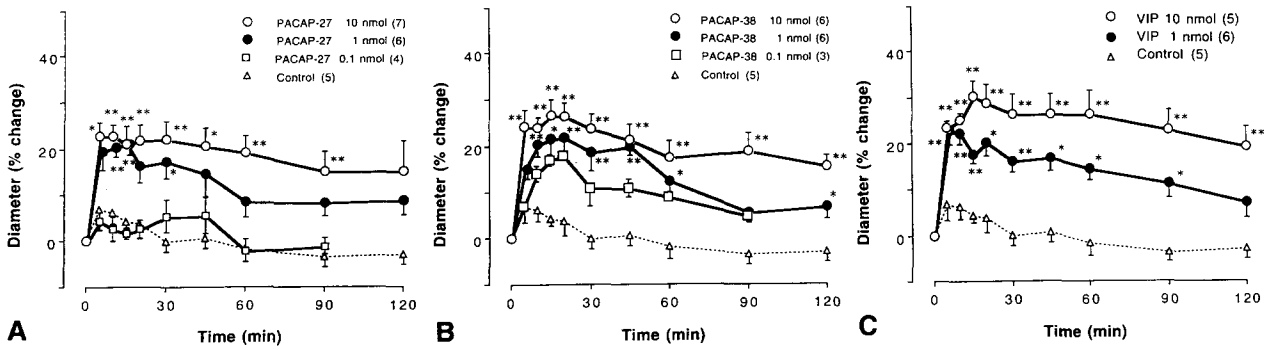

Fig. 2. Time course of the vasodilator response of the canine basilar artery to intracisternal administration of pituitary adenylate cyclase-activating polypeptide (PACAP)-27 (A), PACAP-38 (B) and vasoactive intestinal peptide (VIP) (C). Values are means ± S.E. The number of animals is indicated in parentheses. $^{*}P < 0.05$; $^{**}P < 0.01$ compared with the control group (Scheffe's F test).

was no response to the vehicle. The middle portion of the basilar artery was chosen as the site for the quantitative assessment of the changes in blood vessel diameter. The maximal vasodilation induced by 10 nmol PACAP-27, PACAP-38, and VIP was 23 ± 2, 27 ± 3 and 30 ± 3% of the baseline, respectively. There was no significant difference in the magnitude of vasodilation elicited by these peptides. Significant vasodilation persisted for 90, 120 and 120 min, respectively (Fig. 2). The mean arterial blood pressure remained stable when 1 nmol peptide and 10 nmol VIP were administered, but it significantly increased when 10 nmol PACAPs were given (Table 1).

Intracisternal pretreatment with 10 μmol of N^G-monomethyl-L-arginine for 30 min reduced the diameter of cerebral arteries to 92 ± 1.6% of control ($n = 8$), but did not inhibit the vasodilator effect of 1 nmol PACAP-27 (21.2 ± 2.5%, $n = 6$ vs. 26.0 ± 9.0%, $n = 4$) and PACAP-38 (21.8 ± 3.0%, $n = 6$ vs. 15.0 ± 4.6%, $n = 4$). This pretreatment alone had no effect on mean arterial blood pressure.

Intravenous infusion of the non-peptide vasopressin V_1 receptor antagonist, OPC-21268, did not alter the maximal response of the basilar artery induced by the intracisternal administration of 10 nmol PACAP-27 (22.7 ± 2.4% vs. 28.3 ± 4.6%, $n = 7$). This antagonist alone had no effect on the diameter of the basilar artery and mean arterial blood pressure.

The relationship between the control diameter of major cerebral arteries and the maximal vasodilation elicited by 10 nmol PACAP-27 was examined in the groups treated with or without the vasopressin V_1 receptor antagonist. The diameters of the anterior cerebral (proximal portion), the middle cerebral (proximal portion), the posterior communicating and the anterior spinal arteries (at the C1 level), in addition to the basilar artery, were measured and compared. The smaller arteries, belonging to the anterior circulation of the circle of Willis, showed inversely greater vasodilation (Fig. 3). For example, the anterior cerebral artery, with a mean control diameter of 0.71 ± 0.03 mm, showed a significantly greater vasodilation (48 ± 5%) than the basilar artery (27 ± 2%) with a mean control diameter of 1.12 ± 0.03 mm.

Table 1
Changes in mean arterial blood pressure after intracisternal administration of PACAP-27, PACAP-38 and VIP

Group	n	Baseline MABP (mm Hg)	Changes in MABP (mm Hg)							
			5 min	10 min	20 min	30 min	45 min	60 min	90 min	120 min
Saline	5	127 ± 3	2 ± 1	0 ± 2	0 ± 2	1 ± 2	3 ± 2	2 ± 3	4 ± 4	3 ± 3
PACAP-27										
1 nmol	6	121 ± 5	−1 ± 3	−4 ± 2	−2 ± 1	0 ± 2	1 ± 2	4 ± 3	9 ± 6	10 ± 7
10 nmol	7	125 ± 3	−4 ± 4	2 ± 4	9 ± 4	20 ± 5 [a]	14 ± 7	11 ± 5	7 ± 5	10 ± 5
PACAP-38										
1 nmol	6	144 ± 6	2 ± 2	3 ± 2	5 ± 3	3 ± 1	5 ± 5	7 ± 6	11 ± 5	11 ± 4
10 nmol	6	129 ± 6	7 ± 2	9 ± 3	10 ± 3	12 ± 3 [a]	13 ± 2	15 ± 3	17 ± 4	16 ± 4
VIP										
1 nmol	6	130 ± 6	2 ± 2	−1 ± 2	−1 ± 1	−4 ± 4	2 ± 3	3 ± 4	3 ± 5	3 ± 5
10 nmol	5	131 ± 6	−2 ± 3	−2 ± 3	1 ± 3	3 ± 3	5 ± 4	5 ± 3	6 ± 5	6 ± 4

Values are means ± S.E. n indicates the number of animals. [a] $P < 0.05$, difference from saline group. MABP = mean arterial blood pressure; PACAP = pituitary adenylate cyclase-activating polypeptide; VIP = vasoactive intestinal peptide.

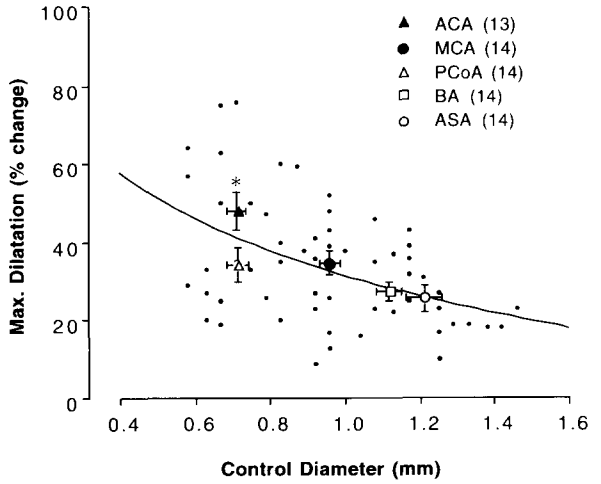

Fig. 3. Relationship between the maximal dilatation of major cerebral arteries and their initial diameter. Individual data can be described by $y = 31 - 66 \log_{10}(x)$ ($R = 0.48$, $P < 0.01$), where y = percent change in diameter and x = diameter of artery prior to the administration of PACAP-27. ACA = anterior cerebral artery; MCA = middle cerebral artery; PCoA = posterior communicating artery; BA = basilar artery; ASA = anterior spinal artery. $^{*}P < 0.05$ compared with BA dilatation (Scheffe's F test).

3.2. Concentration of cyclic AMP in CSF

The basal concentration of cyclic AMP in CSF was 21 ± 2 pmol/ml. The concentration significantly increased 10 min after the intracisternal injection of 1 and 10 nmol PACAP-27 (Table 2).

3.3. Vertebral artery blood flow

The vertebral artery blood flow remained stable after the injection of vehicle into the femoral-vertebral artery shunt. The administration of 10 pmol, 100 pmol, and 1 nmol of PACAPs increased it dose dependently (Fig. 4). The maximal increase in vertebral artery blood flow produced by 100 pmol of VIP ($62 \pm 11\%$) was significantly greater than the increase seen with 100 pmol of PACAP-38 ($29 \pm 4\%$) (Fig. 5). There were no appreciable changes in the mean arterial blood pressure and heart rate following the administration of 10 and 100 pmol PACAPs. Although the VIP receptor antagonist, [Lys^1,Pro^{2,5},Arg^{3,4},Tyr^6]VIP (10 nmol), alone did not produce any significant change in base-

Table 2
Changes in cerebrospinal fluid cyclic AMP concentration after intracisternal administration of PACAP-27

Peptide	n	CSF cyclic AMP (pmol/ml)		
		Control	10 min	30 min
PACAP-27 1 nmol	4	21 ± 2	57 ± 13 [a]	77 ± 15 [b]
PACAP-27 10 nmol	6		87 ± 17 [b]	158 ± 17 [b]

Values are means ± S.E. [a] $P < 0.05$, [b] $P < 0.01$, difference from control values. n indicates the number of animals. PACAP = pituitary adenylate cyclase-activating polypeptide; CSF = cerebrospinal fluid.

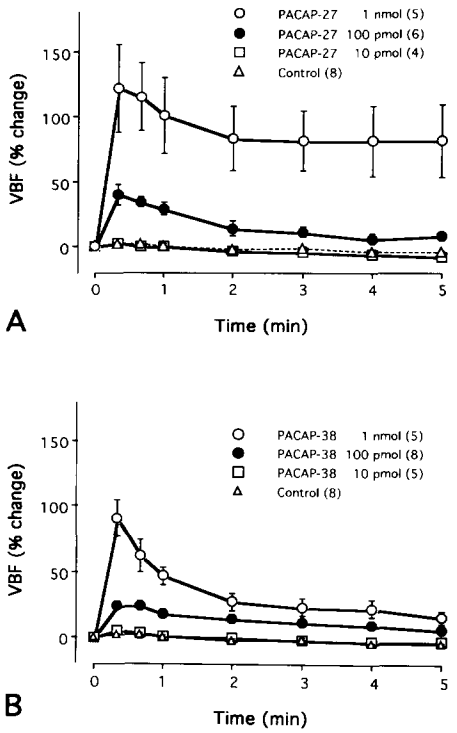

Fig. 4. Vertebral artery blood flow (VBF) changes in response to pituitary adenylate cyclase-activating polypeptide (PACAP)-27 (A) and PACAP-38 (B) administered via the vertebral artery. The vertebral artery blood flow change is expressed as a percentage of the baseline value. Values are means ± S.E. The number of animals is indicated in parentheses. Vertebral artery blood flow changes were significantly greater ($P < 0.05$) at higher doses (100 pmol and 1 nmol) than the corresponding control values from 20 s to 5 min.

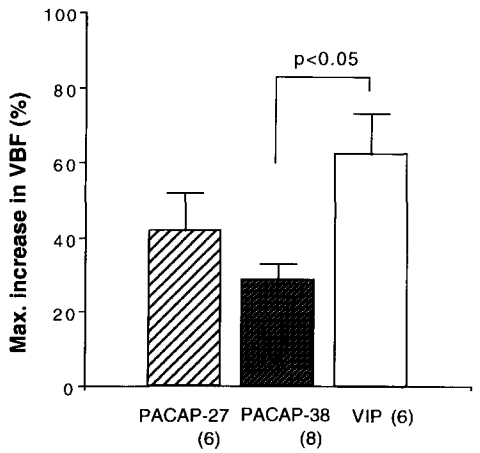

Fig. 5. Comparison of the maximal increase in vertebral artery blood flow (VBF) produced by 100 pmol of pituitary adenylate cyclase-activating polypeptide (PACAP)-27, PACAP-38 and vasoactive intestinal peptide. Values are means ± S.E. The number of animals is indicated in parentheses.

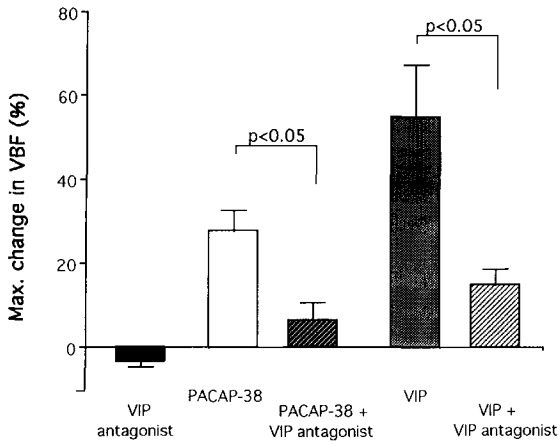

Fig. 6. Effect of the vasoactive intestinal peptide (VIP) receptor antagonist, [Lys[1],Pro[2,5],Arg[3,4],Tyr[6]]VIP, on the vertebral artery blood flow (VBF) increase produced by pituitary adenylate cyclase-activating polypeptide (PACAP)-38 and VIP. 10 nmol of the antagonist was simultaneously administered with 100 pmol of PACAP-38 or VIP into the vertebral artery. Values are means ± S.E. from four animals in each group.

line values for vertebral artery blood flow, it significantly inhibited an increase in vertebral artery blood flow when injected simultaneously with 100 pmol VIP or 100 pmol PACAP-38 (Fig. 6).

4. Discussion

This study has shown that the intracisternal administration of PACAP-27, PACAP-38, and VIP produced a dose-dependent vasodilation of cerebral arteries in anesthetized dogs. The intra-arterial administration of these peptides also produced significant increases in vertebral artery blood flow. While the duration of vasodilation produced by PACAP-38 was longer than that produced by PACAP-27 in angiographic experiments, the increase in vertebral artery blood flow was in a reversed order. We cannot explain why each PACAP showed a slightly different activity in different experiments. As the vertebral artery blood flow reflects more factors including the blood flow in extracranial tissues like meninges or skeletal muscles in addition to the cerebral blood flow, the sensitivity of receptor sites for PACAPs may be slightly different in vessels supplying different regions. However, our findings are relatively consistent with previous data obtained from cerebral blood vessels in different animal species (Uddman et al., 1993; Tong et al., 1993). The relative vasodilator potencies of both PACAP forms have already been demonstrated in a number of models including rabbit aortic rings (Warren et al., 1991), rat tail artery precontracted with phenylephrine (Absood et al., 1992b) and rabbit skin (Warren et al., 1992). These studies showed that PACAP-27 exhibited a similar vasodilator potency as PACAP-38. In addition, intravenous infusion of both forms of PACAP decreased uveal vascular resistance equally in rabbits (Nilsson, 1994). These data suggest that the N-terminal 27 amino acids are sufficient to produce vasodilation, and the C-terminal residues have a slight modulatory effect on biological activity.

The vasodilator potencies of PACAP and VIP have been compared in other studies. These studies have shown a variation in vascular responses between species or regions of vasculature. We found that the vasodilator potency of VIP is slightly greater than that of PACAP by angiographic evaluation, but significantly greater when assessing vertebral artery blood flow. This difference may be due to the fact that vertebral artery blood flow is affected by many factors including the vasodilation in major cerebral arteries. We cannot exclude a secondary vasodilator effect caused by an elevation in intraparencymal metabolism, as it has been shown in baboons that an intracarotid infusion of VIP increases cerebral oxygen consumption with an increase in cerebral blood flow (McCulloch and Edvinsson, 1980). The intracisternal injection of high concentrations of PACAPs induced systemic hypertension accompanied by an elevation in plasma vasopressin. These secondary factors also may influence cerebral blood flow in addition to their effects on general hemodynamics.

Arteries in the basal region of the canine brain responded well to PACAP, but the response to PACAP-27 was greater in the anterior parts of the circle of Willis where the vessel diameters were smaller than those of the basilar, vertebral, or anterior spinal arteries. A concentration gradient of PACAP-27 may have existed in the CSF after injection into the cisterna magna, but the basilar and anterior spinal arteries exposed to a higher concentration of drug showed less vasodilation than arteries rostrally located. A similar phenomenon was observed in bovine pial arteries (Suzuki et al., 1984) and feline cortical arterioles in response to VIP (McCulloch and Edvinsson, 1980). Our finding of a regional difference in cerebrovascular reactivity to PACAP-27 may be related to the finding that pial vessels originating in the rostral portion of the circle of Willis have a richer supply of PACAP fibers (Uddman et al., 1993).

It has been shown that the VIP-induced cerebral vasodilation is independent of endothelial cells (Lee et al., 1984). An intracisternal pretreatment with N^G-monomethyl-L-arginine, an analogue of L-arginine which inhibits nitric oxide synthesis, did not inhibit the vasodilator effect of PACAPs. This finding supports the evidence obtained in peripheral vessels (Minkes et al., 1992) that the vasodilation induced by PACAPs does not involve a nitric oxide-mediated mechanism in

the endothelium, or in nerve fibers innervating blood vessels. PACAP has been shown to relax aortic rings without endothelium and to increase adenylate cyclase activity in rabbit smooth muscle homogenates (Warren et al., 1991). We observed that the intracisternal administration of PACAP-27 increased the concentration of cyclic AMP in CSF, which supports the hypothesis that PACAP-induced vasodilation is mediated via an accumulation of cyclic AMP. As cyclic nucleotides have been observed to leak from cells (Absood et al., 1992a; Fiscus et al., 1987), the increase in CSF cyclic AMP may be mainly derived from nervous tissues such as neurons or glia. However, it may involve a small contribution from vascular tissues in the subarachnoid space.

Membrane preparations obtained from rat blood vessels (aorta, iliac, and femoral arteries and veins) (Nandha et al., 1991) and porcine coronary arteries (Huang et al., 1993) show common binding sites for both ^{125}I-labeled VIP and ^{125}I-labeled PACAP-27. This class of PACAP receptors with shared VIP binding sites has been classified as type II (Gottschall et al., 1990; Shivers et al., 1991). As the VIP receptor antagonist in our experiment suppressed both the PACAP-38- and the VIP-induced increase in the vertebral artery blood flow, we suggest that PACAP acts through type II receptors in blood vessels fed by the vertebral artery. This is in contrast to membranes prepared from brain or anterior lobe of the pituitary gland, which contain high affinity binding sites specific for PACAP. These receptors which are not shared with VIP are classified as type I receptors (Cauvin et al., 1991; Lam et al., 1990; Gottschall et al., 1990; Shivers et al., 1991; Suda et al., 1991). The intracisternal administration of 10 nmol PACAP caused an elevation in arterial blood pressure which may have been mediated via a type I receptor in the central pressor center, since VIP does not share the hypertensive effect.

In conclusion, both forms of PACAP had effects of similar magnitude on both major cerebral arteries and vertebral artery blood flow. VIP was more potent than PACAP in increasing vertebral artery blood flow. The vasoactivity of PACAP was independent of nitric oxide and was probably mediated through receptors coupled to adenylate cyclase. The receptors for PACAP in cerebral blood vessels are different to those in brain parenchyma and appear to have sites shared by VIP. These results support the notion that PACAP plays a role in the neurogenic regulation of local cerebral blood flow.

References

Absood, A., D. Chen and R. Håkanson, 1992a, Neuropeptides of the vasoactive intestinal peptide/helodermin/pituitary adenylate cyclase activating peptide family elevate plasma cAMP in mice: comparison with a range of other regulatory peptides, Regul. Pept. 40, 311.

Absood, A., D. Chen, Z.-Y. Wang and R. Håkanson, 1992b, Vascular effects of pituitary adenylate cyclase activating peptide: a comparison with vasoactive intestinal peptide, Regul. Pept. 40, 323.

Allen, J.M., F. Schon, N. Todd, J.C. Yeats, H.A. Crockard and S.R. Bloom, 1984, Presence of neuropeptide Y in human circle of Willis and its possible role in cerebral vasospasm, Lancet ii, 550.

Arimura, A., A. Somogyvári-Vigh, A. Miyata, K. Mizuno, D.H. Coy and C. Kitada, 1991, Tissue distribution of PACAP as determined by RIA: highly abundant in the rat brain and testes, Endocrinology 129, 2787.

Cauvin, A., P. Robberecht, P. De Neef, P. Gourlet, A. Vandermeers, M.-C. Vandermeers-Piret and J. Christophe, 1991, Properties and distribution of receptors for pituitary adenylate cyclase activating peptide (PACAP) in rat brain and spinal cord, Regul. Pept. 35, 161.

Chan-Palay, V., 1977, Innervation of cerebral blood vessels by norepinephrin, indoleamine, substance P and neurotensin fibers and leptomeningeal indoleamine axons: their roles in vasomotor activity and local alterations of brain blood composition, in: Neurogenic Control of Brain Circulation, eds. C. Owman and L. Edvinsson (Pergamon Press, Oxford) p. 39.

Edvinsson, L., B.B. Fredholm, E. Hamel, I. Jansen and C. Verrecchia, 1985, Perivascular peptides relax cerebral arteries concomitant with stimulation of cyclic adenosine monophosphate accumulation or release of an endothelium-derived relaxing factor in the cat, Neurosci. Lett. 58, 213.

Fiscus, R.R., B.T. Robles, S.A. Waldman and F. Murad, 1987, Atrial natriuretic factors stimulate accumulation and efflux of cyclic GMP in C6-2B rat glioma and PC 12 rat pheochromoctoma cell cultures, J. Neurochem. 48, 522.

Gottschall, P.E., I. Tatsuno, A. Miyata and A. Arimura, 1990, Characterization and distribution of binding sites for the hypothalamic peptide, pituitary adenylate cyclase-activating polypeptide, Endocrinology 127, 272.

Hanko, J., J.E. Hardebo, J. Kåhrström, C. Owman and F. Sundler, 1985, Calcitonin gene-related peptide is present in mammalian cerebrovascular nerve fibers and dilates pial and peripheral arteries, Neurosci. Lett. 57, 91.

Huang, M., H. Shirahase and O.P. Rorstad, 1993, Comparative study of vascular relaxation and receptor binding by PACAP and VIP, Peptides 14, 755.

Kivipelto, L., A. Absood, A. Arimura, F. Sundler, R. Håkanson and P. Panula, 1992, The distribution of pituitary adenylate cyclase-activating polypeptide-like immunoreactivity is distinct from helodermin- and helospectin-like immunoreactivities in the rat brain, J. Chem. Neuroanat. 5, 85.

Köves, K., A. Arimura, A. Somogyvári-Vigh, S. Vigh and J. Miller, 1990, Immunohistochemical demonstration of a novel hypothalamic peptide, pituitary adenylate cyclase-activating polypeptide, in the ovine hypothalamus, Endocrinology 127, 264.

Lam, H.-C., K. Takahashi, M.A. Ghatei, S.M. Kanse, J.M. Polak and S.R. Bloom, 1990, Binding sites of a novel neuropeptide pituitary-adenylate-cyclase-activating polypeptide in the rat brain and lung, Eur. J. Biochem. 193, 725.

Larsson, L.-I., L. Edvinsson, J. Fahrenkrug, R. Håkanson, C.H. Owman, O. Schaffalitzky de Muckadell and F. Sundler, 1976, Immunohistochemical localization of a vasodilatory polypeptide (VIP) in cerebrovascular nerves, Brain Res. 113, 400.

Lee, T.J.-F., A. Saito and I. Berezin, 1984, Vasoactive intestinal polypeptide-like substance: the potential transmitter for cerebral vasodilation, Science 224, 898.

Lundberg, J.M., L. Terenius, T. Hökfelt and M. Goldstein, 1983, High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man, Neurosci. Lett. 42, 167.

McCulloch, J. and L. Edvinsson, 1980, Cerebral circulatory and metabolic effects of vasoactive intestinal polypeptide, Am. J. Physiol. 238, H449.

Minkes, R.K., T.J. McMahon, T.R. Higuera, W.A. Murphy, D.H. Coy and P.J. Kadowitz, 1992, Analysis of systemic and pulmonary vascular responses to PACAP and VIP: role of adrenal catecholamines, Am. J. Physiol. 263, H1659.

Miyata, A., A. Arimura, R.R. Dahl, N. Minamino, A. Uehara, L. Jiang, M.D. Culler and D.H. Coy, 1989, Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells, Biochem. Biophys. Res. Commun. 164, 567.

Miyata, A., L. Jiang, R.D. Dahl, C. Kitada, K. Kubo, M. Fujino, N. Minamino and A. Arimura, 1990, Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP38), Biochem. Biophys. Res. Commun. 170, 643.

Nandha, K.A., M.A. Benito-Orfila, D.M. Smith, M.A. Ghatei and S.R. Bloom, 1991, Action of pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal polypeptide on the rat vascular system: effects on blood pressure and receptor binding, J. Endocrinol, 129, 69.

Nilsson, S.F.E., 1994, PACAP-27 and PACAP-38: vascular effects in the eye and some other tissues in the rabbit, Eur. J. Pharmacol. 253, 17.

Oyama, H., Y. Suzuki, S. Satoh, Y. Kajita, M. Takayasu, M. Shibuya and K. Sugita, 1993, Role of nitric oxide in the cerebral vasodilatory responses to vasopressin and oxytocin in dogs, J. Cereb. Blood Flow Metab. 13, 285.

Shivers, B.D., T.J. Görcs, P.E. Gottschall and A. Arimura, 1991, Two high affinity binding sites for pituitary adenylate cyclase-activating polypeptide have different tissue distributions, Endocrinology 128, 3055.

Suda, K., D.M. Smith, M.A. Ghatei, J.K. Murphy and S.R. Bloom, 1991, Investigation and characterization of receptors for pituitary adenylate cyclase-activating polypeptide in human brain by radioligand binding and chemical cross-linking, J. Clin. Endocrinol. Metab. 72, 958.

Suzuki Y., D. McMaster, K. Lederis and O.P. Rorstad, 1984, Characterization of the relaxant effects of vasoactive intestinal peptide (VIP) and PHI on isolated brain arteries, Brain Res. 322, 9.

Suzuki, Y., M. Shibuya, I. Ikegaki, S. Satoh, M. Takayasu and T. Asano, 1988, Effects of neuropeptide Y on canine cerebral circulation, Eur. J. Pharmacol. 146, 271.

Tong, S., H. Parfenova, M. Shibata, S. Zuckerman, W.M. Armstead and C.W. Leffler, 1993, Pituitary adenylate cyclase-activating polypeptide dilates cerebral arterioles of newborn pigs, Proc. Soc. Exp. Biol. Med. 203, 343.

Uddman, R., P.J. Goadsby, I. Jansen and L. Edvinsson, 1993, PACAP, a VIP-like peptide: immunohistochemical localization and effect upon cat pial arteries and cerebral blood flow, J. Cereb. Blood Flow Metab. 13, 291.

Vigh, S., A. Arimura, K. Köves, A. Somogyvári-Vigh, J. Sitton and C.D. Fermin, 1991, Immunohistochemical localization of the neuropeptide, pituitary adenylate cyclase activating polypeptide (PACAP), in human and primate hypothalamus, Peptides 12, 313.

Warren, J.B., L.E. Donnelly, S. Cullen, B.E. Robertson, M.A. Ghatei, S.R. Bloom and J. MacDermot, 1991, Pituitary adenylate cyclase-activating polypeptide: a novel, long-lasting, endothelium-independent vasorelaxant, Eur. J. Pharmacol. 197, 131.

Warren, J.B., S.W. Larkin, M. Coughlan, R. Kajekar and T.J. Williams, 1992, Pituitary adenylate cyclase activating polypeptide is a potent vasodilator and oedema potentiator in rabbit skin in vivo, Br. J. Pharmacol. 106, 331.

Yamamura, Y., H. Ogawa, T. Chihara, K. Kondo, T. Onogawa, S. Nakamura, T. Mori, M. Tominaga and Y. Yabuuchi, 1991, OPC-21268, an orally effective, nonpeptide vasopressin V_1 receptor antagonist, Science 252, 572.