BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **219,** 59–63 (1996)
ARTICLE NO. 0181

Vasopressor Activities of N-Terminal Fragments of Adrenomedullin in Anesthetized Rat

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Received December 20, 1995

Adrenomedullin (AM) is a vasorelaxant peptide that was recently isolated from human pheochromocytoma. In contrast to human (h) AM, which has vasodepressor activity, a synthetic N-terminal fragment of hAM, hAM-(1-25)-NH_2 showed vasopressor activity in the anesthetized rat. The N-terminal peptides hAM-(1-31)-NH_2, hAM-(1-25)-OH, hAM-(1-21)-NH_2, acetyl-hAM-(16-21)-NH_2, and acetyl-hAM-(16-36)-OH all showed vasopressor activities. The potency of hAM-(1-21)-NH_2, acetyl-hAM-(16-21)-NH_2 was greater than that of hAM-(1-25)-NH_2. Pretreatment with phenoxybenzamine, guanethidine, or reserpine attenuated vasopressor activities of these peptides. These data suggested that vasopressor activity of N-terminal fragment of hAM is due to a stimulation of endogenous catecholamine release.

Adrenomedullin (AM) is a potent vasorelaxant peptide that has recently been isolated from an acid extract of human pheochromocytoma (1). The amino acid sequence of human AM, a 52-amino acid peptide (hAM-(1-52)-NH_2), is Tyr[1]-Arg-Gln-Ser-Met-Asn-Asn-Phe-Gln-Gly-Leu-Arg-Ser[13]-Phe-Gly-Cys[16]-Arg-Phe-Gly-Thr-Cys[21]-Thr-Val-Gln-Lys[25]-Leu-Ala-His-Gln-Ile-Tyr[31]-Gln-Phe-Thr-Asp-Lys[36]-Asp-Lys-Asp-Asn-Val-Ala-Pro[43]-Arg-Ser-Lys-Ile-Ser-Pro-Gln-Gly-Tyr[52]-NH_2 (1). Cultured rat vascular smooth muscle cells possess specific AM receptors, which are functionally coupled to adenylate cyclase (2,3). In a structure-activity relationship study, we demonstrated that synthetic hAM-(1-52)-NH_2 and its N-terminal truncated derivatives [hAM-(13-52)-NH_2, hAM-(16-52)-NH_2] inhibited ^{125}I-hAM binding and stimulated cAMP formation with nearly equal potency. While a peptide hAM-(1-51)-NH_2 in which the C-terminal Tyr[52] had been removed showed both a significantly lower receptor binding activity and cAMP response (3). We have obtained similar results with regards to the structure-activity relationship of the hAM in measurements of the vasorelaxant activity in anesthetized rats (4).

As the hAM-(1-52)-NH_2 has three Lys-residues, we digested hAM with a lysyl endopeptidase (EC 3.4.21.50). In a preliminary study, hAM-(1-25)-OH was the only major product. We determined the biological activity of this peptide on blood pressure. Surprisingly, in contrast to hAM-(1-52)-NH_2 which has vasorelaxant activity, the N-terminal fragment of hAM, hAM-(1-25)-OH showed vasopressor activity. To confirm this vasopressor activity in anesthetized rat, we synthesized hAM-(1-25)-OH, hAM-(1-25)-NH_2 and other related peptides. The present study was designed to determine which amino acid residues are essential for this vasopressor activity and to elucidate the mechanism by which this activity might come about.

MATERIALS AND METHODS

Peptides. Adrenomedullin analogues were synthesized as reported previously (5). Briefly, peptides were synthesized by a solid phase procedure using an Applied Biosystems Model 430A peptide synthesizer on a Boc strategy. The crude products with disulfide bond were purified to homogeneity by reverse phase high-performance liquid chromatography (HPLC). The purity of the products was confirmed by analytical HPLC and amino acid analysis. The amino acid compositions and the net contents of the peptide were determined by amino acid analysis. The purity of each preparation was over 98% of the final purified peptide.

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0006-291X/96 $18.00

Pressor activities. Male Sprague-Dawley rats (260-290 g) were anesthetized with pentobarbital (50 mg/kg, intraperitoneal) and a tracheotomy was performed. Arterial blood pressure was determined directly through a femoral arterial cannula as reported previously (6). Peptides were injected as a bolus (0.5 ml/kg) via a femoral venous cannula. In another series of experiments using a conscious rat, the aortic blood pressure was determined directly without anesthesia or restraint 2–7 days following the insertion of a cannula into the abdominal aorta via the left femoral artery as reported previously (7). The synthetic peptides were injected as a bolus (1 ml/kg) through the other aortic cannula via the right femoral artery. The tip of this cannula was positioned about 5 mm peripheral to the cannula for blood pressure determination. Blood pressure was monitored with an electronic system (Century Technology CP-01, Star Medical PA-011 and San-Ei 8K21, Tokyo). In these experiments, the mean blood pressure level was 90-100 mmHg in anesthetized rats, and 100-120 mmHg in conscious rats. Changes in blood pressure were expressed as the percentage of change in blood pressure compared to that before peptide administration.

Statistical analysis. The results are given as the mean ± S.E. with the number of experiments in parentheses. The statistical significance of the results was evaluated by unpaired Student's *t* test. A value of $P < 0.05$ was taken as being statistically significant.

Drugs. The drugs used and their sources were: phenoxybenzamine hydrochloride (Tokyo Kasei Co., Tokyo, Japan); guanethidine sulfate (Sigma Chemical Co., St. Louis, MO, USA); reserpine (Sigma Chemical Co., St. Louis, MO, USA); [Sar^1, Val^5, Ala^8] Angiotensin II, hAM-(22-52)-NH_2, human calcitonin gene-related peptide (hCGRP) (8-37) (Peptide Institute, Osaka, Japan); pentobarbital sodium (Abbott Laboratories, North Chicago, IL, USA).

RESULTS AND DISCUSSION

The peptides hAM-(1-25)-OH and hAM-(1-25)-NH_2 showed dose-dependent vasopressor activity in both anesthetized (Fig. 1; left record) and conscious (Fig. 1; right record) rats. The vasopressor potencies of the two peptides in conscious rats were greater than that determined in anesthetized rats. While, the difference in potency between the two peptides was not significantly deferent in either case. The time course of pressor effect of hAM-(1-25)-NH_2 is shown in Fig. 2 (left record).

As the potency of both peptides was stronger in conscious state than in the anesthetized state, it is possible that sympathetic activity may influence the sensitivity of the rats to these peptides. To investigate the involvement of sympathetic activity, we assessed the involvement of α-adrenergic receptors on the pressor effects of both hAM-(1-25)-NH_2 and hAM-(1-25)-OH both at a dose of 50 nmol/kg, intravenously (i.v.). To maintain a stable blood pressure, anesthetized rats were used. The α-adrenergic receptor antagonist, phenoxybenzamine (10 μmol/kg, i.v.) reduced the control pressor response of both hAM-(1-25)-NH_2, and hAM-(1-25)-OH by about 30% (Fig. 3). This dose of phenoxybenzamine inhibited almost completely the pressor response to norepinephrine (5 nmol/kg, i.v.) (data not shown). One of the original records is shown in Fig. 2. These data indicated that the release of epinephrine/norepinephrine might be involved. To investigate this, we inhibited the

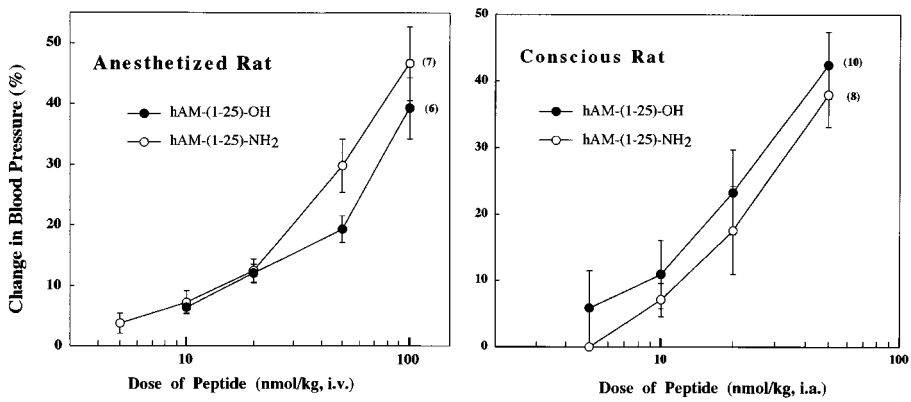

FIG. 1. Dose-vasopressor response curves for hAM-(1-25)-OH (●) and hAM-(1-25)-NH_2 (○) in anesthetized or conscious rats. Values are means ± SEM. Number of experiments is shown in parentheses. Peptides were administered intravenously (i.v.) or intraarterially (i.a.).

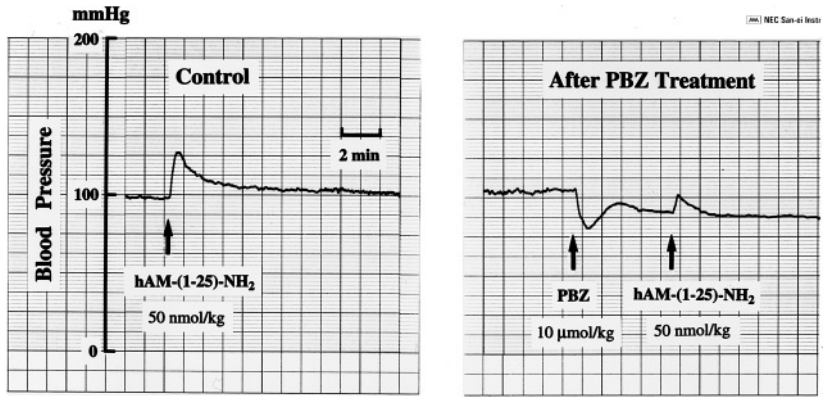

FIG. 2. Inhibitory effect of phenoxybenzamine (PBZ) on the pressor response to hAM-(1-25)-NH_2 in anesthetized rats. A typical control response (left record) and a reduced response after treatment with PBZ (right record) were recorded. Peptide and PBZ were administered intravenously.

effect of endogenous catecholamine release by pretreatment of the rats with guanethidine as reported previously (8). Pretreatment with guanethidine (10 μmol/kg, i.v.) attenuated the pressor response to both hAM-(1-25)-NH_2, and hAM-(1-25)-OH (Fig. 3). We also investigated the effect of adrenergic depletion by reserpine on the pressor effect of these fragments. Rats were pretreated with reserpine (5 mg/kg, intraperitoneal) for two consecutive days as reported previously (9). The pressor responses to both hAM-(1-25)-NH_2, and hAM-(1-25)-OH were reduced by reserpine pretreatment (Fig. 3). These data indicate that the N-terminal fragment of hAM stimulated the release of endogenous catecholamine. The main source of this release might be the sympathetic nerve endings, because the pressor responses were attenuated by reserpine and guanethidine. The involvement of catecholamine release from adrenal medulla will be a subject for further investigation.

We also investigated the influence of another vasopressor peptide, angiotensin II on the pressor response to the N-terminal fragment of hAM. Infusion of the Angiotensin II antagonist, [Sar^1, Val^5, Ala^8] Angiotensin II (50 nmol/kg/min, i.v.) had no significant effect on vasopressor activity of either hAM-(1-25)-NH_2 or hAM-(1-25)-OH (n = 6, data not shown). As hAM-(22-52)-NH_2 and

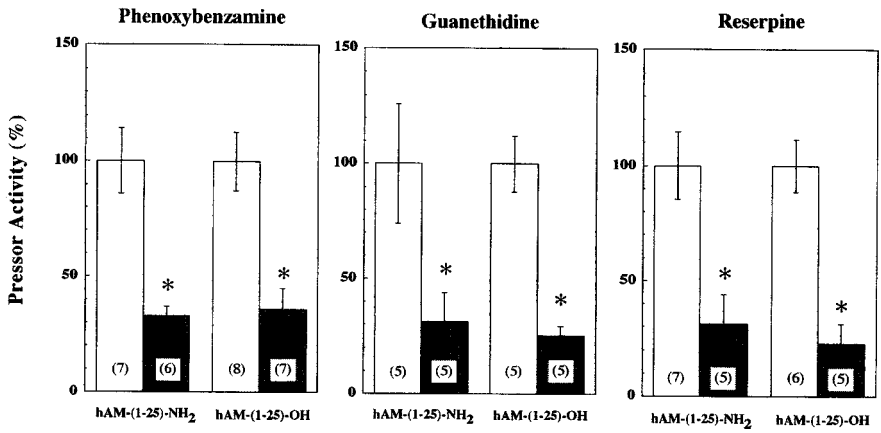

FIG. 3. Inhibitory effect of phenoxybenzamine (10 μmol/kg, intravenously), guanethidine (10 μmol/kg, intravenously), and reserpine (5 mg/kg, intraperitoneal for two days) on the pressor response of hAM-(1-25)-NH_2 and hAM-(1-25)-OH in anesthetized rat. Values are means ± SEM. Number of experiments is shown in parentheses. *$P < 0.05$ compared with the control pressor response.

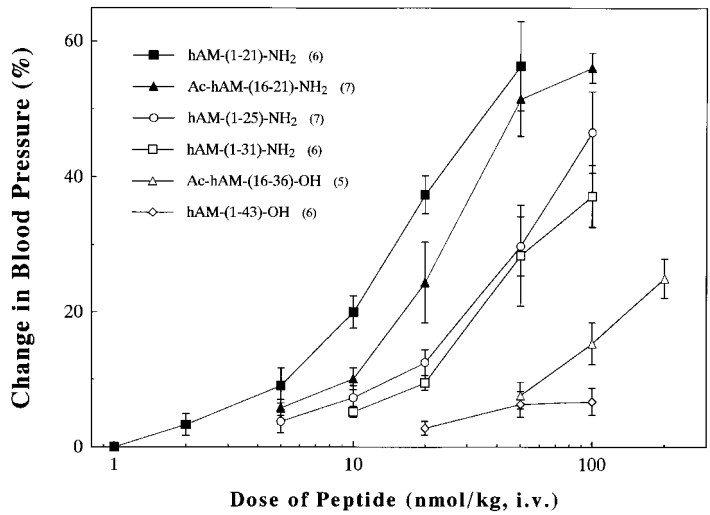

FIG. 4. Dose-vasopressor response curves for hAM-(1-21)-NH_2 (■), Ac-hAM-(16-21)-NH_2 (▲), hAM-(1-25)-NH_2 (○), hAM-(1-31)-NH_2 (□), Ac-hAM-(16-36)-OH (△), and hAM-(1-43)-OH (◇) in anesthetized rat. Details are the same as in Fig. 1.

hCGRP-(8-37) have both been shown to antagonize the effect of hAM (3, 10), we determined the effect of these antagonists on the pressor response to the hAM-N-terminal fragment. Neither hAM-(22-52)-NH_2 (100 nmol/kg/min, i.v.) nor hCGRP-(8-37) (30 nmol/kg/min, i.v.) had an effect on the pressor effect of hAM-(1-25)-NH_2 ($n = 3$, data not shown). These data suggested that the receptor for the N-terminal fragment of hAM might be different from that of hAM-(1-52)-NH_2.

In addition, we synthesized other analogs of hAM-(1-25)-NH_2 and determined vasopressor activity (Fig. 4). The shorter fragment, hAM-(1-21)-NH_2 had a greater potency compared to that of hAM-(1-25)-NH_2. Interestingly, only the ring structure of acetyl(Ac)-hAM-(16-21)-NH_2 had strong vasopressor activity. The elongated C-terminal peptide of this ring moiety, Ac-hAM-(16-36)-NH_2 had a reduced pressor effect. Although hAM-(1-31)-NH_2 was as potent as hAM-(1-25)-NH_2, the elongated C-terminal peptide, hAM-(1-43)-NH_2 had lost its activity. These data highlighted the importance of the six amino acid residues within the ring structure for the pressor activity of N-terminal fragment of hAM. We searched in the data base PRF/SEQDB of the Protein Research Foundation for peptides that contains regions that were at least 83% homologous to these six amino acid residues. The search identified a number of polypeptides, including the epidermal growth factor precursor (11), fibrillin (12), and a number of others. However, no homologous peptides were identified that are known to be related to the cardiovascular system.

In conclusion, the N-terminal fragment of hAM produced a dose-dependent increase in blood pressure in rats. This vasopressor effect might be caused by release of endogenous catecholamine. Furthermore, the ring structure of six amino acid residues was essential for the vasopressor activity of N-terminal fragment of hAM. The mechanisms of action, including the source of endogenous catecholamine should be a subject for further investigation.

ACKNOWLEDGMENTS

We thank Drs. H. Matsuo and K. Kangawa, National Cardiovascular Center Research Institute, for helpful discussion. We also thank Dr. C. Redhead (Max Plank Institute, Germany) for kindly commenting on the manuscript.

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