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Vasorelaxant effect of PACAP-27 on canine cerebral arteries and rat intracerebral arterioles

Masaoki Anzai, Yoshio Suzuki *, Masakazu Takayasu, Yasukazu Kajita, Yoshimasa Mori, Yukio Seki, Kiyoshi Saito, Masato Shibuya

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

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Abstract

The vasorelaxant effects of pituitary adenylate cyclase activating polypeptide (PACAP)-27 were examined and compared with those of PACAP-38 and vasoactive intestinal polypeptide (VIP) on isolated canine cerebral arteries and rat intracerebral arterioles in vitro. The addition of PACAP-27, PACAP-38 or VIP resulted in similar concentration-dependent relaxations in both canine basilar arteries and rat intracerebral arterioles. There were regional differences in the PACAP-27-induced relaxations measured in canine cerebral arteries. The maximum relaxation induced by PACAP-27 was significantly lower in the basilar arteries ($23.0 \pm 5.6\%$) than in the rostrally located arteries (proximal middle cerebral arteries: $45.4 \pm 5.7\%$, anterior cerebral arteries: $55.2 \pm 5.8\%$). The maximum relaxation induced by PACAP-27 in the basilar arteries was significantly enhanced by mechanical removal of the endothelium ($16.4 \pm 4.5\%$ vs. $32.7 \pm 5.8\%$) as well as by pretreatment with indomethacin or aspirin ($12.9 \pm 4.1\%$ vs. $48.7 \pm 6.1\%$ and $46.5 \pm 9.2\%$, respectively). Incubation of canine cerebral arteries with PACAP-27 in vitro resulted in an increased release of prostaglandin $F_{2\alpha}$ in the buffer from 14.5 ± 2.1 pg/min/1 mg vessel to 31.1 ± 4.2 pg/min/1 mg vessel, while other cyclooxygenase cascade metabolites such as prostaglandin E_2, thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$ did not change. These data suggest that the PACAP-27-induced relaxation of canine basilar arteries may be associated with prostaglandin $F_{2\alpha}$ or its precursor, prostaglandin H_2.

Keywords: PACAP (pituitary adenylate cyclase activating polypeptide); Cerebral artery, canine; Intracerebral arteriole, rat; Endothelium-derived contracting factor; Prostaglandin $F_{2\alpha}$

1. Introduction

Pituitary adenylate cyclase activating polypeptide (PACAP) is a novel neuropeptide which was originally isolated from ovine hypothalamus following stimulation of adenylate cyclase in cultured rat anterior pituitary cells (Miyata et al., 1989; Arimura, 1992). PACAP is translated as either a 27 or 38 amino acid polypeptide (PACAP-27 and PACAP-38) (Kimura et al., 1990). The first 28 N-terminal residues of PACAP-38 have 68% structural homology with vasoactive intestinal polypeptide (VIP), but its ability to activate adenylate cyclase in cultured rat anterior pituitary cells is over 1000 times greater than that of VIP (Miyata et al., 1990).

PACAP stimulates many biological activities, including the release of growth hormone, prolactin, adrenocorticotropic hormone, and luteinizing hormone from superfused rat pituitary cells (Miyata et al., 1989), the secretion of amylase from rat pancreas (Mungan et al., 1990), the release of interleukin-6 from cultured rat pituitary cells (Tatsuno et al., 1991), and the secretion of adrenaline from rat chromaffin cells (Watanabe et al., 1992). PACAP also has been reported to induce hypotension following intravenous injection (Nandha et al., 1990; Minkes et al., 1992). Vasodilator effects of PACAP have previously been demonstrated in various vasculatures such as the rabbit aorta (Warren et al., 1991), rat tail arteries (Absood et al., 1992), cat middle cerebral arteries (Uddman et al., 1993) and newborn pig pial arterioles (Tong et al., 1993). Recently, we have found that intracisternal injection of PACAP results in dilatation of the cerebral arteries in dogs while

* Corresponding author. Tel. (052) 741-2111 ext. 2302, fax (052) 731-0638.

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intraarterial injection of PACAP increases vertebral artery blood flow (Seki et al., 1995).

The present study characterized the vasodilator effects of PACAP-27 on isolated canine cerebral arteries and rat intracerebral arterioles. The vasodilator activity of PACAP was partially suppressed by endothelium-derived contractile factor (EDCF), which may be a metabolite(s) generated in the cyclooxygenase cascade.

2. Materials and methods

Our protocol followed the guidelines for the care and use of animals in the physiological sciences as approved by the Physiological Society of Japan.

2.1. Preparation of arterial rings

The experiments were performed on rings (4 mm in length) of basilar, posterior communicating, middle cerebral and anterior cerebral arteries obtained from mongrel dogs of either sex (body weight, 7.5–13.5 kg) anesthetized with pentobarbital sodium (10 mg/kg i.v.) followed by exsanguination.

The rings were placed in Krebs-Henseleit solution (millimolar composition: NaCl 115.0, KCl 4.7, $MgCl_2$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2, glucose 10.0). Each ring was connected to an isometric force transducer (TB-611T, Nihon Kohden Kogyo Co., Tokyo, Japan), suspended in an organ chamber filled with 20 ml of Krebs-Henseleit solution (37°C, pH = 7.4) and gassed with 95% O_2-5% CO_2. Isometric force was recorded continuously on a polygraph.

The rings were allowed to stabilize at a resting tension of approximately 500 mg for 1 h. Each ring was then stretched to the optimal point of its length-tension relation in 65.9 mM potassium chloride, followed by another 1-h period of equilibrium. During this period, the organ chamber fluid was replaced twice. Two or three contractile responses to 65.9 mM potassium chloride were obtained until the responses remained constant. After this procedure, the preparations were allowed to equilibrate for 40 min. Arterial rings were partially contracted with prostaglandin $F_{2\alpha}$ before the addition of test peptides; contractions ranged between 35% and 45% of those induced by 65.9 mM potassium chloride.

2.2. Measuring relaxation of basilar artery rings

Concentration-response curves for PACAP-27, PACAP-38 and VIP were obtained in a cumulative fashion. The relaxation induced by these peptides was expressed as a percentage of the maximal relaxation induced by papaverine hydrochloride (1×10^{-4} M). Concentration-response curves for PACAP-27 also were obtained on the endothelium-denuded rings of basilar arteries or on the rings of basilar arteries which were pretreated with aspirin or indomethacin. Endothelium was removed mechanically using a small wire. The presence or absence of functional endothelium was determined at the start of each experiment from the relaxation response to 1 μM acetylcholine and 100 pM of susbstance P, as described previously (Furchgott and Zawadzki, 1980; Ikegaki et al., 1989). Rings were pretreated with aspirin (3×10^{-4} M) or indomethacin (1×10^{-5} M) for 15 min before PACAP-27 was applied cumulatively.

2.3. Measurement of prostaglandin $F_{2\alpha}$, prostaglandin E_2, thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$

Remaining arteries from the brain were cut into rings (4 mm in length) and divided into two groups of similar weights. Rings from both groups were equilibrated for 2 h in test tubes filled with 10 ml of Krebs-Henseleit solution (37°C, pH = 7.4) and gassed with 95% O_2-5% CO_2. During this period, the buffer solution in each test tube was replaced twice. PACAP-27 (1×10^{-7} M) was then added to one test tube and the same volume of vehicle was added to a second tube (control). After incubation with PACAP-27 for 15 min, the fluid in the test tubes was collected and stored at −80°C until assays were performed.

Concentrations of eicosanoids were measured with slight modifications of the methods described previously (Jaffe et al., 1973; Powell, 1980). Fluid samples were analyzed for eicosanoids using radioimmunoassays (radioimmunoassay kit of [^{125} I]6-keto prostaglandin $F_{1\alpha}$, [^{125} I]prostaglandin E_2 and [^{125}I]-thromboxane B_2; New England Nuclear Corp., Boston, USA; radioimmunoassay kit of [^{3}H]prostaglandin $F_{2\alpha}$; Clinical Assays, Cambridge, USA). Radioactivities of bound [^{125} I]6-keto prostaglandin $F_{1\alpha}$, [^{125}I]prostaglandin E_2 and [^{125} I]thromboxane B_2 were measured in a γ-counter, and those of [^{3}H]prostaglandin $F_{2\alpha}$ were measured in a liquid scintillation counter.

2.4. Preparation of arterioles

Intracerebral arterioles were isolated and cannulated in an organ bath. The changes in vessel diameter in response to the extraluminal administration of agents were measured as previously described (Dacey and Duling, 1982; Takayasu and Dacey, 1989; Takayasu et al., 1993). Briefly, penetrating intracerebral arterioles, 20–80 μm in diameter, were surgically isolated from the first (M1) portion of the middle cerebral artery from the brains of pentobarbital-anesthetized Sprague-Dawley rats (body weight, 300–400 g). The vessels were transferred to a temperature-controlled chamber on the stage of an Olympus inverted micro-

scope and were cannulated at 25°C with glass pipettes. The inner diameter of the vessel was determined using a video dimension analyzer (FOR. A. Model IV-550, Tokyo, Japan). After cannulation, the transmural pressure was set and maintained throughout the experimental protocol at 60 mm Hg via the cannulating pipette. The temperature was increased to 37.5°C, and the vessels were allowed to equilibrate for 45 min in an extraluminal bath at a pH of 7.3. The bathing medium was exchanged every 5 min. During the equilibration period of 30 min, vessels developed spontaneous tone, contracting to 60–70% of their maximum positive diameter.

Vessel responsiveness was then assessed by changing the extraluminal pH from 7.3 to 6.8 and from 7.3 to 7.6. The physiological salt solution used for this preparation was a modified Ringer's solution (composition: NaCl 144.0 mM, KCl 3.0 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1.4 mM, glucose 5.0 mM, pyruvate 2.0 mM, ethylenediaminetetraacetic acid (EDTA) 0.02 mM, 3-(*N*-morpholino)propanesulphic acid (MOPS) 2.0 mM, NaH_2PO_4 1.21 mM, bovine serum albumin 0.9–1.0 g/100 ml). Albumin was not included in the extraluminal solution used to assess vessel responsiveness.

2.5. Measuring vasodilation of arterioles

PACAPs were dissolved in physiological salt solution just prior to use and applied extraluminally to the arterioles after adjustment of the pH to 7.3. The concentration-response curves for PACAP-27 and PACAP-38 were then determined. The magnitudes of the vasodilatation induced by peptides were expressed as percentage changes of the diameter from the control vessel diameter.

2.6. Materials

Synthetic human PACAP-27, human PACAP-38, human VIP and substance P were obtained from the Peptide Institute (Osaka, Japan). Aspirin (acetylsalicylic acid) and indomethacin were obtained from Sigma (St. Louis, MO, USA). Prostaglandin $F_{2\alpha}$ was obtained from Ono Pharmaceutical Company (Osaka, Japan). The radioimmunoassay kits for [^{125}I]prostaglandin E_2, [^{125}I]thromboxane B_2 and [^{125}I]6-keto prostaglandin $F_{1\alpha}$ were obtained from New England Nuclear Corp. (Boston, USA). The radioimmunoassay kit for [^{3}H]prostaglandin $F_{2\alpha}$ was obtained from Clinical Assays (Cambridge, USA). All other chemicals were reagent grade or the best commercially available grade.

2.7. Statistical analysis

The data are expressed as means ± S.E. Differences were analyzed by analysis of variance (ANOVA), followed by Fisher's protected least significant difference multiple-range test or Student's unpaired *t*-test. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Response of canine major cerebral arteries to PACAP-27, PACAP-38 and VIP

PACAP-27 caused a concentration-dependent relaxation in prostaglandin $F_{2\alpha}$-precontracted canine cerebral arteries. Maximum relaxations were obtained with 1×10^{-7} or 3×10^{-7} M PACAP-27 in the basilar arteries (23.0 ± 5.6%). The maximal relaxation in the basilar artery was lower than in other arteries such as the posterior communicating arteries (50.5 ± 3.1%), proximal middle cerebral arteries (45.4 ± 5.7%), distal middle cerebral arteries (77.0 ± 5.7%) and anterior cerebral arteries (55.2 ± 5.8%) ($P < 0.05$) (Fig. 1). PACAP-27, PACAP-38 and VIP caused similar concentration-dependent relaxations in canine basilar arteries. There was no statistically significant difference between the relaxant activities of VIP, PACAP-27 and PACAP-38.

The maximum relaxations induced by PACAP-27 were greater in basilar arteries without endothelium (32.7 ± 5.8%) than in those with endothelium (16.4 ± 4.5%) ($P < 0.05$) (Fig. 2A).

Pretreatment with indomethacin (1×10^{-5} M) or aspirin (3×10^{-4} M) also enhanced the maximum re-

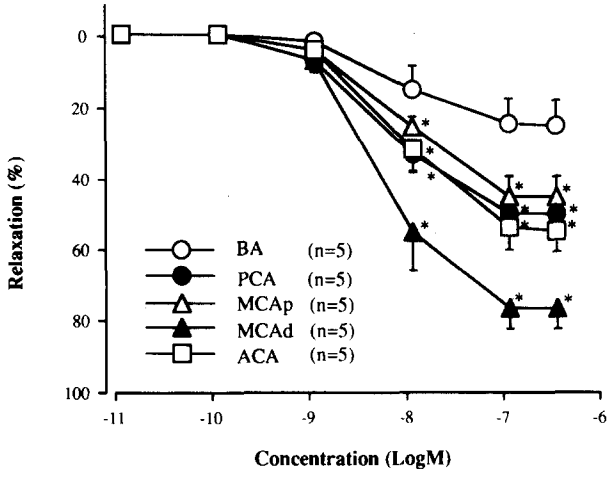

Fig. 1. Regional differences in PACAP-27-induced concentration-dependent relaxations of canine major cerebral arteries precontracted with prostaglandin $F_{2\alpha}$. The relaxation of arteries is expressed as percentage of the maximal relaxation induced by papaverine hydrochloride (1×10^{-4} M). Each point represents the mean ± S.E. The number of experiments is indicated in parentheses. BA = basilar artery, PCA = posterior communicating artery, MCAp = proximal middle cerebral artery, MCAd = distal middle cerebral artery, ACA = anterior cerebral artery. * $P < 0.05$ versus the basilar artery.

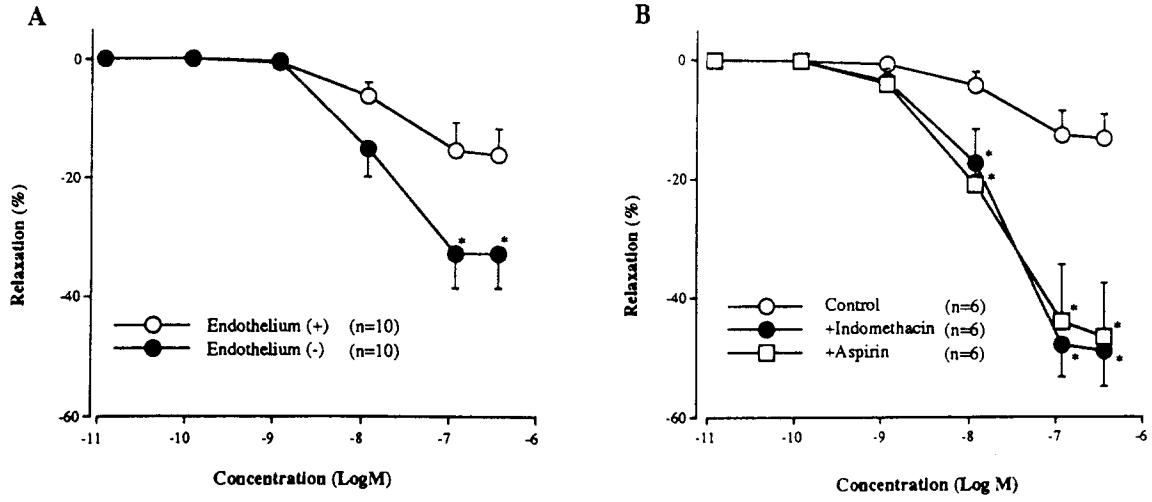

Fig. 2. Effects of removal of endothelium (A) and pretreatment with indomethacin or aspirin (B) on PACAP-27-induced relaxations of canine basilar arteries precontracted with prostaglandin $F_{2\alpha}$. Each point represents the mean ± S.E. The number of experiments is indicated in parentheses. Endothelium (+) = endothelium-intact preparation, Endothelium (−) = endothelium-removed preparation, +Indomethacin = pretreatment with indomethacin (1×10^{-5} M), +Aspirin = pretreatment with aspirin (3×10^{-4} M). * $P < 0.05$.

Table 1
Levels of eicosanoids released from canine major cerebral arteries

	n	Eicosanoid content (pg/min/1 mg vessel)	
		Control	PACAP-27
$PGF_{2\alpha}$	6	14.5 ± 2.1	31.1 ± 4.2 [a]
TXB_2	6	4.9 ± 1.0	6.8 ± 1.1
PGE_2	6	24.8 ± 6.6	29.4 ± 5.0
6-keto $PGF_{1\alpha}$	6	39.8 ± 7.6	50.9 ± 7.8

Values are means ± S.E. *n* indicates number of animals. Eicosanoid levels were determined in in vitro samples of canine major cerebral arteries which were incubated with or without PACAP-27 (1×10^{-7} M). $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; TXB_2, thromboxane B_2; PGE_2, prostaglandin E_2; 6-keto $PGF_{1\alpha}$, 6-keto prostaglandin $F_{1\alpha}$. Significantly different from control: [a] $P < 0.01$.

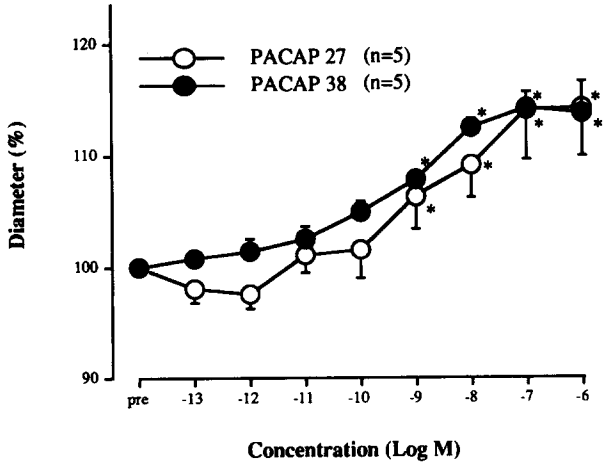

Fig. 3. Curves for concentration-dependent relaxation induced by PACAP-27 and PACAP-38 in rat intracerebral arterioles. Each point represents the mean ± S.E. Vessel diameters are expressed as percentage of the control diameter. * $P < 0.05$.

laxation induced by PACAP-27, from 12.9 ± 4.1% to 48.7 ± 6.1% and 46.5 ± 9.1%, respectively ($P < 0.05$) (Fig. 2B).

3.2. Response of rat intracerebral arterioles

PACAP-27 and PACAP-38 caused similar concentration-dependent relaxation of rat intracerebral arterioles. Maximum relaxations were obtained with 1×10^{-7} to 1×10^{-6} M PACAPs (PACAP-27, 114.3 ± 9.7%; PACAP-38, 114.4 ± 1.3%). There was no statistically significant difference between the relaxant activities of PACAP-27 and PACAP-38 (Fig. 3).

3.3. Measurement of eicosanoids

The concentration of prostaglandin $F_{2\alpha}$ released from artery ring samples to which PACAP-27 was added was more than 2-fold greater than in control samples (31.1 ± 4.2 vs. 14.5 ± 2.1 pg/min/1 mg vessel) ($P < 0.01$) (Table 1). In contrast, there were no statistically significant differences in the concentrations of thromboxane B_2, prostaglandin E_2 or 6-keto prostaglandin $F_{1\alpha}$ in samples treated or not treated with PACAP-27.

4. Discussion

Although heterogeneity of vasomotor responses to various vasoactive substances has been well-described for cerebral vascular beds (Suzuki et al., 1992; Takayasu et al., 1993), we found that PACAP produces a similar vasodilator response in isolated canine pial cerebral

arteries and in rat intracerebral arterioles. These data are compatible with our previous in vivo findings (Seki et al., 1995) that the intracisternal administration of PACAP-27 and PACAP-38 produces a dose-dependent vasodilatation of cerebral arteries as assessed by angiography and that the intra-arterial administration of these peptides increases vertebral artery blood flow. These results suggest that PACAP increases cerebral blood flow by dilating both large cerebral arteries and microvessels.

Immunohistochemical studies showed that PACAP-containing fibers are expressed around cerebral arteries and pial arterioles in the cat (Uddman et al., 1993) and around ovine cerebral small blood vessels (Koves et al., 1990). The anterior portion of the circle of Willis has a richer supply of PACAP fibers than its posterior portion in cat pial arteries (Uddman et al., 1993), probably co-localizing with VIP or other neurotransmitters in parasympathetic fibers. These findings suggest that PACAP may act as a transmitter in perivascular nerve fibers and modulate local cerebral blood flow. Our results demonstrate that the vasodilator effects of PACAP-27 are greater in the anterior portion of the circle of Willis than in the posterior parts. This finding is also consistent with our previous in vivo cerebral artery data (Seki et al., 1995). Additionally, we found a regional difference in the sensitivity of bovine pial arteries to VIP (Suzuki et al., 1984). Thus, the reactivity of cerebral arteries to PACAP appears to correlate to the density of PACAP in the perivascular neural fibers.

The potency of the vasodilatation produced by PACAP-27, PACAP-38 and VIP has been compared in several studies (Warren et al., 1991, 1992; Nandha et al., 1990, Minkes et al., 1992; Uddman et al., 1993; Santiago and Kadowitz, 1993; Cheng et al., 1993; Absood et al., 1992). These studies have shown that the relaxant activity of PACAP-27, PACAP-38 and VIP varied among species, regions of vessels and methods of administration. In our previous in vivo study, the intracisternal administration of VIP and PACAP produced similar vasodilator activities in the basilar arteries. Although the vasodilator activity of VIP was slightly greater than that of PACAP in the present pial artery ring study, there was no statistically significant difference between these peptides. In agreement with the previous in vitro study with rat intracerebral arterioles (Dacey et al., 1988), we now showed that VIP and PACAP have similar vasodilator activities in the cerebral arterioles. These results suggest that the vasodilator activity of PACAP is almost similar to that of VIP in the cerebral vasculatures.

We observed tachyphylaxis following repeated application of PACAP-27, PACAP-38 and VIP to canine cerebral arteries (data not shown). The increase in vertebral artery blood flow induced by PACAP-27 was inhibited by a VIP receptor antagonist (Seki et al., 1995). These findings suggest that these peptides may share common receptors in canine cerebral arteries. The binding sites shared by PACAP and VIP, which have been termed 'type II', also have been demonstrated in vascular smooth muscle cell membranes (Nandha et al., 1990; Huang et al., 1993) as well as in lung, liver, prostate gland and cultured sphenocytes (Gottschall et al., 1990; Lam et al., 1990; Robberecht et al., 1991; Shivers et al., 1991). Another type of binding site, termed 'type I', which binds PACAP with much higher affinities than VIP, has been demonstrated in the brain, anterior lobe of the pituitary gland, adrenal gland and testis (Suda et al., 1991; Gottschall et al., 1990, 1991; Lam et al., 1990; Ohtaki et al., 1990; Shivers et al., 1991; Cauvin et al., 1991).

Previous studies have shown that PACAP produces endothelium-independent relaxation in the cat aorta, iliac artery and rabbit aorta (Warren et al., 1991; Cheng et al., 1992). An inhibitor of nitric oxide synthesis, N^G-monomethyl-L-arginine (L-NMMA), does not affect vasodilatation by PACAP-27 (Seki et al., 1995). In the present study, the removal of endothelium significantly enhanced the relaxation induced by PACAP-27, and pretreatment with a cyclooxygenase inhibitor, aspirin or indomethacin, also enhanced PACAP-induced vasorelaxation. These results suggest that PACAP-27-induced vasorelaxation is partially suppressed by vasocontractile products from the cyclooxygenase pathway in the endothelium. Although this endothelium-derived contracting factor (EDCF) has yet to be identified, it is distinct from endothelin (Vanhoutte, 1989; Auch-Schwelk et al., 1989). The eicosanoids, thromboxane A_2 and prostaglandin H_2, are potential candidates for this factor (Altiere et al., 1985; Buzzard et al., 1993; Ito et al., 1991). When the levels of eicosanoids released from canine cerebral arteries following PACAP-27 administration were compared with those in the controls, the levels of thromboxane B_2, prostaglandin E_2 and 6-keto prostaglandin $F_{1\alpha}$ were not affected, but prostaglandin $F_{2\alpha}$ levels increased significantly. Prostaglandin $F_{2\alpha}$, prostaglandin H_2 and thromboxane A_2 are known to induce strong vasocontraction in cereral vessels, while prostaglandin I_2 or prostaglandin E_2 are vasodilators (Toda et al., 1988). These findings suggest that the release of vasocontractile eicosanoids, prostaglandin $F_{2\alpha}$ itself and/or its precursor, prostaglandin H_2, may be involved in inhibiting PACAP-27-induced relaxation in the canine basilar artery.

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