PRELIMINARY REPORT

An Accelerated Increase of Plasma Adrenomedullin in Acute Asthma

Masakazu Kohno, Takao Hanehira, Kazuto Hirata, Takashi Kawaguchi, Kyoichi Okishio, Hiroaki Kano, Hiroshi Kanazawa, and Junichi Yoshikawa

A novel vasorelaxant peptide, adrenomedullin (AM), has been isolated from the acid extract of human pheochromocytoma. We have recently shown that AM inhibits histamine- and acetylcholine-induced bronchoconstriction in anesthetized guinea pigs in vivo, and this bronchodilatory effect is long-lasting. Here, we measured plasma AM concentrations in nine patients with an acute attack of bronchial asthma. The results were compared with values in 30 age-matched normal control subjects and seven age-matched stable asthmatic patients. The mean AM concentrations of patients with an acute asthma attack (98 ± 22 pg/mL) were clearly higher than those of normal control subjects (18 ± 2 pg/mL) and stable asthmatic patients (21 ± 3 pg/mL). Reverse-phase high-performance liquid chromatography (HPLC) showed that the major component of plasma immunoreactive AM in patients with an asthma attack and in normal subjects equally corresponded to authentic human AM(1-52). Our results suggest that plasma AM is markedly increased in many of the patients during an acute attack of bronchial asthma, but it is not observed in stable asthmatic patients. Although this report is preliminary, the observed increase of circulating AM during an acute asthma attack may represent a compensatory mechanism against the bronchoconstriction, probably through its bronchodilatory action.

A NOVEL VASORELAXANT peptide, adrenomedullin (AM), has been isolated from the acid extract of human pheochromocytoma.[1] This 52–amino acid peptide has one intracellular disulfide bond and shows homology with calcitonin-gene–related peptide (CGRP).[1] It has been demonstrated that intravenous injection of AM causes a potent and long-lasting hypotensive effect in anesthetized rats, and that this peptide binds to specific receptors on platelet membranes to increase intracellular cyclic adenosine 3′, 5′-monophosphate (AMP).[1] Subsequently, AM is shown to be present not only in adrenal medulla but also in plasma.[2] Furthermore, this peptide is found to stimulate cyclic AMP formation[3-5] and inhibit proliferation and migration[6,7] in vascular smooth muscle cells.

Recently, we have shown that AM inhibits histamine- and acetylcholine-induced bronchoconstriction in anesthetized guinea pigs in vivo.[8] In the current study, we measured plasma AM concentrations in patients with an acute attack of bronchial asthma. The results were compared with values in age-matched normal control subjects and stable asthmatic patients. We also characterized immunoreactive AM in plasma obtained from patients during an acute asthma attack and from normal control subjects.

SUBJECTS AND METHODS

Between January and December 1995, we recruited nine patients with an acute bronchial asthma attack and seven stable asthmatic patients for this study in our institution and Tsuji Hospital. The asthmatic patients, all of whom had a history of wheezing and reversible airway obstruction, were clinically diagnosed as previously described.[9] Patients with cardiac and renal failure were excluded from the study, since plasma AM concentrations are found to be high in patients with congestive heart failure or renal failure.[10,11] Thirty normotensive healthy subjects served as controls.

At the point of blood sampling, both stable asthmatic patients and patients with an acute asthma attack did not receive any relieving bronchodilator medications or corticosteroids. However, all patients were receiving sustained-release theophyllin or long-acting oral β_2-agonists.

A blood sample (5 mL) was drawn immediately into ice-chilled tubes containing Trasylol (5×10^5 kallikrein inactivator units/L) and EDTA (1 g/L). Plasma was separated by centrifugation for 10 minutes at 4°C and immediately frozen and stored at −80°C until radioimmunoassay.

Immunoreactive AM was extracted from plasma as described previously.[12] Briefly, 2 mL plasma was diluted with 3 mL 4% acetic acid. After centrifugation, the solution was pumped at the rate of 1 mL/min through a Sep-Pak C18 cartridge (Millipore, Milford, MA). After the cartridge had been washed with distilled water, the adsorbed peptides were eluted with 4 mL 50% acetonitrile containing 0.1% trifluoroacetic acid. After evaporation with a centrifugal evaporator (model RD-31; Yamato Scientific, Tokyo, Japan), the dry residue was dissolved in an assay buffer. The recovery rate was calculated by addition of three different amounts of cold human AM (1-52) (5, 20, and 100 pg/mL [0.8, 3.3, and 16.6 pmol/L]) to plasma pretreated with the Sep-Pak C18 cartridge. The recovery rate was 75% ± 4%.

The plasma immunoreactive AM concentration was measured with an antibody against synthetic human AM(1-52) and ^{125}I-human AM(1-52) (Peninsula Laboratories, Belmont, CA) as described previously.[12] This antibody does not show any cross-reactivity with human AM(13-52), rat AM(1-50), human CGRP, endothelin-1, α-human atrial natriuretic peptide(1-28), brain natriuretic peptide-32, or C-type natriuretic peptide-22. Radioimmunoassay was performed in the assay buffer of 0.01 mol/L sodium phosphate, pH 7.4, containing 0.05 mol/L sodium chloride, 0.1% bovine serum albumin, 0.1% Nonidet P-40, and 0.01% sodium azide as described previously.[12] The effective range of the standard curve was between 2 and 200 pg human AM per assay tube. The

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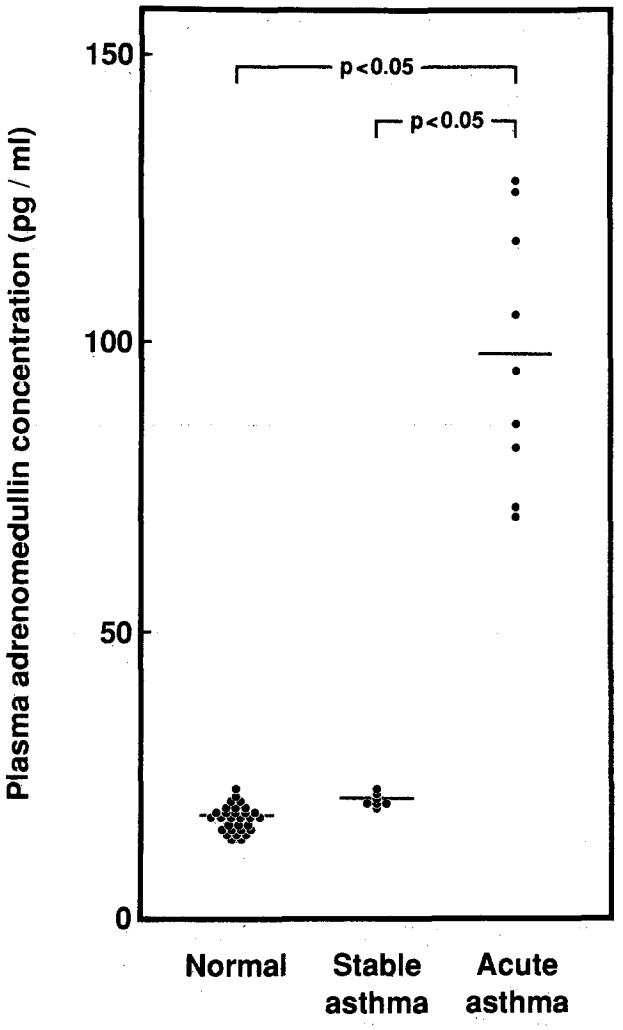

Fig 1. Plasma immunoreactive AM concentrations in normal healthy subjects, patients with stable asthma, and patients with an acute attack of asthma.

50% intercept was 19 pg human AM. The interassay variation was 12%, and intraassay variation 5%.

Reverse-phase high-performance liquid chromatography (HPLC) was performed with an octadecylsilica column (0.46 × 25 cm, TSK-gel, ODS-80; TOSOH, Tokyo, Japan) eluted with a linear gradient of acetonitrile from 10% to 80% (10% to 60%, 35 minutes; 60% to 80%, 10 minutes; and 80%, 5 minutes) in 0.1 mol/L sodium chloride with a flow rate of 1 mL/min as described previously.[12] One-milliliter fractions were collected and assayed by radioimmunoassay. For chromatographic analysis of immunoreactive AM, 30 mL pooled plasma of asthmatic patients and normotensive control subjects was separated and treated by reverse-phase HPLC.

Statistical analysis was made by Scheffe's test for multiple comparisons, preceded by ANOVA.[13] Values are indicated as the mean ± SD.

RESULTS AND DISCUSSION

Figure 1 shows plasma AM concentrations in 30 normal subjects (mean age, 49 ± 6 years), seven stable asthmatic patients (46 ± 14 years), and nine patients with acute asthma attack (47 ± 14 years). The mean concentrations of the three groups were 18 ± 2, 21 ± 3, and 98 ± 22 pg/mL, respectively. The mean concentrations in patients with an acute asthma attack were markedly higher than in normal subjects and stable asthmatic patients.

Figure 2 shows reverse-phase HPLC profiles of immunoreactive AM in extracts of pooled plasma from normal subjects and patients with acute asthma. AM immunoreactivity in the plasma of both groups equally consisted of one major component, which was eluted in the position of synthetic human AM(1-52).

In the current study, we showed that circulating AM concentrations were markedly elevated during the stress of an acute asthma attack and its major component was human AM(1-52). To our knowledge, this is the first report concerning plasma AM concentrations in bronchial asthma. The precise cause of the increase in plasma AM concentration during an acute asthma attack remains to be estab-

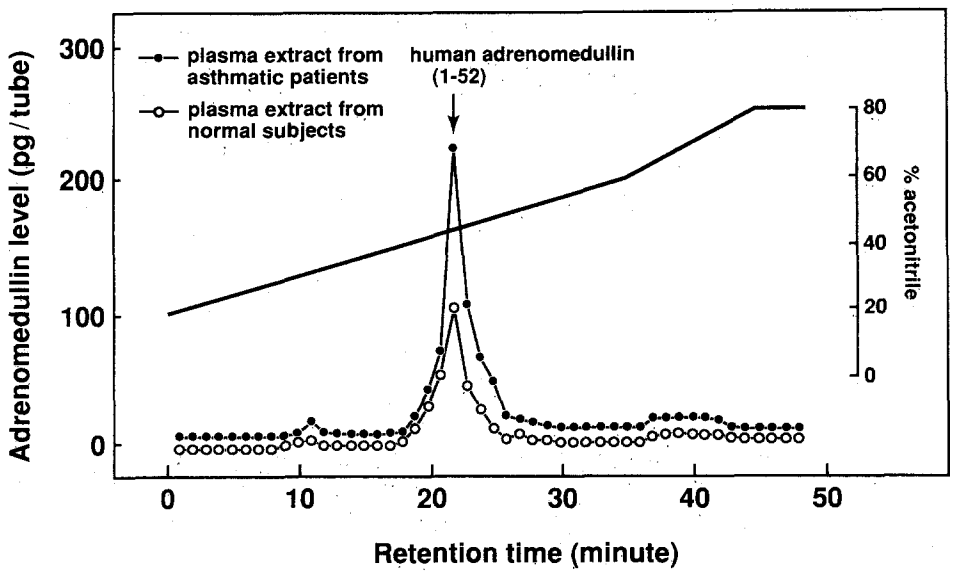

Fig 2. Profiles by reverse phase HPLC of immunoreactive AM in extracts of pooled plasma from patients with an acute asthma attack and normal healthy subjects. Arrow shows elution position of standard human AM(1-52).

lished, but an increase in secretion from the adrenal medulla is one likely possibility. Catecholamines are also secreted by the adrenal medulla and function as circulating hormones. High concentrations of plasma catecholamines have been found in some patients with acute asthma. Therefore, AM, like catecholamines, may be secreted from the adrenal medulla during an acute attack of asthma, since cosecretion of AM and catecholamines by cultured bovine adrenal medullary cells has been recently demonstrated.[14] Furthermore, plasma AM was recently demonstrated to be increased by ergometric exercise in healthy subjects.[15] In this report, AM levels during exercise were correlated with plasma norepinephrine levels. This finding may also support the possibility that sympathetic stimulation participates in AM secretion.

Markedly elevated circulating AM may counteract increased systemic blood pressure during an acute asthma attack through its potent hypotensive activity.[1] In fact, AM is shown to potently stimulate the formation of cyclic AMP[3,4] and to inhibit the production of vasoconstrictive peptide endothelin-1[16] in vascular smooth muscle cells via its specific receptors. Furthermore, we have recently shown that AM inhalation inhibits histamine- and acetylcholine-induced bronchoconstriction in anesthetized guinea pigs in vivo and this bronchodilatory effect is long-lasting.[8,17] Actually, AM inhalation significantly inhibited histamine (10^{-3} mol/L)-induced bronchoconstriction in a concentration-dependent manner between 10^{-9} and 10^{-6} mol/L for longer than 35 minutes.[8] Although we have no direct evidence, these observations raise the hypothesis that some of the elevated circulating AM during asthma attack reaches the airway lumen and induces bronchodilation. In this respect, the elevated concentrations of circulating AM during asthma attack may represent a compensatory mechanism to offset further bronchoconstriction. However, further studies on the exact origin of circulating AM and its releasing mechanism and pathophysiological roles in bronchial asthma are necessary.

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REFERENCES

1. Kangawa K, Kawamoto M, Ichiki Y, et al: Adrenomedullin: A novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun 192:553-560, 1993

2. Ichiki Y, Kitamura K, Kangawa K, et al: Distribution and characterization of immunoreactive adrenomedullin in human tissue and plasma. FEBS Lett 338:6-10, 1994

3. Ishizaka Y, Tanaka M, Kitamura K, et al: Adrenomedullin stimulates cyclic AMP formation in rat vascular smooth muscle cells. Biochem Biophys Res Commun 200:642-646, 1994

4. Eguchi S, Hirata Y, Kano H, et al: Specific receptors for adrenomedullin in cultured rat vascular smooth muscle cells. FEBS Lett 340:226-230, 1994

5. Kohno M, Yokokawa K, Yasunari K, et al: Stimulation by the novel vasorelaxant peptide adrenomedullin of cyclic AMP formation in cultured rat mesangial cells. Metabolism 44:10-12, 1995

6. Kano H, Kohno M, Yasunari K, et al: Adrenomedullin as a novel antiproliferative factor of vascular smooth muscle cells. J Hypertens 14:209-213, 1996

7. Horio T, Kohno M, Kano H, et al: Adrenomedullin as a novel antimigration factor of vascular smooth muscle cells. Circ Res 77:660-664, 1995

8. Kanazawa H, Kurihara N, Hirata K, et al: Adrenomedullin, a newly discovered hypotensive peptide, is a potent bronchodilator. Biochem Biophys Res Commun 205:251-254, 1994

9. Kamoi H, Kurihara N, Fujiwara H, et al: The macroline antibacterial roxithromycin reduces bronchial hyperresponsiveness and superoxide anion production by polymorphonuclear leukocytes in patients with asthma. J Asthma 32:191-197, 1995

10. Kato J, Kobayashi K, Etoh T, et al: Plasma adrenomedullin concentration in patients with heart failure. J Clin Endocrinol Metab 81:180-183, 1996

11. Ishimitsu T, Nishikimi T, Saito Y, et al: Plasma levels of adrenomedullin, a newly identified hypotensive peptide, in patients with hypertension and renal failure. J Clin Invest 94:2158-2161, 1994

12. Kohno M, Hanehira T, Kano H, et al: Plasma adrenomedullin concentrations in essential hypertension. Hypertension 27:102-107, 1996

13. Wallenstein S, Zucker CL, Fleiss JL: Some statistical methods useful in circulation research. Circ Res 47:1-9, 1980

14. Katoh F, Niina H, Kitamura K, et al: Ca^{2+}-dependent cosecretion of adrenomedullin and cathecholamines mediated by nicotinic receptors in bovine cultured adrenal medullary cells. FEBS Lett 348:61-64, 1994

15. Kohno M, Kano H, Horio T, et al: Inhibition of endothelin production by adrenomedullin in the vascular smooth muscle cells. Hypertension 25:1185-1190, 1995

16. Tanaka M, Kitamura K, Ishizaka Y, et al: Plasma adrenomedullin in various diseases and exercise-induced changes in adrenomedullin in healthy subjects. Intern Med 34:728-733, 1995

17. Kanazawa H, Kawaguchi T, Fujii T, et al: Comparison of bronchodilator responses to adrenomedullin and proadrenomedullin *N*-terminal 20 peptide. Life Sci 57:PL241-PL245, 1995