Pituitary Adenylate Cyclase-Activating Polypeptide Dilates Cerebral Arterioles of Newborn Pigs (43609)

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Abstract. **The actions of the 38- and 27-amino acid forms of synthetic pituitary adenylate cyclase-activating polypeptide (PACAP-38 and PACAP-27) on cerebral arterioles were tested in anesthetized newborn pigs equipped with closed cranial windows. The diameter changes of pial arterioles to topical PACAP were measured and cortical periarachnoid cerebrospinal fluid samples were collected for measurement of cAMP. The 38- and 27-amino acid forms of PACAP produced similar dose-dependent vasodilations. The increases of pial arteriolar diameter produced by PACAP-38 were 6 ± 1%, 15 ± 2%, 23 ± 3%, and 38 ± 3%, and those produced by PACAP-27 were 6 ± 1%, 15 ± 2%, 27 ± 5%, and 38 ± 8% at 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} *M*, respectively. Arteriolar diameter began to increase 1–2 min and reached a maximum 6–8 min after topical application of PACAP. Vasoactive concentrations of PACAP-38 and PACAP-27 increased cerebrospinal fluid cAMP levels dose dependently. Thus, PACAP-38 and PACAP-27 appear to stimulate cerebral adenylate cyclase and are potent dilators of newborn pigs pial arterioles.**

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide that was originally isolated from ovine hypothalamus and found to stimulate adenylate cyclase in rat anterior pituitary cell cultures (1). Two forms of PACAP, with either 38 or 27 amino acids (PACAP-38 and PACAP-27), have been described (1, 2). PACAP-27 corresponds to the amino-terminal 27 residues of PACAP-38 (2). Both forms are derived from a 176-amino acid precursor (3). PACAP is a member of the secretin/glucagon/vasoactive intestinal peptide family. The N-terminal 1–28 residues of PACAP-38 have 68% homology with vasoactive intestinal peptide, but its adenylate cyclase-stimulating activity in pituitary cell cultures is 1,000–10,000 times greater than vasoactive intestinal peptide (1). Although PACAP-immunoreactive neuronal elements are widely distributed in the rat (4), sheep (5), pig (6), monkey, and human (7), PACAP is more abundant in brain than in other tissues, at least in rats (8).

A variety of biologic actions of PACAP have been demonstrated, including release of growth hormone, prolactin, corticotropin, and luteinizing hormone from pituitary cells (1). PACAP stimulated the secretion of adrenaline from adrenal medulla (9) and enhanced pancreas exocrine (10) and endocrine secretion (11). Both PACAP-38 and -27 were reported to have similar hypotensive actions after their intravenous injections (12).

The aim of the present study was to investigate the cerebral microvascular effects of PACAP in newborn piglets since PACAP has been found in nerve fibers around cerebral blood vessels (5).

Materials and Methods

Peptides. Synthetic PACAP-38 and PACAP-27 were purchased from Sigma Chemical Co. Stock PACAP was dissolved in deoxygenated distilled water

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at 10^{-5} M and stored at -60°C. PACAP in dilutions of 10^{-9}-10^{-6} M were made with artificial cerebrospinal fluid (CSF) immediately before each experiment.

Cranial Windows. Newborn piglets (1–5 days old, $n = 12$) were anesthetized with ketamine hydrochloride (33 mg/kg, im) and acepromazine (3.3 mg/kg, im) and maintained on α-chloralose (initially 50 mg/kg, iv, followed by 7 mg/kg/hr). The animals were intubated and ventilated with air. Catheters were inserted in the femoral vein for infusion of anesthetic and fluid, and in the femoral artery for recording blood pressure and monitoring blood gases and pH. Body temperature was maintained between 37°C and 38°C with a heating pad. The scalp was retracted, and a hole 2 cm in diameter was made in the skull over the parietal cortex. The dura was cut without touching the brain and retracted over the bone. A stainless steel and glass cranial window was placed in the hole and cemented into place with dental acrylic. The space under the window was filled with piglet artificial CSF (150 mEq Na^+/liter, 3 mEq K^+/liter, 2.5 mEq Ca^{2+}/liter, 1.2 mEq Mg^{2+}/liter, 132 mEq Cl^-/liter, 3.7 mM glucose, 6 mM urea, and 25 mEq HCO_3^-/liter) through needles incorporated into the sides of the window. Artificial CSF was warmed to 37°C and bubbled with a gas mixture of 6% CO_2 and 6% O_2 in N_2 and typically showed pH, PCO_2, and PO_2 in the range of 7.33–7.36, 41–46 mm Hg, and 43–50 mm Hg, respectively. After infusion of artificial CSF under the window, a dissecting microscope was set above the window and the image of the exposed cortical surface was magnified and projected onto a monitor via a video camera. Pial arteriolar diameter was measured with an in-line electrical micrometer. The volume of artificial CSF directly under the window was about 500 μl and was contiguous with the periarachnoid space.

Experimental Protocol. The exposed cortical surface was allowed to rest for 20 min after an initial artificial CSF infusion under the window. To determine the baseline values, artificial CSF in the volume of 5 ml was flushed under the window, and changes in pial arteriolar diameter were taken every minute for 10 min. At the end of the measurements, 300 μl of CSF were sampled from under the window by slowly infusing artificial CSF into an inlet port of the window and allowing the CSF to drip freely into a collection tube from outlet port. The samples were stored at -60°C for determination of cAMP. Artificial CSF containing PACAP-38 or PACAP-27 at 10^{-9}, 10^{-8}, 10^{-7}, or 10^{-6} M was sequentially infused under the window, and diameter changes of pial arterioles to each concentration were taken every minute for 10 min. CSF was sampled for determination of cAMP levels at the end of each 10-min period. In the control animals, artificial CSF without PACAP was placed under the window, measurements were taken, and samples were collected as above in the treatment animals.

Cyclic AMP Assay. Cortical periarachnoid CSF samples were analyzed for cAMP using radioimmunoassay (cAMP [^{125}-I]-scintillation proximity assay system; Amersham). Acetylation of CSF samples with a 2:1 mixture of triethylamine and acetic anhydride was performed immediately before assay to increase the sensitivity of the method (analysis range, 2–128 fmol cAMP/tube). The radioimmunoassay method used simultaneous addition of sample, [^{125}-I]-cAMP, rabbit cAMP antibody, and anti-rabbit second antibody bound to scintillant-incorporated fluoromicrospheres. Samples (unknown) were mixed overnight on an orbital shaker (200 rpm) at room temperature. To determine the amount of [^{125}-I]-cAMP bound to the light-producing fluoromicrospheres, the samples were counted in a β-scintillation counter. All unknowns were assayed at two dilutions.

Statistical Analysis. Comparisons among populations were accomplished using analysis of variance and Fisher probable least significant difference. The 95% confidence level ($P < 0.05$) was considered statistically significant. All values are presented as means ± SE of absolute values or as percentage of change from baseline values.

Results

Vasodilation. Arterial blood pH, PCO_2, PO_2, and mean arterial blood pressure were unchanged throughout the course of the experimental protocol (Table I). Topically applied PACAP produced concentration-dependent dilation of pial arterioles (Fig. 1 and Table I). The dilations to PACAP-38 and PACAP-27 were similar at all doses. As shown in Figures 2 and 3, the vasodilation began 1–2 min and reached a maximum 6–8 min after topical application of these polypeptides. The vasodilation lasted for 30 to 60 min if PACAP were not flushed from under the cranial window.

Both PACAP-38 and PACAP-27 produced concentration-dependent increases of cAMP in CSF (Fig. 4 and Table I) over the same concentration range that produced the vasodilation.

Discussion

The principal findings of this study were that PACAP-38 and PACAP-27 increased cAMP in cortical CSF and dilated pial arterioles when applied to the cortical surface of newborn pig brains.

To the best of our knowledge, there are no previous reports on the effects of PACAP on cerebral circulation *in vivo*. Nevertheless, the present observations are consistent with those reported on peripheral vessels. Nandha *et al.* (12) reported that intravenous administration of bolus doses of PACAP-38 and PACAP-27 induced similar, rapid, dose-dependent decreases in systemic blood pressure because of peripheral vasodilation in anesthetized rats. Similarly, Warren *et al.* (13) demonstrated that PACAP-38 and PACAP-27 were of

Table I. Blood Gases, pH, Mean Arterial Blood Pressure, Pial Arteriolar Diameter, and Cortical Periarachnoid CSF cAMP Concentrations before and during Treatment with PACAP

	PACAP-38 (*M*)					PACAP-27 (*M*)				
	Control	10^{-9}	10^{-8}	10^{-7}	10^{-6}	Control	10^{-9}	10^{-8}	10^{-7}	10^{-6}
Pial arteriolar diameter (μm)	101 ± 6	110 ± 6	120 ± 5[a]	131 ± 6[a]	142 ± 9[a]	103 ± 3	105 ± 4	114 ± 3[a]	121 ± 5[a]	136 ± 6[a]
Mean arterial blood pressure (mm Hg)	83 ± 2	83 ± 2	83 ± 2	83 ± 2	83± 2	80 ± 2	79 ± 1	79 ± 2	80 ± 3	80 ± 2
PaO_2 (mm Hg)	95 ± 3		94 ± 5			95 ± 6		90 ± 4		
$PaCO_2$ (mm Hg)	31 ± 1		33 ± 1			32 ± 1		32 ± 1		
pH	7.45 ± 0.01		7.46 ± 0.01			7.49 ± 0.02		7.49 ± 0.02		
cAMP (pmol/ml)	1.7 ± 0.3	3.2 ± 1.8	6.2 ± 1.8[a]	5.5 ± 1.3[a]	7.2 ± 1.2[a]	1.4 ± 0.3	2.0 ± 0.3	2:6 ± 0.4	4.4 ± 1.2[a]	5.5 ± 0.9[a]

[a] $P < 0.05$ compared with the control value.

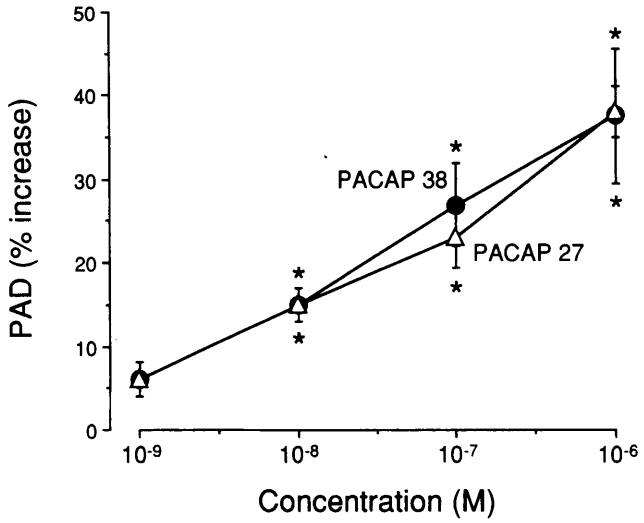

Figure 1. Cumulative dose-response curves of increases in pial arteriolar diameter (PAD) after PACAP-38 and PACAP-27 infusion under the cranial window. Diameter changes at various PACAP doses were compared with those of the corresponding baseline value, and expressed as percentage increase ($n = 6$; mean ± SE). *$P < 0.05$, compared with no increase.

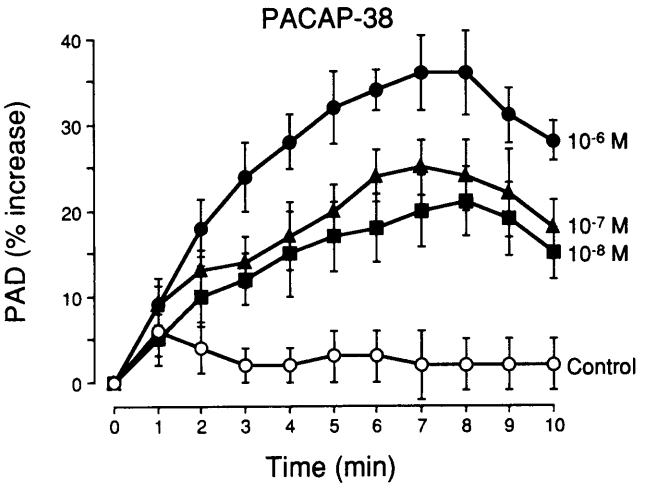

Figure 2. Increases in pial arteriolar diameter (PAD) produced by infusions of PACAP-38 under the cranial window. Increases were significantly greater ($P < 0.05$) than zero and the corresponding control values at all three doses from 2 to 10 min ($n = 6$ each).

equal potency as vasodilators of preconstricted aortic rings *in vitro*.

Immunohistochemical studies (4–7) demonstrated that PACAP is a neuropeptide with wide tissue distribution. Nerve fibers displaying PACAP immunoreactivity were found around blood vessels and among bundles of smooth muscle cells in the tracheobronchial wall of pigs (6) and around blood vessels in ovine hypothalamus (5). Radioreceptor binding studies (12) demonstrated that ^{125}I-labeled PACAP-27 bound to membranes prepared from rat aorta, iliac artery, femoral artery, and vein. Taken together, the present results and these previous observations indicate that PACAP could take part in the regulation of cerebral and peripheral vascular tone. The physiologic significance of PACAP on the cerebral circulation cannot be determined from the present experiments. Certainly the presence of PACAP-containing nerve fibers around cerebral vessels suggests substantial concentrations could be produced at junctions between these fibers and the vascular smooth muscle. However, until receptor blockers are available, demonstration that dilation in response to a particular stimulus involves PACAP cannot be made. Furthermore, stimuli that cause PACAP release from these neurons have not been demonstrated.

Since, in the present study, PACAP-38 and

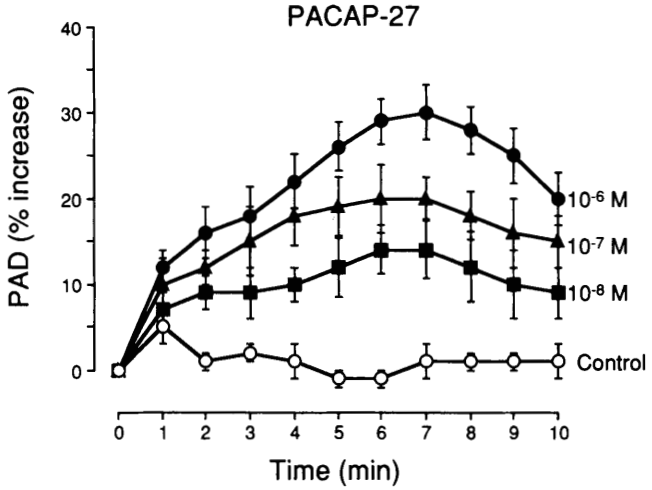

Figure 3. Increases in pial arteriolar diameter (PAD) produced by infusions of PACAP-27 under the cranial window (mean ± SE). Increases were significantly greater ($P < 0.05$) than zero and the corresponding control values at all three doses from 2 to 10 min ($n = 6$ each).

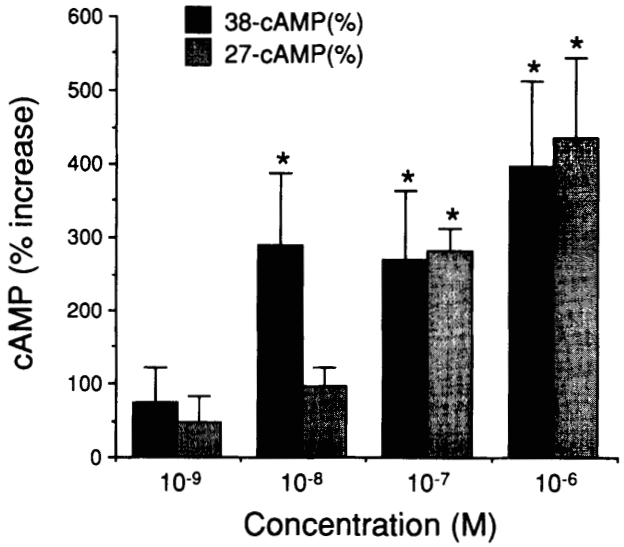

Figure 4. Effects of PACAP-38 and PACAP-27 on concentrations of cAMP in cortical periarachnoid CSF. Values are compared with the corresponding control levels (no PACAP), and expressed as percentage increase (mean ± SE, $n = 6$). *$P < 0.05$, compared with no increase.

PACAP-27 appeared to be equipotent in inducing vasodilation, it is likely that the N-terminal first 27 amino acids are most important in the vasoreactivity of PACAP-38. This is somewhat surprising since Ohtaki *et al.* (14) used ^{125}I-PACAP-27 as radioligand and demonstrated that bovine brain had a 57-kDa-specific PACAP receptor and the receptor was found to have higher affinity for PACAP-38 than PACAP-27. Cauvin *et al.* (15), using ^{125}I-PACAP-38 as radioligand, demonstrated the presence of both high and low affinity binding sites for PACAP-38 and of low affinity binding sites for PACAP-27 in rat brain cortical membranes. Possibly the low affinity binding sites, with similar affinity for PACAP-27 and -38, are predominant activators of adenylate cyclase in vascular smooth muscle. Regardless, Arimura *et al.* (8) also found that PACAP-38 represented the major portion of total PACAP immunoreactivity in rat brain and peripheral tissues.

In the present study, we assayed cAMP levels in the cortical CSF as an indirect indicator of the adenylate cyclase activity of the underlying surface. Activation of adenylate cyclase causes increased intracellular concentrations of cAMP that affect cellular function. Cyclic nucleotides are removed by phosphodiesterase metabolism and by expulsion from the cells. Measurement of cyclic nucleotides in extracellular fluid provides a mechanism for repeatedly detecting changes in cyclic nucleotide production. Cyclic AMP in cortical periarachnoid CSF should be considered solely as an indicator of intracellular cAMP and not as a potential paracrine factor. The sources of the increased cortical periarachnoid CSF cAMP produced by PACAP-38 and PACAP-27 were not determined in the present study. However, they potentially include all cell types found under the window: vascular smooth muscle, endothelium, glia, and neurons. Most of these cell types have been shown to increase cAMP via activation of adenylate cyclase by PACAP (1, 13, 16).

Tatsuno *et al.* (17) demonstrated that PACAP-induced interleukin 6 production from pituitary cell cultures was prevented by preincubating pituitary cells with a specific inhibitor of cAMP-dependent protein kinase A-H89. Schafer *et al.* (18) showed that PACAP-receptor complex was associated with a GTP-binding protein. The present study found a close association between vasodilation and increased cAMP after PACAP application. Relaxation of cat aorta and iliac artery segments by PACAP was endothelium independent and was not affected by indomethacin, glibenclamide, or nitro-L-arginine methyl ester (19). Therefore, PACAP-induced vasodilation appears to be independent of cyclooxygenase metabolites, endothelium-derived relaxing factor, or activation of K^+-ATP channels.

In summary, PACAP-38 and PACAP-27 are potent vasodilators of the newborn pig cerebral microcirculation. Physiologic roles of PACAP in control of cerebral circulation remain to be demonstrated.

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