Proadrenomedullin NH_2-terminal 20 Peptide, a New Product of the Adrenomedullin Gene, Inhibits Norepinephrine Overflow from Nerve Endings

Tatsuo Shimosawa, Yasushi Ito, Katsuyuki Ando, Kazuo Kitamura,* Kenji Kangawa,‡ and Toshiro Fujita
*Fourth Department of Internal Medicine, University of Tokyo School of Medicine, Tokyo 112, Japan; *First Department of Internal Medicine, Miyazaki Medical College, Miyazaki 889-16, Japan; and ‡National Cardiovascular Center Research Institute, Osaka 565, Japan*

Abstract

Proadrenomedullin NH_2-terminal 20 peptide (PAMP) and adrenomedullin, which are derived from proadrenomedullin, exhibit remarkable hypotensive action. We investigated the effect of PAMP and adrenomedullin on peripheral sympathetic neural transmission. Using perfused rat mesenteric arteries, PAMP (0, 1, 5, and 10 pmol/ml) decreased norepinephrine overflow by periarterial electrical nerve stimulation in a dose-dependent fashion (0.244±0.043, 0.231±0.048, 0.195±0.061, and 0.168±0.051 ng/gram tissue weight: NS, $P < 0.05$, and $P < 0.02$, respectively). In contrast to PAMP, adrenomedullin (1, 5, and 10 pmol/ml) did not change it. In contrast, vasoconstrictive response of mesenteric arteries to exogenous norepinephrine was significantly attenuated by 10 pmol/ml of adrenomedullin but not by the same dose of PAMP. Calcitonin gene-related peptide (8-37) [CGRP(8–37)], a CGRP receptor antagonist, inhibited the vasodilatory effect of adrenomedullin but could not suppress the sympathoinhibitory effect of PAMP. Neither a nicotinic antagonist, hexamethonium, nor a presynaptic α_2 antagonist, yohimbine, blocked the sympathoinhibitory effect of PAMP. Thus, it suggests that PAMP and adrenomedullin, which are derived from the same gene, exhibit different hypotensive mechanisms: PAMP inhibits neural transmission at peripheral sympathetic nerve ending, although adrenomedullin directly dilates vascular smooth muscle, possibly through CGRP-like receptor. (*J. Clin. Invest.* 1995. 96:1672–1676.) Key words: norepinephrine • norepinephrine overflow • vasodilation • hypotensive action • rat

Address correspondence to Toshiro Fujita, M.D., The Fourth Department of Internal Medicine, University of Tokyo School of Medicine, 3-28-6 Mejirodai Bunkyo-ku Tokyo 112, Japan. Phone: 81-3-3943-1151; FAX: 81-3-3942-5690.

Received for publication 8 February 1995 and accepted in revised form 19 June 1995.

1. *Abbreviations used in this paper:* AM, adrenomedullin; CGRP, calcitonin gene-related peptide; PAMP, proadrenomedullin NH_2-terminal 20 peptide.

J. Clin. Invest.

0021-9738/95/09/1672/05 $2.00
Volume 96, September 1995, 1672–1676

Introduction

Adrenomedullin (AM),[1] originally isolated from pheochromocytoma tissue, has been shown to exhibit strong hypotensive action (1). The DNA sequence encoding AM precursor, preproadrenomedullin, has been identified in human as well as rat tissue (2, 3). Preproadrenomedullin consists of 185 amino acids, and cleavage at the signal peptide between Thr^{21} and Ala^{22} yields a truncated propeptide with 164 peptides which contains AM. Three typically paired basic amino acids, which represent sites for proteolytic processing signals, are found in the proadrenomedullin (2, 3). The first paired basic amino acid, Lys^{43}-Arg^{44}, is a representative site for proteolytic cleavage, giving the product named as proadrenomedullin NH_2-terminal 20 peptide (PAMP) (2, 3). Although it was reported recently that tissue concentration of PAMP is exceedingly high in adrenal medulla, PAMP is also detectable in plasma and other tissues such as right atrium, kidney, and brain (4). This distribution of PAMP indicates its physiological role in circulation control.

AM has been reported to decrease blood pressure remarkably, associated with a fall in total peripheral resistance in anesthetized rat (5). Moreover, the vasodilator action of AM has been shown in the ex vivo experiment with perfused mesenteric arteries (6). Thus AM decreases blood pressure mainly by vasodilation. Consistently, AM increased heart rate and cardiac output through vasodilation-induced reflex compensatory mechanisms. As in AM, PAMP decreased blood pressure in anesthetized rat (7). Interestingly, the reduction of blood pressure with PAMP was not associated with increment of heart rate and cardiac output (8). It suggests that it might be due to the absence of reflex tachycardia which is related to centrally mediated baroreflex; otherwise, PAMP suppresses the peripheral sympathetic tone, which, in turn, causes vasodilation. AM shares slight homology with calcitonin gene-related peptide (CGRP) and its vasodilatory effect is inhibited by CGRP antagonist CGRP(8-37) in isolated rat mesenteric artery (6). On the other hand, PAMP has an apparently different structure from AM and CGRP (2), suggesting that AM and PAMP are two distinct products of the AM gene with vasodilating action. It led us to the speculation that PAMP and AM have two different vasodilatory mechanisms. In contrast to CGRP, vasodilation with PAMP may occur not due to its direct effect on vascular smooth muscle but also indirect effects such as sympathetic inhibition. However, the effect of PAMP on peripheral sympathetic tone has not been examined.

Thus, we investigated the effect of PAMP and AM on pe-

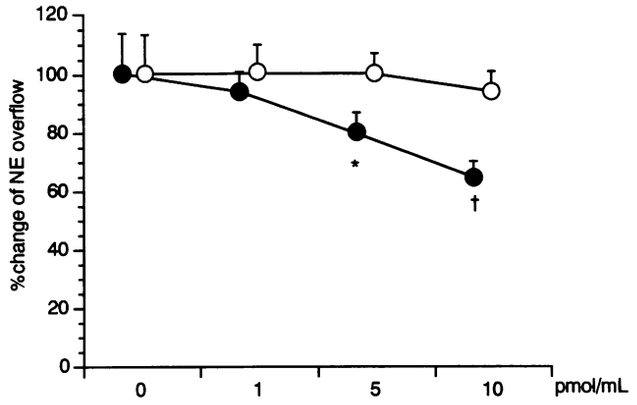

Figure 1. Percent changes of NE overflow with periarterial electrical stimulation. Periarterial electrical stimulation (8 Hz, 1 min) was given on mesenteric artery perfusion preparation of rats. Perfusate was collected for 2 min. Total NE in perfusate was measured. PAMP significantly decreased NE overflow (*closed circles*) in a dose-dependent fashion but AM did not affect it (*open circles*). * $P < 0.05$, † $P < 0.02$ vs. control buffer (0 pmol/ml PAMP or AM).

ripheral sympathetic nerve activity and vascular tone in this study. As a result, we found that PAMP suppressed peripheral sympathetic tone whereas AM antagonized vasoconstrictor effect of norepinephrine (NE).

Methods

Male Sprague-Dawley rats at 10 wk of age were used. Rat mesenteric arteries were prepared by a modification of Castellucci's method (9). The entire intestines were discarded and the mesenteric arteries were quickly connected to the perfusion apparatus. The preparations were perfused with a Krebs-Henseleit solution by use of a peristaltic pump (Minipulse 2; Gilson Medical Electronics SA, Villiers-le-Bel, France) at a rate of 2 ml/min. Constituents of the solution were as follows (mmol/liter): NaCl, 114.5; KCl, 4.6; KH_2PO_4, 1.4; $MgSO_4$, 2.4; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 5.6. The solution was continuously oxygenated with a gas mixture of 95% O_2–5% CO_2 at 37°C. A 30-min equilibration period was allowed before starting each experiment.

Norepinephrine overflow by electrical stimulation. A platinum electrode placed around the periarterial plexus of the mesenteric artery was used to stimulate the postganglionic sympathetic nerve fibers. A standard electrical stimulus of 8 Hz for 1-min duration was given every 15 min. The perfusate through the mesenteric vascular preparation was collected into tubes containing 10 mg EDTA as final concentration of 2.5 mg/ml for measurement of NE by high performance liquid chromatography (10). NE was exactly measurable from 1–10,000 pg/ml and NE concentrations in the effluent of stimulated arteries are ~ 100–500 pg/ml. Samples were collected every 2-min period before and after nerve stimulation. NE overflow was defined as NE content of perfusates per wet tissue weight.

Drug administration. Rat PAMP, rat AM in doses of 1, 5, and 10 pmol/ml, or 100 pmol/ml of CGRP(8-37), a CGRP receptor antagonist (Peptide Institute, Osaka, Japan) (6), was applied for 5 min before electrical stimulation. Yohimbine, a presynaptic α_2 receptor antagonist (11), in a dose of 100 pmol/ml, or 100 nmol/ml of hexamethonium, a nicotinic antagonist (12), was applied for 10 min before electrical stimulation.

As control, two electrical stimulations every 15 min were given. In experiments for dose dependency of PAMP or AM, one electrical stimulation was given for each dose every 15 min. Two electrical stimulations were applied with CGRP(8-37) and then once with 5 and 10 pmol/ml of PAMP which were coinfused with CGRP(8-37). Two electrical stimulations were given with yohimbine alone and two with both yohimbine and 10 pmol/ml of PAMP. Hexamethonium was applied in buffer with 1.2 mM of potassium, in which chloride was replaced with NaCl. The way to apply hexamethonium and PAMP was the same as in experiments with yohimbine.

Table I. NE Overflow with Periarterial Electrical Stimulation

	Control	1 pmol/ml	5 pmol/ml	10 pmol/ml
PAMP	0.244±0.043*	0.231±0.048	0.195±0.061‡	0.168±0.051§
AM	0.225±0.044*	0.213±0.030	0.215±0.030	0.203±0.028
PAMP + CGRP (8–37) (100 pmol/ml)	0.245±0.040‖	—	0.185±0.031‡	0.162±0.041§

NE overflow was expressed as nanograms/gram tissue weight. Values are shown as means±SEM. * Control experiments were done without PAMP or AM. ‖ Control experiments were done with CGRP alone. ‡ $P < 0.05$ and § $P < 0.01$ by repeated measures of ANOVA.

Vasoconstrictor response to exogenous NE. NE (Sigma Chemical Co., St. Louis, MO) in a dose of 1, 3, 10, and 100 μmol in 100 μl Krebs-Henseleit buffer was injected as a bolus using a microinjector. The change of perfusion pressure was recorded by a pressure transducer (model TP-200T, Nihon Kohden, Tokyo, Japan) connected to a thermal array recorder (model WS-641G; Nihon Kohden, Tokyo, Japan). NE in each dose was injected before and 5 min after perfusion with PAMP (10 pmol/ml) or AM (10 pmol/ml). Also effects of PAMP or AM were examined under 5-min presence of CGRP(8-37) in a concentration of 100 pmol/liter.

Statistical analysis. Data were presented as means±SEM. NE overflows were averaged and statistically analyzed by repeated measurements of ANOVA for analysis of dose dependency and for antagonizing effect of CGRP(8-37). Unpaired and paired *t* tests were applied for effect of yohimbine and hexamethonium. Change in perfusion pressure at each dose of NE before and after the agents was calculated and statistically analyzed by repeated measurements of ANOVA. *P* value < 0.05 was considered to be significant.

Results

Effect of PAMP or AM on NE overflow. NE overflow by six consecutive electrical stimulations was not different (0.245±0.042, 0.260±0.049, 0.241±0.051, 0.253±0.034, 0.228±0.061, and 0.242±0.053 ng/gram tissue weight, respectively; $n = 6$). Average tissue weight was 3.56±0.88 grams. Both the NE overflow and pressor responses induced by electrical stimulation were completely abolished by the addition of guanethidine (10^{-5} M) and tetrodotoxin (10^{-7} M) to the perfusate (NE; not detectable), suggesting that it adequately reflects the activity of the sympathetic nerves. PAMP at the dose of 0, 1, 5, and 10 pmol/ml decreased NE overflow dose dependently (Fig. 1) (100.0±13.4, 94.9±6.07, 81.7±5.3, and 65.9±4.6%: NS, $P < 0.05$, $P < 0.02$ compared with control, respectively; $n = 8$) but AM in a dose of 0, 1, 5, and 10 pmol/ml did not affect it (100.0±12.9, 100.3±9.7, 100.0±6.8, and 94.7±5.9%: NS; $n = 7$). The absolute value of NE overflow and its change by PAMP or AM are shown in Table I.

Effect of PAMP or AM on pressor response to NE. Fig. 2 represents typical recording of the effects of PAMP or AM on pressor response by exogenous NE. Neither PAMP nor AM changed basal perfusion pressure. Pressor response to 1, 3, and

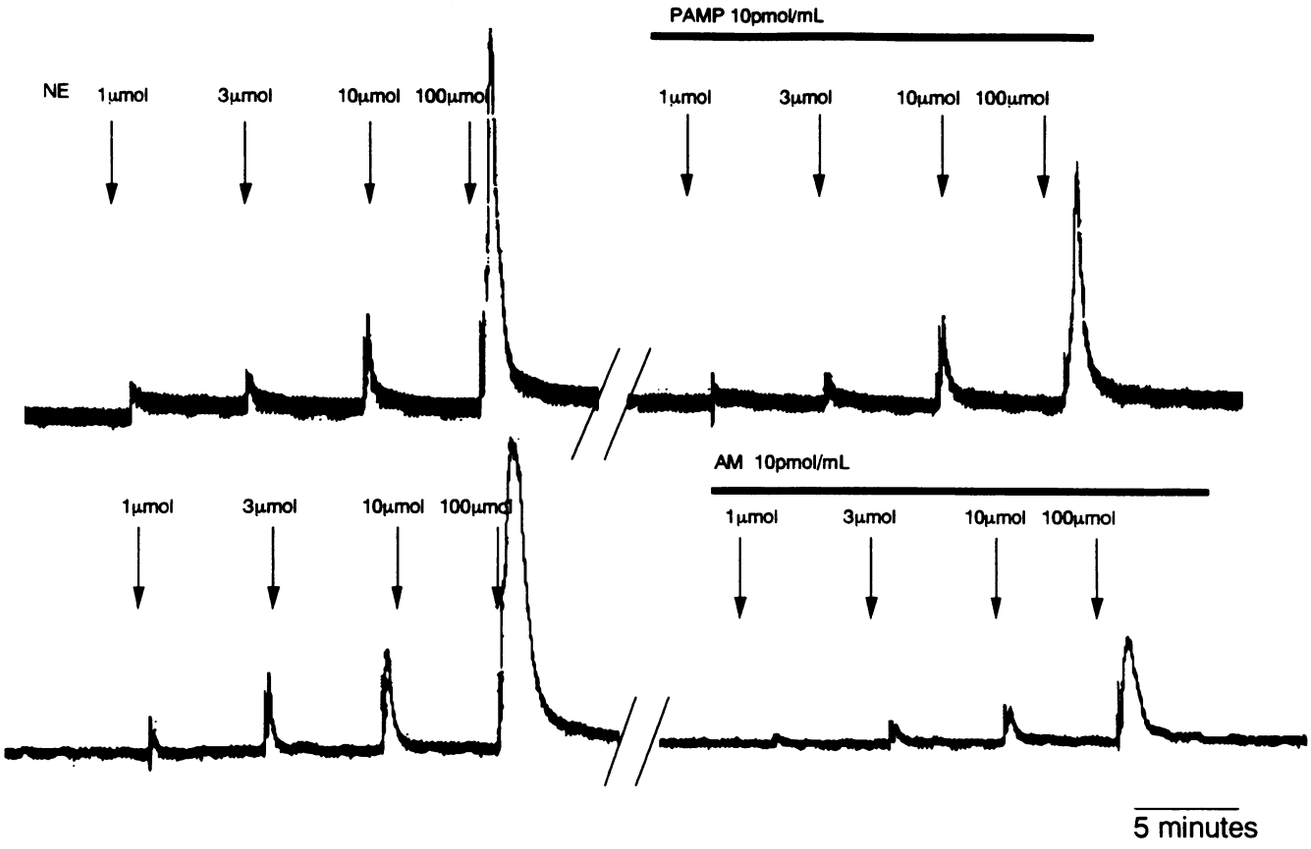

Figure 2. Typical recording of pressor response by exogenous NE. One of six experiments is shown. NE (1, 3, 10, and 100 μmol)-evoked pressor responses were shown in a dose-dependent fashion. Pressor response with 1, 3, and 10 μmol of NE was not significantly suppressed with the treatment of 10 pmol/ml of PAMP, whereas they were significantly suppressed with the same dose of AM. The pressor response with 100 μmol of NE was slightly but significantly suppressed by PAMP and AM.

10 μmol of NE was not suppressed by 10 pmol/ml of PAMP, although it was significantly suppressed by 10 pmol/ml of AM (Table II). Pressor response to 100 μmol of NE was significantly suppressed by either 10 pmol/ml of PAMP or 10 pmol/ml of AM.

Effect of CGRP(8-37). It has been reported that the vasodilating effect of AM is antagonized by CGRP antagonist CGRP(8-37) (6). CGRP(8-37) alone affected neither NE overflow (0.233±0.032 vs. 0.245±0.040 ng/gram tissue weight: NS; $n = 6$) nor pressor response to exogenous NE in any doses (Table II). As shown in Table II, CGRP(8-37) completely inhibited the effect of AM ($n = 7$) which is consistent with a previous report (6). However, CGRP(8-37) neither reversed the vasodilating effect of PAMP on 100 μmol of exogenous NE ($n = 7$) (Table II) nor blocked sympathoinhibitory effect of PAMP in doses of 5 and 10 pmol/ml (100.0±9.4, 80.2±6.3, 62.5±5.6%: $P < 0.05$, $P < 0.02$ compared with control, respectively; $n = 8$) (Fig. 3). The absolute values of the effect of CGRP(8-37) plus PAMP on NE overflow are shown in Table I. NE overflow did not differ between PAMP alone and PAMP with CGRP(8-37) in both doses.

Table II. Percent Change of Pressor Response by Exogenous NE

NE (μmol)	1	3	10	100
AM	67.9±6.9%*	69.1±7.7%*	68.2±9.4%*	42.6±9.6%*
PAMP	101.7±8.6%	110.4±4.5%	96.4±9.3%	76.4±4.5%*
CGRP (8–37)	101.0±8.4%	108.4±4.7%	97.4±6.3*	96.4±4.2%
CGRP (8–37) + AM‡	103.7±8.2%	100.4±7.5%	95.4±7.8%	97.4±6.5%
CGRP (8–37) + PAMP‡	102.7±7.3%	105.4±5.3%	98.4±8.5%	74.4±6.5%*

Pressor responses to exogenous NE were measured with each preparation and percent changes of pressor response by agents were calculated. ‡ AM or PAMP after CGRP. Values are shown as means±SEM. * $P < 0.01$ by repeated measurements of ANOVA. AM, 10 pmol/ml; PAMP, 10 pmol/ml; and CGRP (8–37), 100 pmol/ml.

Effect of yohimbine. Yohimbine is an antagonist of presynaptic α_2 receptor (11). Pretreatment with yohimbine stimulated NE overflow (Table III). Inhibitory effect of PAMP on NE overflow was not blocked by coinfusion with yohimbine ($n = 8$) (Table III). Percent change of NE overflow by PAMP was not different between with or without (Table II) yohimbine.

Effect of hexamethonium. Hexamethonium is an antagonist of nicotinic receptor (12), which has been demonstrated to exist at postganglionic nerve endings (13–15). Hexamethonium reduced NE overflow and PAMP further reduced NE overflow ($n = 7$) (Table IV). Moreover, the inhibitory effect of PAMP in low potassium buffer was the same level between with and without hexamethonium.

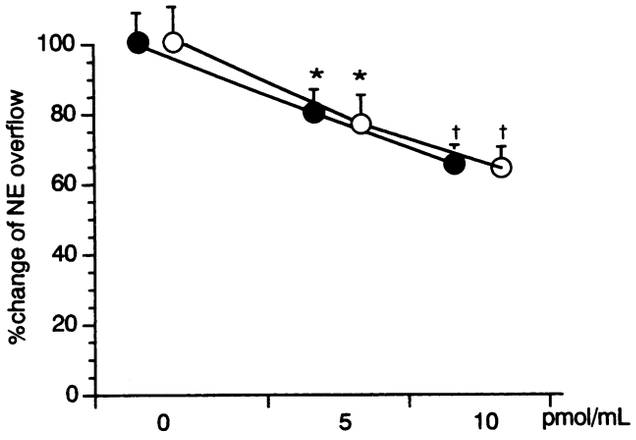

Figure 3. Effect of CGRP(8-37), an antagonist of CGRP, on sympathoinhibitory effect of PAMP. Stimulation and collection of NE was done as indicated in Fig. 1. Percent changes of NE overflow were calculated: the effect of PAMP after CGRP(8-37) (*open circles*) was compared with that without CGRP(8-37) (*closed circles*). There are no significant differences in percent changes of NE overflow between with and without CGRP, suggesting that CGRP(8-37) could not block sympathoinhibitory effect of PAMP in doses of 5 and 10 pmol/ml. * $P < 0.05$, † $P < 0.02$ compared with control by repeated measurements of ANOVA.

Discussion

AM is a novel hypotensive peptide isolated from pheochromocytoma cell. This peptide was discovered by monitoring elevation of cellular cAMP (1). A cDNA clone of AM precursor was isolated from the pheochromocytoma cDNA library and its nucleotide sequence was determined. The preproadrenomedullin consists of 185 amino acids including AM sequence. In addition to AM, proadrenomedullin contains a unique 20-residue sequence followed by Gly-Lys-Arg in the NH_2-terminal region. This peptide was named PAMP. Its carboxy terminus is amidated (2–4).

In the previous studies, both PAMP in a range of 10–50 nmol/kg doses and AM in a range of 0.1–1.0 nmol/kg doses have shown hypotensive effect (5, 8, 7). Although AM decreases blood pressure mainly due to direct vasodilating effect, hypotensive mechanism of PAMP has not been elucidated. In this study, with isolated rat mesenteric arteries, we show for the first time that the hypotensive mechanism of PAMP is mainly due to decreased sympathetic nerve transmission.

In this study, we investigated sympathetic nerve activity at mesenteric arteries, which are known to receive approximately one-fifth of the cardiac output and, thus, could be important as blood pressure–regulating sites (16). Periarterial electrical stimulation is known to evoke NE release and subsequently induces vasoconstrictor response. The present observation that guanethidine and tetrodotoxin completely abolished pressor response indicates that electrical stimulation induces intraarterial sympathetic nerve activation. Thus, newly defined peptide PAMP exhibited sympathoinhibitory effect.

Since we did not directly measure NE release in this study, there are two possibilities in mechanism for PAMP to reduce NE overflow which indicate sympathetic neurotransmission: decreases in NE release itself and increases in NE reuptake to nerve terminal or other tissue. Our preliminary data showed that pretreatment with cocaine, a specific neuronal NE uptake blocker (17), or deoxycorticosterone, a specific nonneuronal NE uptake blocker (18), did not affect the effect of PAMP on NE overflow (72.3±4.3 and 73.3±3.7%, respectively). These data suggest that PAMP suppresses sympathetic neurotransmission through the direct inhibition of NE release rather than the increased NE reuptake.

Table III. Effect of Yohimbine on NE Overflow and on Inhibitory Effect of PAMP

	Control	Yohimbine	Yohimbine + PAMP
NE (ng/gram tissue weight)	0.191±0.016	0.235±0.042*	0.156±0.019*‡
Percent change of NE overflow	—	119.5±10.1 (vs. control)	74.3±8.2 (vs. yohimbine)

* $P < 0.01$ vs. control by paired t test, ‡ $P < 0.01$ vs. yohimbine by paired t test. PAMP, 10 pmol/ml.

On the other hand, vasodilating action of AM was inhibited by CGRP(8-37) as reported previously (6) and sympathetic inhibitory action of PAMP was not affected by CGRP(8-37). Moreover, the attenuation of pressor response by the highest dose of NE with PAMP could not be reversed by CGRP(8-37). It was reported that AM shares slight homology with CGRP and that their receptors might be in the same family (6). In contrast to AM, our result suggests that PAMP does not share a receptor similar to CGRP.

There are several studies that show the presence of nicotinic receptor at postganglionic sympathetic nerve endings of heart (13), vas deferens (14), and spleen (15). Since nicotine induces NE release at postganglionic sympathetic nerve ending by inducing sodium and calcium influx (13–15), it led us to the speculation that PAMP can decrease NE release in some way through nicotine receptor. In the present study, therefore, we investigated the role of nicotinic receptor in the action of PAMP at sympathetic nerve ending: PAMP-induced reduction of NE overflow was not changed by pretreatment of hexamethonium possessing the blocking action of nicotinic receptor (11). On the other hand, the previous study using bovine chromaffin cell showed that high dose of PAMP (1 nmol/ml) reduced carbacol-induced sodium influx and thus suggests that PAMP may negatively interact with nicotinic receptor (19). Although this inconsistency between the results of the present study and that of the previous one (19) is still unknown, it might be attributable to the difference of PAMP used, the difference of species (bovine and rat), difference of experimental condition such as ex vivo and in vitro, or difference of stimulation (electrical and pharmacological). Moreover, we showed that yohimbine, a specific α_2 receptor antagonist (12), also did not block the effect of PAMP in reducing NE overflow. Thus, our data suggest that PAMP may reduce NE overflow in some way other than interacting with nicotinic or α_2 receptor. Further studies are required to elucidate specific receptor of PAMP.

Plasma AM and PAMP levels in healthy volunteers were reported previously to be in the order of femtomoles per milliliter and plasma AM level has been reported to increase only ~ 1.5 times higher in hypertensives (4, 20). AM is also known

Table IV. Effect of Hexamethonium on NE Overflow and on Effect of PAMP

	Control 1	PAMP	Control 2	HEX	HEX + PAMP
NE (ng/gram tissue weight)	0.278±0.020	0.193±0.014*	0.267±0.031	0.237±0.073*	0.189±0.041*‡
Percent change of NE	—	70.3±4.5 (vs. control 1)	—	90.1±6.3 (vs. control 2)	73.3±9.1 (vs. HEX)

* $P < 0.01$ vs. control by paired t test, ‡ $P < 0.01$ vs. hexamethonium by paired t test. PAMP alone and HEX + PAMP were statistically not different by unpaired t test. PAMP, 10 pmol/ml; HEX; hexamethonium (100 nmol/ml).

to be present in several organs in the order of 0.1–2 fmol/mg in heart atrium, ventricle, kidney, and lung besides adrenal medulla where AM is present ~ 50 fmol/mg (1). Thus, AM may act as a local hormone to protect from high blood pressure (20, 21). PAMP is also reported to exist not only in adrenal medulla (~ 13 fmol/mg) but also in atrium, kidney, and brain in the range of 0.01–5 fmol/mg, which are important organs to regulate circulatory system (4). Indeed, in the present study, the dose of PAMP was higher than plasma PAMP level (4), leading to the possibility that it might be effective only in a pharmacological dose. However, PAMP is reported to be cosecreted with catecholamine (19). Moreover, by RNA blotting, mRNA of AM, which can also detect mRNA of PAMP, was reported to exist in both vascular smooth muscle and endothelial cells (22, 23). These data indicate that PAMP may be a local hormone and its concentration at sympathetic nerve terminal might be much higher than plasma level.

In conclusion, the hypotensive effect of PAMP is mainly due to the inhibition of sympathetic neural transmission at nerve ending rather than its direct vasodilating action on vascular smooth muscle.

References

1. Kitamura, K., K. Kangawa, M. Kawamoto, Y. Ichiki, S. Nakamura, H. Matsuo, and T. Eto. 1993. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem. Biophys. Res. Commun.* 192:553–560.

2. Kitamura, K., J. Sakata, K. Kangawa, M. Kojima, H. Matsuo, and T. Eto. 1993. Cloning and characterization of cDNA encoding a precursor for human adrenomedullin. *Biochem. Biophys. Res. Commun.* 194:720–725.

3. Sakata, J., T. Shimokubo, K. Kitamura, S. Nakamura, K. Kangawa, H. Matsuo, and T. Eto. 1993. Molecular cloning and biological activities of rat adrenomedullin, a hypotensive peptide. *Biochem. Biophys. Res. Commun.* 195:921–927.

4. Washimine, H., K. Kitamura, Y. Ichiki, Y. Yamamoto, K. Kangawa, H. Matsuo, and T. Eto. 1994. Immunoreactive proadrenomedullin N-terminal 20 peptide in human tissue, plasma and urine. *Biochem. Biophys. Res. Commun.* 202:1081–1087.

5. Ishiyama, Y., K. Kitamura, Y. Ichiki, S. Nakamura, O. Kida, K. Kangawa, and T. Eto. 1993. Hemodynamic effects of a novel hypotensive peptide, human adrenomedullin, in rats. *Eur. J. Pharmacol.* 241:271–273.

6. Nuki, C., H. Kawasaki, K. Kitamura, M. Takenaga, K. Kangawa, T. Eto, and A. Wada. 1993. Vasodilator effect of adrenomedullin and calcitonin gene-related peptide receptors in rat mesenteric vascular beds. *Biochem. Biophys. Res. Commun.* 196:245–251.

7. Kitamura, K., K. Kangawa, Y. Ishiyama, H. Washimine, Y. Ichiki, M. Kawamoto, N. Minamino, H. Matsuo, and T. Eto. 1994. Identification and hypotensive activity of proadrenomedullin N-terminal 20 peptide (PAMP). *FEBS Lett.* 351:35–37.

8. Lippton, H., Y. Gao, B. Lin, J. Heaton, J. Ferrara, M. DeVito, T. Granger, J. Pigott, J. K. Chang, and A. Hyman. 1994. Prodepin: a new product of the adrenomedullin (ADM) gene has systemic vasodilator activity. *Life Sci.* 54:L409–L412.

9. Shimosawa, T., K. Ando, A. Ono, K. Takahashi, M. Isshiki, M. Kanda, E. Ogata, and T. Fujita. 1992. Insulin inhibits norepinephrine overflow from peripheral sympathetic nerve ending. *Biochem. Biophys. Res. Commun.* 188:330–335.

10. Ito, Y., H. Noda, M. Isaka, K. Ando, Y. Sato, and T. Fujita. 1989. Norepinephrine responsiveness in patients with borderline hypertension under three different sodium balances. *Clin. Exp. Hypertens.* A1:363–370.

11. Tsuda, K., Y. Kusuyama, T. Hano, M. Kuchii, I. Nishio, and Y. Masuyama. 1984. Alteration of presynaptic α_2-mediated inhibition of norepinephrine release in perfused mesenteric arteries of young and adult spontaneously hypertensive rats. *J. Hypertens.* 2(Suppl. 3):95–97.

12. Nedergaard, O., and J. Schrold. 1977. The mechanism of action of nicotine on vascular adrenergic neuroeffector transmission. *Eur. J. Pharmacol.* 42:315–329.

13. Löffelholz, K. 1970. Autoinhibition of nicotinic release of noradrenaline from postganglionic sympathetic nerves. *Naunyn-Schmiedebergs Arch. Pharmakol.* 267:49–63.

14. Jayasundar, S., and M. M. Vohra. 1977. Mechanism of nicotine induced release of noradrenaline from adrenergic nerve endings. *Br. J. Pharmacol.* 61:183–188.

15. Kirpekar, S. M., A. G. Garcia, and J. C. Prat. 1980. Action of nicotine on sympathetic nerve terminals. *J. Pharmacol. Exp. Ther.* 213:133–138.

16. Nichols, A., A. Wilson, and C. Hiley. 1985. Effects of sympathectomy with 6-hydroxydpoamine on cardiac output and its distribution in the rat. *Eur. J. Pharmacol.* 109:263–268.

17. Pitts, D. K., and J. Marwah. 1988. Autonomic actions of cocaine. *Can. J. Physiol. Pharmacol.* 67:1168–1176.

18. Salt, P. 1979. Inhibition of noradrenaline uptake in the isolated rat heart by steroids, clonidine and methoxylated phenylethylamines. *Eur. J. Pharmacol.* 20:329–340.

19. Katoh, F., K. Kitamura, H. Niina, R. Yamamoto, H. Washimine, K. Kangawa, Y. Yamamoto, H. Kobayashi, T. Eto, and A. Wada. 1995. Proadrenomedullin N-terminal 20 peptide (PAMP), an endogenous anticholinergic peptide: its exocytotic secretion and inhibition of catecholamine secretion in adrenal medulla. *J. Neurochem.* 64:459–461.

20. Ishimitsu, T., T. Nishikimi, Y. Saito, K. Kitamura, T. Eto, K. Kangawa, H. Matsuo, T. Omae, and H. Matsuoka. 1994. Plasma levels of adrenomedullin, a newly identified hypotensive peptide, in patients with hypertension and renal failure. *J. Clin. Invest.* 94:2158–2161.

21. Nishikimi, T., K. Kitamura, Y. Saito, K. Shimada, T. Ishimitsu, M. Takamiya, K. Kangawa, H. Matsuo, T. Eto, T. Omae, and H. Matsuoka. 1994. Clinical studies on the sites of production and clearance of circulating adrenomedullin in human subjects. *Hypertension (Dallas).* 24:600–604.

22. Sugo, S., N. Minamino, H. Shoji, K. Kangawa, K. Kitamura, T. Eto, and H. Matsuo. 1994. Production and secretion of adrenomedullin from vascular smooth muscle cells: augmented production by tumor necrosis factor-alpha. *Biochem. Biophys. Res. Commun.* 203:719–726.

23. Sugo, S., N. Minamino, K. Kangawa, K. Miyamoto, K. Kitamura, J. Sakata, T. Eto, and H. Matsuo. 1994. Endothelial cells actively synthesize and secrete adrenomedullin. *Biochem. Biophys. Res. Commun.* 201:1160–1166.