Plasma Levels of Adrenomedullin, a Newly Identified Hypotensive Peptide, in Patients with Hypertension and Renal Failure

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Abstract

Adrenomedullin is a potent hypotensive peptide newly discovered in pheochromocytoma tissue by monitoring its elevating activity on platelet cAMP. We measured plasma concentration of adrenomedullin in patients with essential hypertension and chronic renal failure. As compared with normal subjects, plasma adrenomedullin was increased by 26% ($P < 0.05$) in hypertensives without organ damage and by 45% ($P < 0.005$) in those with organ damage. The increase in plasma adrenomedullin was more prominent in renal failure than in hypertension. Renal failure patients with plasma creatinine of 1.5–3, 3–6, and > 6 mg/dl had higher plasma adrenomedullin levels than healthy subjects by 78% ($P < 0.05$), 131% ($P < 0.001$), and 214% ($P < 0.001$), respectively. Moreover, adrenomedullin showed intimate correlations with norepinephrine, atrial natriuretic peptide, and cAMP in plasma ($r = 0.625$, $P < 0.001$; $r = 0.656$, $P < 0.001$; and $r = 0.462$, $P < 0.001$; respectively). Thus, plasma adrenomedullin is supposed to increase in association with changes in sympathetic nervous activity and body fluid volume in hypertension and renal failure. Considering its potent vasodilator effect, adrenomedullin may be involved in the defense mechanism preserving the integrity of the cardiovascular system in these disorders. (*J. Clin. Invest.* 1994. 94:2158–2161.) Key words: kidney failure • norepinephrine • atrial natriuretic factor • cyclic AMP • human

Introduction

Adrenomedullin (AM)[1] is a peptide from human pheochromocytoma tissue newly discovered by monitoring its stimulating action on platelet cAMP production (1). The peptide consists of 52 amino acids with an intramolecular disulfide bond forming a ring structure of six residues, and shares slight homology with calcitonin gene-related peptide (CGRP), a potent hypotensive peptide. Like CGRP, intravenous injection of AM elicits a strong and long-lasting hypotensive effect in anesthetized rats (1, 2). The vasodilator action of AM has been also demonstrated in the ex vivo experiment using perfused mesenteric vessels (3).

With regard to the regional distribution of AM, the adrenal gland prominently expresses mRNA of AM, as expected, from the origin of tissue in which this peptide was discovered. However, besides the adrenal, considerable mRNA expression has been recognized in much larger organs such as the heart, kidney, and lung (4). Moreover, a significant level of AM has been identified in human plasma by means of specific RIA coupled with liquid chromatography (1, 5). These findings suggest the possibility of AM as a new circulating hormone participating in regulation of the cardiovascular system.

Among numerous studies to be done for the purpose of understanding the pathophysiological implications of AM, it seems of primary importance to determine the behavior of this peptide in various cardiovascular diseases. In this present study, we measured the circulating level of AM in patients with hypertension or renal failure, and investigated the relations to other known cardiovascular hormones.

Methods

The study population consisted of 17 normal subjects (9 males, 8 females) aged 29 to 62 yr (42±2 yr), 35 patients with essential hypertension (18 males, 17 females) aged 27 to 74 yr (55±3 yr), and 29 nondialyzed patients with stable chronic renal failure (19 males, 10 females) aged 23 to 77 yr (57±5 yr). Hypertension was defined as elevated blood pressure exceeding 150/90 mmHg for three consecutive measurements over a period of 4 wk. Secondary causes of hypertension were ruled out through a comprehensive checkup. They had either not received antihypertensive drugs or, if they had, the drug had been stopped for at least 2 wk. The patients with chronic renal failure had plasma creatinine concentration > 1.5 mg/dl. Their causes of renal failure were chronic glomerulonephritis in 15 patients, nephrosclerosis in 5, diabetic nephropathy in 4, polycystic kidney in 2, and repetitive

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1. *Abbreviations used in this paper:* AM, adrenomedullin; ANP, atrial natriuretic peptide; CGRP, calcitonin gene-related peptide; TFA, trifluoroacetic acid; WHO, World Health Organization.

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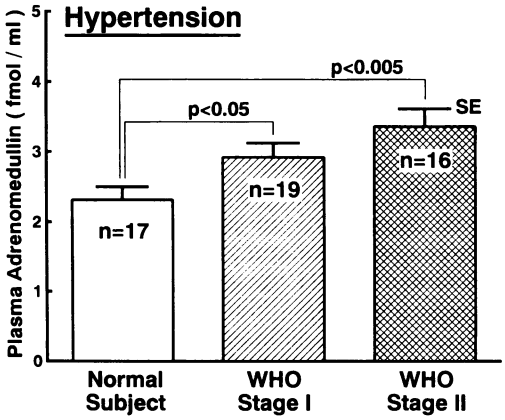

Figure 1. Plasma level of adrenomedullin in patients with essential hypertension. Analysis of variance resulted in an F value of 5.68, $P < 0.01$.

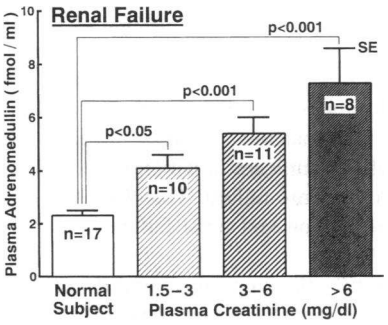

Figure 2. Plasma level of adrenomedullin in patients with chronic renal failure. Analysis of variance resulted in an F value of 12.41, $P < 0.001$.

pyelonephritis due to vesicoureteral reflux in 2 patients. Diuretics or calcium channel blockers may have been used for their edema or blood pressure control, however, patients given other classes of antihypertensive drug were excluded. The normal subjects and patients with essential hypertension were maintained in mild sodium restriction, 7–8 g NaCl/d, for 2 wk or more. In renal failure patients, sodium intakes may have varied from 3 to 7 g NaCl per day according to their disease state.

Antecubital venous blood (20 ml) was taken during morning hours, 0700–0900, after an overnight fast and 30 min of supine rest. The blood was transferred to two tubes ice chilled, one containing EDTA (1 mg/ml) and the other supplemented aprotinin (500 U/ml) in addition. Plasma was separated by centrifugation at 4°C, and stored at −80°C until assayed. The aprotinin plasma was used for the assays of AM and α-human atrial natriuretic peptide (ANP).

Plasma AM concentration was measured by specific RIA after extraction and purification as previously described (5). Briefly, 2 ml of plasma was applied to conditioned Sep-Pak C18 cartridge (Millipore Corp., Waters Chromatography, Milford, MA), and the column was sequentially washed with 5 ml of isotonic saline, 5 ml of 0.1% (vol/vol) trifluoroacetic acid (TFA), and 5 ml of 20% (vol/vol) acetonitril in 0.1% TFA. Then, the absorbed material was eluted with 4 ml of 50% (vol/vol) acetonitril, and the eluate was lyophilized. The residue was dissolved in 0.3 ml of 50 mM phosphate buffer (pH 7.4), and was submitted to RIA using the radioiodinated AM and antiserum raised against synthetic AM in rabbits. Plasma ANP was measured using ShionoRIA ANP assay kit (Shionogi & Co., Ltd., Osaka, Japan) (6). Plasma levels of epinephrine and norepinephrine were determined by the automated analyzer including HPLC system (HLC-725CA; Tosoh, Co. Ltd., Tokyo, Japan) (7). Cyclic AMP in plasma was measured using Yamasa cyclic AMP assay kit (Yamasa Shoyu, Co., Ltd., Chiba, Japan) (8, 9). Plasma renin activity and plasma aldosterone concentration were determined by RIA, and plasma creatinine by colorimetry.

Data were given as mean±SE. Comparisons between groups of patients were performed by analysis of variance followed by modified t test of Dunnett. Linear regression analysis was used to evaluate correlations between variables. A P value less than 0.05 was considered statistically significant.

Results

The plasma level of AM was 2.3±0.2 fmol/ml in normal subjects. Fig. 1 shows plasma AM concentrations in patients with essential hypertension when they were classified according to the World Health Organization (WHO) criteria for organ damage. WHO stage I patients without signs of organ damage had significantly higher plasma AM than normal subjects, 2.9±0.2 fmol/ml, $P < 0.05$. WHO stage II patients accompanied by left ventricular hypertrophy, proteinuria, or optic fundus lesions showed even higher plasma AM concentration, 3.4±0.3 fmol/ml, $P < 0.005$ vs. normal subjects. Although the average age was higher in the hypertensive group than in the normal group, plasma AM was still significantly increased in the age-matched group of essential hypertension ($n = 14$, age 42±3 yr), 3.3±0.2 fmol/ml, $P < 0.005$ vs. normal subjects. Fig. 2 presents plasma AM concentrations in patients with chronic renal failure when the patients were classified according to their plasma creatinine levels. The renal failure patients with 1.5–3, 3–6, and > 6 mg/dl plasma creatinine showed higher plasma AM than normal subjects; 4.1±0.5, 5.3±0.6, and 7.3±1.3 fmol/ml, respectively. Thus, the plasma AM level was elevated with increasing plasma creatinine concentration. The renal failure group were also older than the normal group. However, the age-matched group of chronic renal failure ($n = 12$, age 42±3 yr) still showed increased plasma AM, 4.9±0.7 fmol/ml, $P < 0.005$ vs. normal group.

Table I lists the correlations between plasma AM and other humoral factors concerning the circulation system. Although AM was discovered in pheochromocytoma, the plasma level of AM did not correlate to that of epinephrine, mainly derived from the adrenal medulla. However, an intimate relationship was observed between plasma AM and norepinephrine levels. Moreover, plasma AM closely correlated to plasma ANP. There was also a significant correlation between AM and cAMP in plasma. In comparison to these, correlations of AM to renin and aldosterone were not significant.

Discussion

In this study, it was demonstrated that the plasma AM level was increased in patients with essential hypertension or chronic

Table I. Correlations between Plasma Adrenomedullin and Other Humoral Factors Relating to Cardiovascular System

Parameters	Correlation coefficient (r)	P Value
Plasma norepinephrine concentration	0.625	< 0.001
Plasma epinephrine concentration	0.018	NS
Plasma renin activity	0.055	NS
Plasma aldosterone concentration	0.216	NS
Plasma ANP concentration	0.656	< 0.001
Plasma creatinine concentration	0.583	< 0.001
Plasma cAMP concentration	0.462	< 0.001

renal failure. Furthermore, this increase of plasma AM was closely correlated with elevation of norepinephrine, ANP, and cAMP in plasma. The average age was higher in hypertension and renal failure groups than in normal subjects. However, even in comparison between age-matched groups, increases in plasma AM were still highly significant in patients with essential hypertension and chronic renal failure. Therefore, the elevated plasma AM in hypertension and renal failure is not explained by the effect of aging.

Too little is known to infer the mechanism and the significance of increased AM in hypertension and renal failure. Although the adrenal medulla abundantly contains AM (1), significant correlation was not observed between AM and epinephrine in plasma. Secretions of these two humoral factors to circulating blood may not always be parallel. In addition, considering that not only the adrenal medulla but also the heart, lung, and kidney have been shown to express a considerable amount of mRNA (4), production of AM by these organs may have masked the correlation between AM and epinephrine derived from the adrenal. It also may be possible that the circulating AM is derived mainly from these large organs rather than the small adrenal. In patients with essential hypertension, plasma AM was increased even in WHO stage I group. At the present time, the major source of circulating AM is unknown, however, it is possible that AM is released in response to high blood pressure.

There seems to be an intimate relationship between circulating AM and norepinephrine. Therefore, plasma AM is supposed to increase in states in which the sympathetic nervous system is enhanced. The activation of the sympathetic nervous system may be also associated with an increase in plasma cAMP level. The correlation between AM and ANP in plasma was also highly significant. Because the plasma ANP level has been shown to rise when body fluid volume is increased either acutely or chronically (10–12), it seems possible that plasma AM is also increased in response to the blood volume expansion. The elevated plasma AM levels in hypertensive patients with organ damage and patients with renal failure may reflect increased body fluid volume caused by decreased capacity of the kidney for sodium excretion in these patients.

In patients with chronic renal failure, plasma AM was increased in correlation with the plasma creatinine concentration. In patients with essential hypertension, plasma AM was higher in WHO stage II group with signs of organ damage than in stage I group without them. Although the plasma creatinine concentrations were not different between the two groups, renal function may have been impaired to some extent in the WHO Stage II group. Therefore, the increased plasma levels of AM in these patients may be a result of decreased clearance by the kidney, like other low molecular weight peptides such as insulin and parathyroid hormone (13–15). Although the precise mechanism by which AM is metabolized remains to be elucidated, plasma concentration of AM was not significantly reduced after passing the kidney in our blood samplings from hypertensive patients who had undergone percutaneous catheterization (16). Furthermore, the half-life of AM in plasma seems less than a couple of minutes when injected intravenously into rats (17). These suggest that increased plasma AM in hypertension and renal failure cannot be ascribed simply to reduced renal function.

Although the physiological role of AM is unclear, the potent hypotensive action of AM suggests its pathophysiological implication in the cardiovascular system. Assuming the implication of AM in the integrity of the cardiovascular system, AM may be involved in the protective mechanism against blood pressure elevation and body fluid volume expansion, since AM is increased in hypertension and renal failure. It is of interest that there was an intimate correlation between AM and cAMP in plasma, because cAMP is known to play a profoundly important role as a second messenger of various hormones in the regulation of the circulatory system, its vascular tone, platelet aggregation, and cardiac function (18, 19). The effect of norepinephrine through β-adrenoceptor may have been concerned with this correlation (20), and actually, there was a significant correlation between plasma norepinephrine and cAMP to a similar extent ($r = 0.472, P < 0.001$). However, it is possible that the stimulative action of AM on cAMP production also contributes to the positive correlation between the two factors. The hypotensive effect of AM is mainly mediated by reduction of peripheral vascular resistance. Concomitantly with this vasodilator action, Ishiyama et al. (2) have observed an increase in cardiac output without a change in heart rate after intravenous injection of AM in anesthetized rats. This inotropic action on the heart also may be due to increased cAMP by AM (21). Taken together, it is speculated that an increase in AM may contribute to preserving regional blood flow by dilating resistance vessels and improving cardiac function in states of hypertension and renal failure, and thereby may slow formation and progression of a vicious cycle in these disorders.

In conclusion, we have demonstrated that plasma AM increases in essential hypertension and chronic renal failure with advancing severity of the disease. The intimate correlations of AM with norepinephrine and ANP in plasma suggest that elevation of AM level is related to increases in sympathetic nervous activity and body fluid volume. In addition, AM may influence the circulating cAMP level. Considering the potent vasorelaxing action of AM, the increase in AM may be involved in the defense mechanism against further blood pressure elevation, and the possibly cardiotonic action may contribute to preventing further body fluid retention.

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