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Role of PAC₁ receptor in adrenal catecholamine secretion induced by PACAP and VIP in vivo

STÉPHANE LAMOUCHE AND NOBUHARU YAMAGUCHI

Groupe de Recherche sur le Système Nerveux Autonome, Faculté de Pharmacie,
Université de Montréal, Montréal, Québec, Canada H3C 3J7

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Role of PAC₁ receptor in adrenal catecholamine secretion induced by PACAP and VIP in vivo. *Am J Physiol Regulatory Integrative Comp Physiol* 280: R510–R518, 2001.—The present study was conducted to investigate the functional implication of the pituitary adenylate cyclase-activating polypeptide (PACAP) type I (PAC₁) receptor in the adrenal catecholamine (CA) secretion induced by either PACAP-27 or vasoactive intestinal polypeptide (VIP) in anesthetized dogs. PACAP-27, VIP, and their respective antagonists were locally infused to the left adrenal gland via the left adrenolumbar artery. Plasma CA concentrations in adrenal venous and aortic blood were determined by means of a high-performance liquid chromatograph coupled with an electrochemical detector. Adrenal venous blood flow was measured by gravimetry. The administration of PACAP-27 (50 ng) resulted in a significant increase in adrenal CA output. VIP (5 µg) also increased the basal CA secretion to an extent comparable to that observed with PACAP-27. In the presence of PACAP partial sequence 6–27 [PACAP-(6–27); a PAC₁ receptor antagonist] at the doses of 7.5 and 15 µg, the CA response to PACAP-27 was attenuated by ~50 and ~95%, respectively. Although the CA secretagogue effect of VIP was blocked by ~85% in the presence of PACAP-(6–27) (15 µg), it remained unaffected by VIP partial sequence 10–28 [VIP-(10–28); a VIP receptor antagonist] at the dose of 15 µg. Furthermore, the CA response to PACAP-27 did not change in the presence of the same dose of VIP-(10–28). The results indicate that PACAP-(6–27) diminished, in a dose-dependent manner, the increase in adrenal CA secretion induced by PACAP-27. The results also indicate that the CA response to either PACAP-27 or VIP was selectively inhibited by PACAP-(6–27) but not by VIP-(10–28). It is concluded that PAC₁ receptor is primarily involved in the CA secretion induced by both PACAP-27 and VIP in the canine adrenal medulla in vivo.

pituitary adenylate cyclase-activating polypeptide-27 and -(6–27); vasoactive intestinal polypeptide-(10–28); medulla; dogs

PITUITARY ADENYLATE cyclase-activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) are neuropeptides widely distributed in the central and peripheral nervous systems. Both of them are members of the vasoactive intestinal polypeptide/secretin/glucagon

family of peptides. PACAP was first isolated from the ovine hypothalamus and can be found in two biologically active isoforms depending on the number of amino acid residues, PACAP-27, and PACAP-38 (19, 20). VIP is a 28-amino acid polypeptide originally isolated from porcine intestine and shares 68% homology with PACAP-27 in their amino acid sequence (20, 22). PACAP and VIP possess a wide variety of biological activities, particularly a potent secretagogue effect on adrenal catecholamine secretion both in vitro (18, 28) and in vivo models (28, 31). PACAP exerts tissue-specific effects by interacting with at least three distinct G protein-coupled receptors: the PAC₁, VPAC₁, and VPAC₂ receptors (11). The PAC₁ receptor is highly selective for both isoforms of PACAP and has only a weak affinity for VIP (11). On the other hand, the VPAC receptors possess a high affinity for both VIP and PACAP (11). The adrenal gland contains high concentrations of PACAP (2, 28) as well as PAC₁ receptor (28). Despite the fact that pharmacological characterization of the PAC₁ and VPAC receptors has been well documented in various tissues in vitro (28), studies in vivo on the functional involvement and the relative importance of these receptors in the adrenal medulla have been limited in number. Therefore, the aims of the present study were to demonstrate the functional existence of PAC₁ receptor involved in PACAP-induced adrenal catecholamine secretion and to clarify the relative importance of PAC₁ and VPAC receptors in the secretion of adrenal catecholamines induced by VIP under in vivo conditions.

METHODS

Preparation of animals. Adult mongrel dogs (26.82 ± 1.78 kg; *n* = 35), fasted overnight but allowed free access to water, were anesthetized with pentobarbital sodium (30 mg/kg iv, followed by 4 mg/kg as needed; MTC Pharmaceutical, Cambridge, ON). Respiration was controlled through an endotracheal tube, with room air delivered by a respirator (model 607; Harvard, South Natick, MA). Body temperature of each dog was monitored and kept constant at 37.5 ± 0.5°C by a thermoregulator (model 74; Yellow Springs Instruments, Yellow Springs, OH) connected to a heating pad throughout the experiment. Physiological saline was slowly adminis-

Address for reprint requests and other correspondence: N. Yamaguchi, Faculté de Pharmacie, Université de Montréal, C.P. 6128, Succursale "Centre-ville," Montréal, Québec, Canada H3C 3J7 (E-mail: nobuharu.yamaguchi@umontreal.ca).

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tered intravenously during the whole period of the experiment to prevent dehydration. The pH of physiological saline was adjusted at 7.38 immediately before use. Both femoral arteries were cannulated; the right femoral artery was used to measure aortic pressure, and the left femoral artery was used to obtain aortic blood samples.

Preparation of local intra-arterial drug infusion into the left adrenal gland. The experimental model used in this study has previously been validated in our laboratory and reported in full detail elsewhere (31). Briefly, after a median laparotomy, a catheter (PE-50) was inserted into the left adrenolumbar artery from the peripheral end toward the gland and then advanced, so that the tip of the catheter reached under the gland or to a level close to the adrenolumbar arterial-aortic junction. All visible branches arising from the adrenolumbar artery toward the outside of the gland were ligated to prevent undesired drug diffusion into the systemic circulation. The volume of this catheter was fixed at 0.25 ml, and the catheter was connected to a double-infusion pump (model 55-2226, Harvard).

Preparation of an extracorporeal adrenal venous circuit. A specially shaped catheter (PE-240) was inserted into the left adrenolumbar vein through the left femoral vein. The catheter was tied at the adrenoabdominal-vena cava junction. The left adrenolumbar vein distal to the gland was ligated to obtain actual adrenal venous blood. The volume of this catheter was fixed at 1.5 ml. Venous blood from the gland was continuously drained into a small blood reservoir to facilitate blood sampling. Venous blood in the reservoir was returned to the dog by a perfusion pump (Masterflex model 7016-52, Cole-Parmer Instrument, Chicago, IL) through a catheter inserted into the right femoral vein at a perfusion rate adjusted to a stabilized initial venous flow. After all surgical procedures were completed, heparin sodium was administered at 200 U/kg iv and then at 100 U/kg every hour. The dog was then allowed a stabilization period of ~60 min.

Measured parameters. Mean aortic pressure and heart rate were measured and recorded with a polygraph system (model RM-6000, Nihon-Kohden, Tokyo, Japan). Aortic and left adrenal venous blood were simultaneously sampled into graded, chilled tubes for catecholamine analyses. Adrenal venous blood flow was determined by a gravimetric method at each sampling time point (31). Hematocrit was measured in all adrenal venous blood samples. Blood (1.5 ml) was transferred to a centrifuge tube containing 30 μ l of preservative solution (pH 6.5) consisting of ethylene glycol-bis(β -amino-ethyl ether)-*N,N,N',N'*-tetraacetic acid (95 mg/ml) and glutathione (60 mg/ml). Blood samples were immediately centrifuged at 4°C for 5 min at 14,000 revolutions/min with a refrigerated centrifuge (model 5402, Eppendorf, Hamburg, Germany). Plasma was stored at -80°C until assayed. Plasma concentrations of adrenaline and noradrenaline were quantified by means of an isocratic high-performance liquid chromatographic system (Gilson, Villiers-Le-Bel, France) coupled with an electrochemical detector "Coulchem II" (model 5200; ESA, Bedford, MA; Ref. 31). At the end of each experiment, the left adrenal gland was removed and weighed. Adrenal catecholamine data were obtained in net catecholamine output calculated as follows: net output of adrenal catecholamine ($\text{ng} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ of gland) = $([\text{CA}]_{\text{AV}} - [\text{CA}]_{\text{AO}}) \times \text{BF}_{\text{AV}} \times (1 - \text{Hct}_{\text{AV}}) / \text{wet weight of gland}$, where $[\text{CA}]_{\text{AV}}$ is plasma catecholamine concentration in adrenal venous blood, $[\text{CA}]_{\text{AO}}$ is plasma catecholamine concentration in aortic blood, BF_{AV} is adrenal venous blood flow, and Hct_{AV} is adrenal venous blood hematocrit.

Experimental protocol. The present study consisted of two series of experiments. The first series of experiments was to

determine the functional existence of the PAC₁ receptor in the PACAP-27-induced catecholamine secretion. The first group (28.1 ± 4.8 kg; $n = 6$) of this series served as the control, receiving a vehicle (saline 0.9%, pH 7.38) to ensure the reproducibility of the adrenal catecholamine responses to PACAP-27 (Sigma Chemical, St-Louis, MO). The two identical doses of PACAP-27 (50 ng with concentration of 0.1 $\mu\text{g}/\text{ml}$) were administered with an interval of 30 min. One minute after the initial control sample was taken, the vehicle was infused via the catheter inserted into the left adrenolumbar artery at a rate of 0.25 ml/min for 4 min. At the third minute, PACAP-27 was added to the vehicle infusion line through a three-way stopcock connected to the adrenolumbar arterial catheter. Thus the vehicle and PACAP-27 were simultaneously administered during the last minute of the infusion. At the end of the 4-min infusion, the administration of both vehicle and PACAP-27 was discontinued. Adrenal venous blood samples were obtained for precisely 1 min during the infusion at 1, 2, 3, and 4 min followed by sample collections at 1, 2, 4, 10, 15, and 30 min after the secession of the infusion. The dead volume of the adrenal arterial (0.25 ml) and venous (1.5 ml) catheters was taken into account in relation to the infusion rate and adrenal venous blood flow, respectively. Aortic blood samples (1.5 ml each) were simultaneously obtained during adrenal venous sample collections at these sampling time points. The sample obtained 30 min after the secession of the first infusion served as control for the subsequent intervention. The second administrations of the vehicle and PACAP-27 as well as the sequence of blood sample collections followed exactly the same protocol described for the first bloc of the experiment.

In the second (27.3 ± 2.9 kg; $n = 6$) and the third (25.1 ± 0.7 kg; $n = 6$) group of the first series, the catecholamine secretagogue response to PACAP-27 (50 ng) was tested in absence and presence of PACAP partial sequence 6-27 [PACAP-(6-27); Sigma Chemical], a selective PAC₁ receptor antagonist (24). The second and third group received 7.5 and 15 μg of PACAP-(6-27), respectively. During the first bloc of the protocol, the dog received the vehicle followed by PACAP-27. In the second bloc, the infusion of either 7.5 or 15 μg of PACAP-(6-27), instead of saline, was started 3 min before PACAP-27 and continued during the fourth minute. At the fourth minute, the infusion of PACAP-27 was started so that the antagonist and the agonist were administered simultaneously. Thus the timing of drug administrations and the sequence of sample collections of adrenal venous and aortic blood were exactly the same as those described for the control group.

The second series consisted of three groups to investigate the relative importance of PAC₁ and VPAC receptors in catecholamine release induced either by PACAP-27 or VIP. In the first group (24.5 ± 2.2 kg; $n = 6$), the effect of PACAP-27 (50 μg) was tested in absence and presence of 15 μg of VIP partial sequence 10-28 [VIP-(10-28); Sigma Chemical], a nonselective VPAC receptor antagonist (27). In the second (25.7 ± 3.4 kg; $n = 6$) group, the catecholamine secretagogue effect of VIP (5 μg) was evaluated in absence and presence of VIP-(10-28) (15 μg). Similarly, the third group (27.4 ± 5.0 kg; $n = 5$) served to test the effect of VIP (5 μg) in absence and presence of PACAP-(6-27) (15 μg). In all groups of this series, the experimental protocols for the sequence of local administrations of the vehicle and the drugs as well as the timing of sample collections of adrenal venous and aortic blood were exactly the same as those described for the first series. Each of the experimental protocols described for both series was applied only once on each animal.

The doses of PACAP-(6–27) used in this study were selected according to several dose-finding experiments with doses ranging from 1.5 to 15 µg. The dose of VIP-(10–28) (15 µg) was used according to the same molar equivalence with the higher dose of PACAP-(6–27). The doses of PACAP-27 and VIP were selected on the basis of the dose-response relationships of each peptide previously reported from our laboratory (17, 31). The dose of 5 µg of VIP was used in the present study, as this dose of VIP produced catecholamine responses to an extent similar to that caused by 50 ng of PACAP-27 (17, 31).

The animal research committee of the Université de Montréal has approved the experimental protocol. The animals used in this study have been cared for and used in accordance with the principles of the *Guide to the Care and Use of Experimental Animals* published by the Canadian Council on Animal Care.

Statistical analyses. The statistical evaluations and the calculations of an area under the curve were carried out using a statistical software package (SigmaStat for Windows, version 2.03; SPSS, Chicago, IL). Differences over an experimental period within the same subjects were assessed by an analysis of variance for repeated measures followed by multiple comparisons with one control using the Dunnett's method. Catecholamine responses to the agonists in absence and presence of the antagonists in the same subjects were analyzed using the paired *t*-test. This test was made on the net amount of catecholamines released during the first 5 min after the onset of the agonist infusion obtained from the area under the curve of the catecholamine output. Comparisons between different groups were conducted by the use of one-way analysis of variance followed by the Bonferroni *t*-test. When applicable, a preliminary logarithmic transformation was used to satisfy the condition of a normal distribution of variance (30).

RESULTS

Catecholamine secretion in response to PACAP-27. The initial basal values for adrenal venous and aortic catecholamines, mean aortic pressure, heart rate, left adrenal venous blood flow, and hematocrit obtained from six separate groups are summarized in Table 1. These values were not statistically different between groups. Aortic catecholamine levels, mean aortic pres-

sure, heart rate, and hematocrit remained relatively stable during and after the local administrations of any drug tested. In the control group receiving the vehicle (saline), the administration of PACAP-27 at the dose of 50 ng increased both adrenal venous catecholamine concentration and blood flow without significantly affecting circulating catecholamine concentrations in aortic blood. Consequently, the output of adrenal epinephrine and norepinephrine increased significantly in response to PACAP-27 (Fig. 1A). The onset of catecholamine response to PACAP-27 was rapid, and the increased catecholamine output returned toward the corresponding preinfusion control levels within ~10 min after the secession of drug infusion (Fig. 1A). The time course of catecholamine output as well as the net amount of catecholamines released during the first 5 min after the onset of PACAP-27 infusion were highly reproducible upon the repetition of the same dose of PACAP-27 with an interval of 30 min (Fig. 1, A and B).

Catecholamine response to PACAP-27 in the presence of PACAP-(6–27). In the second and third group of the first series, the catecholamine response to the first infusion of PACAP-27 was identical as in the first group (Figs. 1A, 2A, 3A). In the presence of a low dose (7.5 µg) of PACAP-(6–27), the PACAP-27-induced catecholamine output still significantly increased, but the amplitude was markedly attenuated (Fig. 2A). Consequently, the net amount of epinephrine and norepinephrine released during the first 5 min after the onset of PACAP-27 infusion was significantly reduced (Fig. 2B). A more pronounced inhibition was obtained in the group receiving the higher dose (15 µg) of PACAP-(6–27) (Fig. 3, A and B). The PACAP-27-induced catecholamine secretion was thus diminished in a dose-dependent manner when compared between three different groups receiving the vehicle or 7.5 or 15 µg of PACAP-(6–27) (Fig. 4). The inhibition obtained with the higher dose of PACAP-(6–27) (~95%) was significantly greater than that found with the lower dose (~50%; Fig. 4).

Table 1. Initial values for adrenal venous and aortic catecholamines, MAP, HR, left AV-BF, hematocrit, and postmortem wet weight of left adrenal gland in group receiving either PACAP-27 or VIP in presence of various drugs

Parameters	First Series			Second Series		
	Gr 1: PACAP-27 + Vehicle (n = 6)	Gr 2: PACAP-27 + PACAP-(6–27) (7.5 µg; n = 6)	Gr 3: PACAP-27 + PACAP-(6–27) (15 µg; n = 6)	Gr 1: PACAP-27 + VIP-(10–28) (15 µg; n = 6)	Gr 2: VIP + PACAP-(6–27) (15 µg; n = 5)	Gr 3: VIP + VIP-(10–28) (15 µg; n = 6)
AV-EPI, ng/ml	29.1 ± 11.1	30.9 ± 17.6	32.8 ± 17.6	16.9 ± 3.2	13.3 ± 5.4	15.6 ± 5.4
AV-NE, ng/ml	3.9 ± 1.5	7.4 ± 4.3	5.4 ± 2.3	1.3 ± 0.6	2.2 ± 0.9	1.4 ± 0.3
AO-EPI, ng/ml	0.07 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.06 ± 0.02
AO-NE, ng/ml	0.42 ± 0.12	0.47 ± 0.04	0.42 ± 0.07	0.18 ± 0.03	0.32 ± 0.07	0.32 ± 0.08
MAP, mmHg	151 ± 10.4	142.9 ± 9.3	153.4 ± 14.0	153.4 ± 14.0	145.7 ± 10.7	146.1 ± 9.0
HR, beats/min	166.8 ± 7.9	164.5 ± 6.3	171.3 ± 16.5	181.3 ± 7.4	150.8 ± 10.3	167.0 ± 9.8
AV-BF, ml/min/g	3.8 ± 0.6	4.0 ± 0.8	4.1 ± 0.7	3.5 ± 0.3	4.9 ± 0.8	3.7 ± 0.4
AV-Hct, %	46.2 ± 0.7	45.9 ± 2.4	49.3 ± 2.8	47.9 ± 1.8	47.1 ± 2.5	45.3 ± 1.1
LAG, g	1.22 ± 0.22	1.22 ± 0.16	1.10 ± 0.16	1.06 ± 0.01	1.14 ± 0.16	0.96 ± 0.08

Values are means ± SE. Gr, group; n, number of dogs in each group; AV-EPI, adrenal venous epinephrine; AV-NE, adrenal venous norepinephrine; AO-EPI, aortic epinephrine; AO-NE, aortic norepinephrine; MAP, mean aortic pressure; HR, heart rate; AV-BF, adrenal venous blood flow; AV-Hct, adrenal venous hematocrit; LAG, wet weight of left adrenal gland.

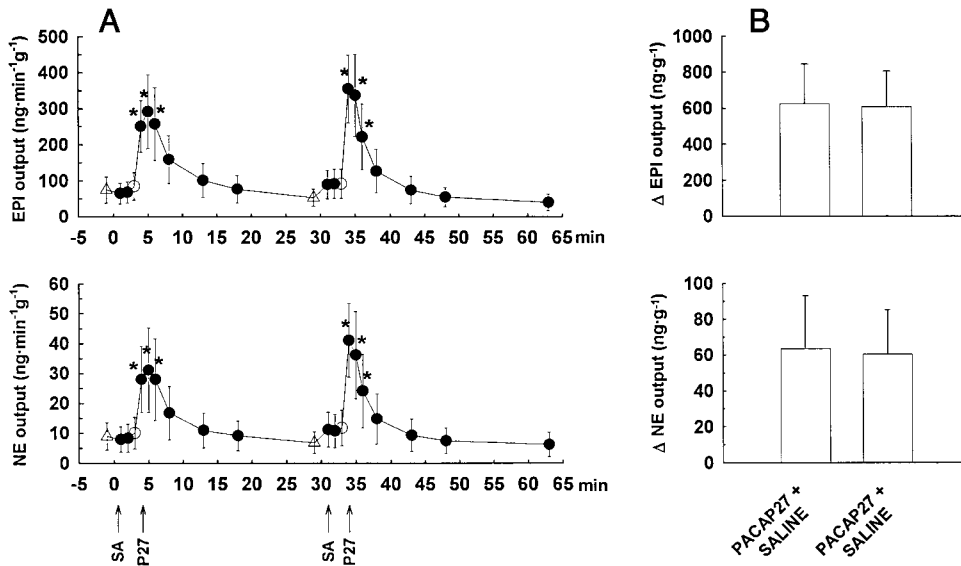


Fig. 1. A: adrenal epinephrine (EPI) and norepinephrine (NE) output in response to repeated administrations of PACAP-27 (P27, 50 ng) given at 3 and 33 min in control group receiving vehicle [saline (SA)] as indicated by arrows; Δ and \circ , control values observed immediately before administration of vehicle and PACAP-27, respectively; \bullet , values observed during and after infusion of vehicle and PACAP-27. $*P < 0.05$ vs. corresponding control value indicated by \circ ($n = 6$). B: net amount of EPI (Δ EPI output) and NE (Δ NE output) released during first 5 min after onset of each infusion of PACAP-27 as shown in A.

Effects of VIP-(10–28) and PACAP-(6–27) on VIP-induced catecholamine secretion. In the first group of the second series receiving VIP-(10–28) (15 μ g), the net quantity of catecholamines released during the first 5 min after the onset of the administration of PACAP-27 (50 ng) remained unchanged (Fig. 5). Similarly, the presence of VIP-(10–28) (15 μ g) did not affect the increase in adrenal catecholamine release induced by VIP with the dose of 5 μ g (Fig. 6). However, the VIP-induced catecholamine secretion was significantly inhibited by $\sim 80\%$ in the presence of PACAP-(6–27) with the same dose that blocked the catecholamine response to PACAP-27 (Fig. 7).

Both PACAP-(6–27) and VIP-(10–28) showed, by themselves, a slight secretagogue effect on the adrenal medulla at the doses used in the present study. For example, catecholamine output significantly increased with the higher dose of PACAP-(6–27) (Fig. 3A). Sim-

ilarly, epinephrine output increased from 10.7 ± 2.4 to 58.8 ± 23.0 $\text{ng}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ ($P < 0.05$; $n = 6$) in the presence of VIP-(10–28). These observations suggest that both antagonists possess the partial agonistic property in the canine adrenal medulla in vivo, as previously shown with PACAP6–38 in rat cerebellar neuroblasts in vitro (9). However, the initially elevated catecholamine output induced by these antagonists was short lasting and returned to the control level before the infusion of either PACAP-27 or VIP was started.

DISCUSSION

The present results indicate that local infusion of PACAP-27 into the left adrenal gland resulted in a significant increase in adrenal medullary catecholamine secretion in a highly reproducible manner when

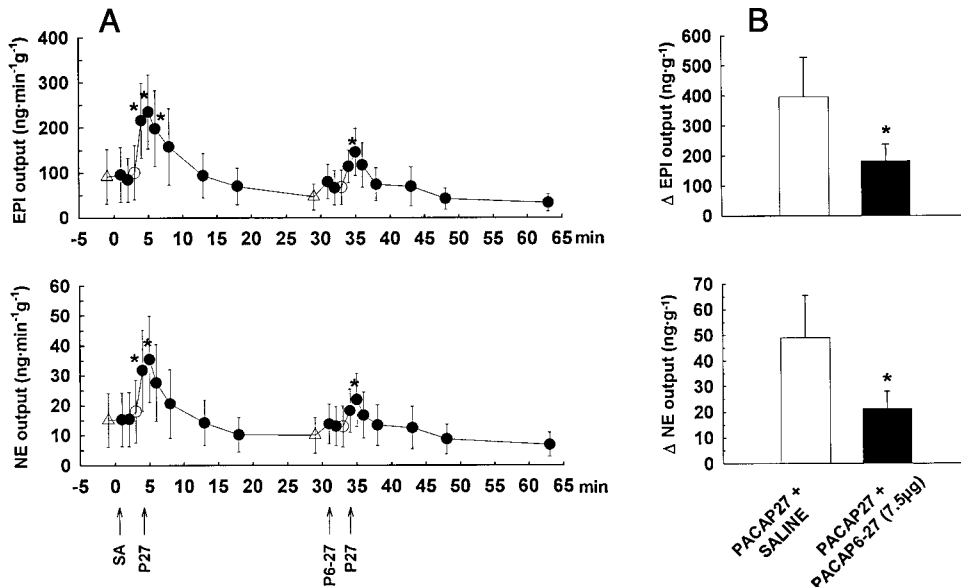
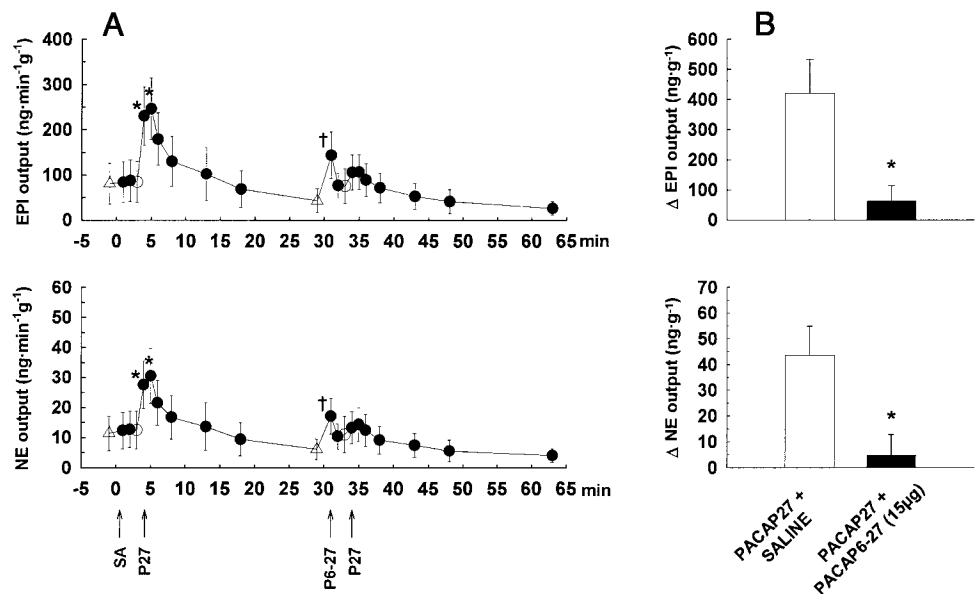


Fig. 2. A: EPI and NE output in response to repeated administrations of PACAP-27 (50 ng) given at 3 and 33 min in group receiving PACAP partial sequence 6–27 [PACAP-(6–27); P6–27, 7.5 μ g] as indicated by arrows; Δ and \circ , control values observed immediately before administration of vehicle (saline) or PACAP-(6–27) and PACAP-27, respectively; \bullet , values observed during and after infusion of vehicle, PACAP-27, or PACAP-(6–27). $*P < 0.05$ vs. corresponding control value indicated by \circ ($n = 6$). B: Δ EPI output and Δ NE output released during first 5 min after onset of each infusion of PACAP-27 (50 ng) in the absence (open bars) and presence of PACAP-(6–27) (filled bars) with dose of 7.5 μ g. $*P < 0.05$ vs. corresponding vehicle control indicated by open bars ($n = 6$).

Fig. 3. A: EPI and NE output in response to repeated administrations of PACAP-27 (50 ng) given at 3 and 33 min in group receiving PACAP-(6-27) (15 μ g) as indicated by arrows; Δ and \circ , control values observed immediately before administration of vehicle (saline) or PACAP-(6-27) and PACAP-27, respectively; \bullet , values observed during and after infusion of vehicle, PACAP-27 or PACAP-(6-27). * $P < 0.05$ vs. corresponding control value indicated by \circ ($n = 6$). † $P < 0.05$ vs. corresponding control value indicated by Δ ($n = 6$). B: Δ EPI output and Δ NE output released during first 5 min after onset of each infusion of PACAP-27 (50 ng) in the absence (open bars) and presence of PACAP-(6-27) (filled bars) with dose of 15 μ g. * $P < 0.05$ vs. corresponding vehicle control indicated by open bars ($n = 6$).



the same dose was repeated with an interval of ~ 30 min. The results also show that the secretion of adrenal catecholamines induced by either PACAP-27 or VIP was significantly blocked by PACAP-(6-27), a selective PAC₁-receptor antagonist. However, neither PACAP-27- nor VIP-induced catecholamine secretion was affected by VIP-(10-28), a nonselective VPAC receptor antagonist. The study suggests that the PAC₁ receptor may be functionally involved in locally regulating the adrenal catecholamine secretion induced by either PACAP-27 or VIP in the canine adrenal medulla in vivo.

It has been well documented that PACAP is a potent secretagogue of adrenal catecholamines both in vitro and in vivo models (28). An immunofluorescence study revealed the existence of PACAP immunoreactivity in splanchnic nerve terminals in the rat adrenal medulla (13, 21) and in sensory nerve fibers ending on chromaffin cell (4). In the isolated, perfused rat (1) and porcine (26) adrenal gland, it has also been shown that endogenous PACAP was released into the perfusate during splanchnic nerve stimulation. These observations are compatible with the hypothesis that PACAP may play a role as a peptidergic neurotransmitter or neuromodulator in the adrenal gland (1, 4, 26). With respect to the postsynaptic binding sites for PACAP, various studies on the biochemical and pharmacological receptor characterizations indicate that PACAP exerts its biological action by stimulating PAC₁, VPAC, or both receptors (28). The presence of PAC₁ receptors has also been shown in human adrenal chromaffin cells in vitro (33). The functional pharmacological antagonism, however, has not yet been clearly demonstrated in the adrenal medulla in vivo. In the present study, it is evident that the increase in catecholamine output in response to PACAP-27 was blocked by PACAP-(6-27) in a dose-dependent manner. PACAP-(6-27) has been shown to specifically antagonize the PACAP-27-induced response (adenylate cyclase activation) in rat pancreatic

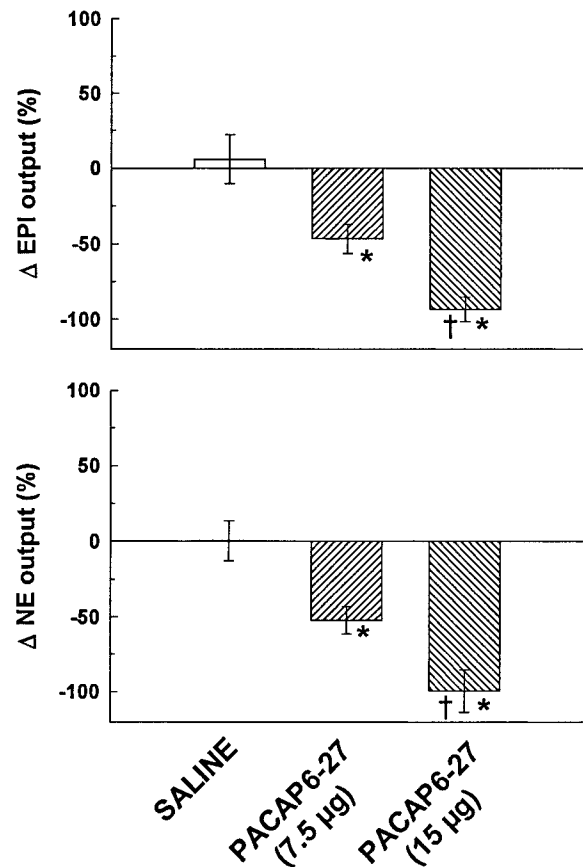


Fig. 4. Percent changes in Δ EPI output and Δ NE output released during first 5 min after onset of second infusion of PACAP-27 (50 ng) in 3 separate groups. Open bars, catecholamine responses to PACAP-27 in absence of PACAP-(6-27) (saline control group). Filled bars with up-right and down-right diagonal lines, catecholamine responses to PACAP-27 in presence of PACAP-(6-27) with the dose of 7.5 and 15 μ g, respectively. * $P < 0.05$ vs. value obtained from vehicle control group (saline). † $P < 0.05$ vs. value obtained from group receiving 7.5 μ g of PACAP-(6-27). Original data are shown in Figs. 1, 2, and 3.

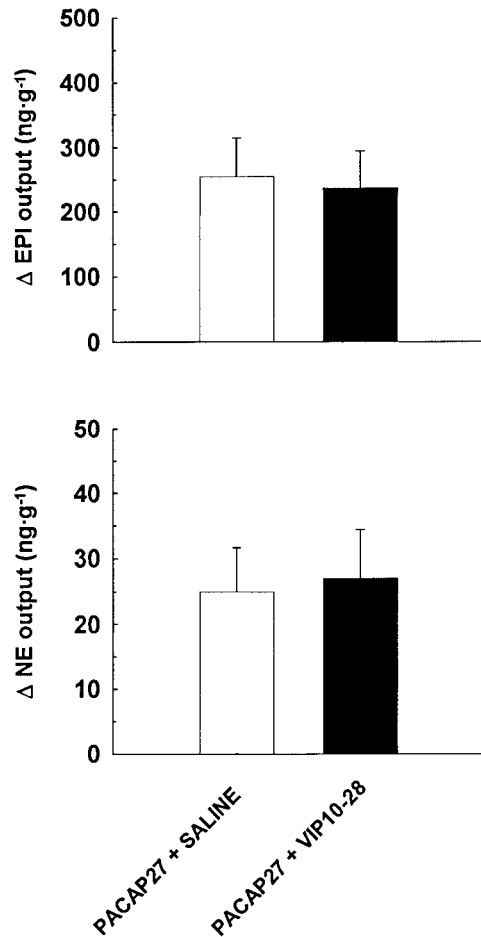


Fig. 5. Δ EPI output and Δ NE output released during first 5 min after onset of each infusion of PACAP-27 (50 ng) in the absence (open bars) and presence of 15 μ g of vasoactive intestinal polypeptide partial sequence 10–28 [VIP-(10–28)] (filled bars; $n = 6$).

cell membranes (24) as well as in human neuroblastoma cell membranes (23). Furthermore, it has been well documented that the adrenal gland contains a higher concentration of the PAC₁ receptor compared with that of VPAC receptor (28). It should also be noted that the present data clearly show that VIP-(10–28), a VPAC receptor antagonist (27), did not affect the catecholamine response to PACAP-27 at the dose equivalent to that of PACAP-(6–27) in their molar basis. Consequently, it is most likely that the observed inhibition by PACAP-(6–27) of the catecholamine response to PACAP-27 results from the specific antagonism at the level of PAC₁ receptor in the adrenal medulla.

It may be of further interest that the catecholamine response to PACAP-27 was almost completely blocked by the higher dose of PACAP-(6–27). We have previously observed that the similar catecholamine response to PACAP-27 was inhibited by nifedipine, an L-type Ca²⁺-channel blocker, to an extent merely of ~50% of the control response (8). However, nifedipine with the same dose that only partially inhibited the catecholamine response to PACAP-27 completely blocked the catecholamine release induced by BAY K-8644, a direct acting L-type Ca²⁺-channel activator,

under the similar experimental condition (8). Thus an increase in Ca²⁺ influx via the dihydropyridine-sensitive L-type Ca²⁺ channel could be functionally implicated in PACAP-27-induced catecholamine secretion both in vitro (15) and in vivo (8). Nevertheless, the present finding that the blockade of PAC₁ receptor resulted in the almost complete inhibition of the catecholamine response to PACAP-27 strongly suggests the principal role for PAC₁ receptor in mediating the secretion of catecholamines induced by PACAP-27. The major contribution of PAC₁ receptor to PACAP-induced catecholamine secretion has been further demonstrated in recent studies in the isolated, perfused porcine adrenal gland (26) as well as in PC12 cells from the rat adrenal medulla (25).

Many previous studies have indicated that VIP triggers catecholamine secretion in a variety of experimental models including cultured chromaffin cells (3) and isolated, perfused rat adrenal gland (18), as well as in the canine adrenal gland in vivo (6, 31). VIP has been localized in both splanchnic nerve terminals and chromaffin cell (16). Furthermore, VIP could be released into the venous effluent in response to direct splanchnic nerve stimulation in the canine adrenal gland in vivo (7). Although these observations are compatible with the view that VIP plays a role of neurotransmitter

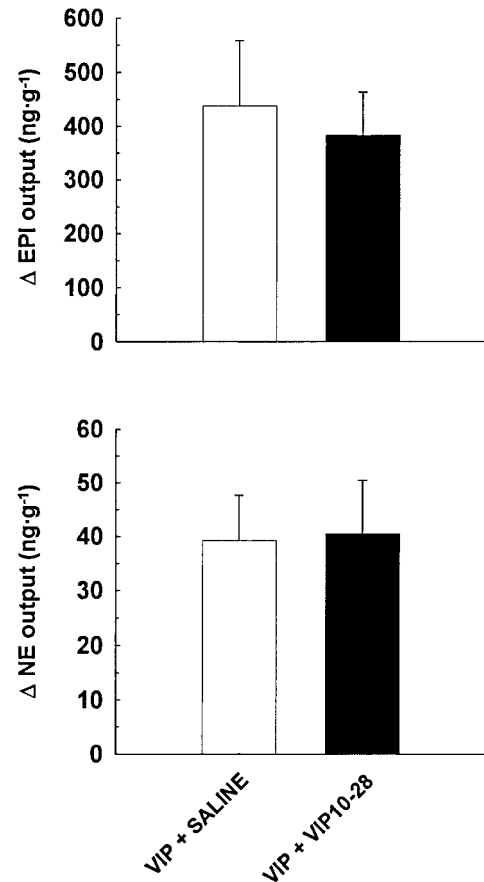


Fig. 6. Δ EPI output and Δ NE output released during first 5 min after onset of each infusion of VIP (5 μ g) in the absence (open bars) and presence of 15 μ g of VIP-(10–28) (filled bars; $n = 6$).

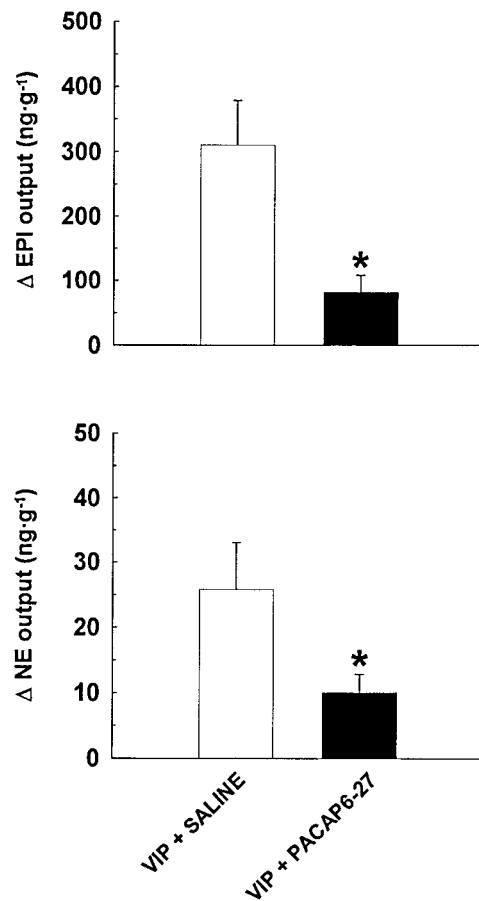


Fig. 7. Δ EPI output and Δ NE output released during the first 5 min after the onset of each infusion of VIP (5 μg) in absence (open bars) and presence of 15 μg of PACAP-(6–27) (filled bars). * $P < 0.05$ vs. corresponding vehicle control as indicated by open bars ($n = 5$).

in the adrenal medulla (29), a role for the VPAC receptor in the adrenal catecholamine response to VIP still remains obscure.

VIP-(10–28) has been widely used as a VIP-receptor antagonist in a variety of tissues and organs including HT29 colonic adenocarcinomic cell line (27), the sinus node of the canine heart (12), and the guinea pig and rat tenia coli and gastric fundus strips (10). Nevertheless, VIP-(10–28) did not affect the catecholamine response to VIP to any significant extent in the present study. Furthermore, we have previously observed that another VIP antagonist, [Lys¹,Pro^{2,5},Arg^{3,4},Tyr⁶]VIP, also failed to block the release of catecholamines induced by VIP in the similar experimental setup in vivo (6). These observations suggest that the failure of the VIP antagonists in blocking the catecholamine secretion induced by VIP may result, most probably, from the absence of specific receptor for VIP in the canine adrenal medulla. In support of this view, it has previously been reported that the specific receptors for VIP (VPAC₁ and VPAC₂) were undetectable in the adrenal medulla (14). Yet, it should be borne in mind that the inhibitory effect of all VIP antagonists cannot consistently be observed in different tissues and models (5). Another possible explanation may be the dose of VIP-

(10–28), which could be insufficient to exert its blocking action on the VPAC receptor. This is, however, less likely to occur, as the catecholamine response to VIP could be significantly blocked by a molar dose of PACAP-(6–27) equivalent to that of VIP-(10–28). In this context, it may be of particular importance to note that VIP usually requires a dose in the range of micro-moles to exert its secretagogue effect on the adrenal medulla, while PACAP only needs a dose in the range of nanomoles to achieve a comparable secretory effect. Thus the ratio of the effective dose of PACAP vs. that of VIP would be roughly 1:1,000. This difference in the effective doses is most likely to result from the PAC₁-receptor affinity, which is $\sim 1,000$ times higher for PACAP compared with that for VIP (11). In this regard, a physiological role for VIP may be less likely than that for PACAP to play in the adrenal medulla. It is of interest that, in the present study, VIP-(10–28) failed to inhibit the catecholamine response to PACAP-27, a finding that further suggests the absence of VIP-specific receptor in the canine adrenal medulla, because PACAP-27 also possesses a high affinity to both VPAC₁ and VPAC₂ receptors (11). Taken together, these observations are compatible with the hypothesis that VIP exerts its secretagogue effect via the activation of the PAC₁ receptor in the canine adrenal medulla.

In conclusion, the present study was carried out to investigate the functional implication of PAC₁ receptor in vivo and the relative importance of PAC₁ and VPAC receptors involved in the adrenomedullary response to PACAP and VIP. The data indicate that the catecholamine response to either PACAP-27 or VIP was significantly blocked by PACAP-(6–27), a specific PAC₁-receptor antagonist. However, VIP-(10–28), a non-selective VPAC-receptor antagonist, did not affect the catecholamine secretion induced by either PACAP-27 or VIP. It is suggested that the VPAC receptor may not functionally be involved in the local regulation of adrenal catecholamine secretion induced by PACAP and VIP. We conclude that the PAC₁ receptor plays a major role in mediating the secretion of catecholamines induced by either PACAP or VIP in the canine adrenal medulla in vivo.

Perspectives

The present study demonstrates that the PAC₁ receptor plays a primary functional role in mediating the catecholamine secretion induced by exogenous PACAP-27 and VIP in the canine adrenal medulla in vivo. However, the question as to whether endogenous PACAP and VIP in the adrenal medulla could be functionally implicated in pathophysiological situations still remains unanswered. Can these neuropeptides be released in the adrenal gland resulting in modulations of the medullary and cortical functions during the sympathoadrenal activation in response to physiological and/or pathophysiological alterations of the body? To answer, at least in part, to this question, endogenous VIP and PACAP have been shown to be released

in response to direct splanchnic nerve stimulation in the canine adrenal gland in vivo (7) and in vitro (S. Lamouche and N. Yamaguchi), respectively. Yet, the potential release of these neuropeptides in response to more pathophysiological perturbations such as severe hypotension, hemorrhage, or hypoglycemia remains to be confirmed. On the other hand, we have recently shown that the adrenomedullary reactivity was significantly enhanced by exogenous PACAP during either direct splanchnic nerve stimulation (17) or insulin-induced hypoglycemia (32). Taken together, these observations are compatible with the hypothesis that both endogenous PACAP and VIP could be released and stimulate the PAC₁ receptor and thereby support or facilitate the cholinergic functions in the adrenal medulla during pathophysiological activation of the sympathoadrenal system. However, this hypothesis has to be confirmed before fully accepting the physiological relevance of endogenous PACAP, VIP, and PAC₁ receptor in regulating catecholamine secretion from the adrenal medulla.

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