ORIGINAL ARTICLE

Aldosterone antisecretagogue and antihypertensive actions of adrenomedullin in patients with primary aldosteronism

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Adrenomedullin (AM) is located in the zona glomerulosa of the adrenal cortex and is considered to suppress aldosterone release. To determine the effect of AM in primary aldosteronism (PA), we infused AM ($2.5 \text{ pmol kg}^{-1} \text{ min}^{-1}$) for 27 h, followed by a 15-h recovery period, in a control group (essential hypertensives with plasma aldosterone levels $\leq 100 \text{ pg ml}^{-1}$, $n=7$) and in a PA group ($n=5$). The control group was also infused with vehicle. Hemodynamic, hormonal, oxidative and inflammatory responses were studied. AM infusion caused similar and steady decreases in blood pressure and several markers for arteriosclerosis (for example, pulse wave velocity) in both groups. Interestingly, AM infusion suppressed aldosterone release to values within the normal range in the PA group (300.0 ± 58.4 to $111.6 \pm 13.5 \text{ pg ml}^{-1}$, $P < 0.01$). In the control group, aldosterone release suppression was significant but limited (81.7 ± 9.1 to $47.9 \pm 9.9 \text{ pg ml}^{-1}$, $P < 0.01$). The adrenocorticotrophic hormone–cortisol system was not changed by AM infusion. Brain natriuretic peptide was cumulatively increased by prolonged AM infusion in both groups, probably because of cardiac overload. AM did not affect oxidative markers. In addition, a mild but significant increase in C-reactive protein (CRP) mediated by interleukin-6 was observed during AM infusion in every participant, without exception. This pathway might participate in CRP elevation in cardiovascular disease. In summary, AM seems to have an essential role in the suppression of aldosterone release in PA. AM may be an important modulator in PA, and intermediate-term (3 h) AM infusion could be used as an alternative renin-stimulating/aldosterone-suppressing test for PA detection.

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INTRODUCTION

Adrenomedullin (AM) is a potent hypotensive peptide found ubiquitously in tissues and organs, especially in cardiovascular tissues, the kidneys, lungs and endocrine glands. AM has multiple functions in a wide range of tissues and acts mainly as a vasodilatory and proliferation-inhibitory factor.¹ AM was initially identified in the adrenal medulla, but similar densities of AM were detected in the zona glomerulosa of the adrenal cortex, where AM suppresses aldosterone release.^{2,3} In addition, expression of AM and its receptor was detected in Conn's adenoma cells, and AM exerted aldosterone antisecretagogue action and proliferative effects on cultured Conn's adenoma cells.⁴ These findings suggest that endogenous AM may be an important modulator of aldosterone release in normal and pathogenic hyperaldosteronism. In addition, AM antagonized aldosterone-induced vascular or cardiac remodeling^{5,6} and suppressed aldosterone-induced oxidative stress in a malignant hypertensive model.⁷ The characteristics that define this compound, from its release to alterations in target organs, suggest that AM may be an endogenous anti-aldosterone factor, especially in the cardiovascular system.

The effects of exogenous AM administration on aldosterone release were varied in experimental animal and human studies.⁸ Short-term AM administration to healthy volunteers,⁹ hypertensive patients¹⁰ and patients with renal insufficiency¹¹ caused vigorous stimulation of renin release, but did not change aldosterone levels; consequently, the aldosterone/renin ratio decreased. AM suppressed aldosterone levels in secondary hyperaldosteronism, such as experimental heart failure in sheep⁸ or congestive heart failure in patients.¹² These data suggest that AM may be a functional antagonist of aldosterone release, but further evidence is required. We investigated the effects of AM in patients with primary aldosteronism (PA) to confirm the antagonistic effect of AM against aldosterone.

METHODS

Study subjects

Initially, essential hypertensive patients were recruited for baseline data collection for future translational research on the clinical applications of AM. After a comprehensive screening, seven hypertensive subjects who showed normal aldosterone release (plasma aldosterone $\leq 100 \text{ pg ml}^{-1}$; control group) were

Figure 1 Experimental protocol. After a comprehensive examination of all participants, AM ($2.5 \text{ pmol min}^{-1} \text{ kg}^{-1}$) or vehicle was intravenously administered for 27 h, followed by a 15-h post-infusion period. Arrows indicate hemodynamic and blood sample assessments.

administered AM. Five patients with PA, owing to an aldosterone-producing adenoma (PA group), were then recruited to receive AM. The control group was administered vehicle after an interval of at least 1 month. All participants were admitted to our hospital and subjected to 2 days of comprehensive examinations, including urine and blood tests, chest X-ray, ECG, echocardiography, echo scan of the carotid artery and a brain MRI. Patients with narrowing or obstruction of extra- and/or intracranial major arteries, renal failure (serum creatinine $>1.2 \text{ mg per } 100 \text{ ml}$), heart failure (left ventricular ejection fraction $<50\%$), coronary heart disease, peripheral artery diseases, collagen diseases or active infections were excluded. All patients with PA had cleared the diagnostic criteria for PA, as proposed by the Japan Endocrine Society (http://square.umin.ac.jp/endocrine/rinsho_juyo/index.html). Potential adrenal adenomas were confirmed by CT and/or MRI. This study was approved by the ethics committee of the institute. All participants gave their written informed consent.

Preparation of human AM

Chemically synthesized human AM was purchased from the Peptide Institute (Osaka, Japan). The homogeneity of human AM was confirmed by reverse-phase high-performance liquid chromatography and amino acid analysis. AM was dissolved in distilled water with 3.75% D-mannitol and 0.05% aminoacetic acid and then sterilized by passing through a $0.22\text{-}\mu\text{m}$ filter (Millipore, Bedford, MA, USA). The chemical nature and level of human AM in vials were verified by reverse-phase high-performance liquid chromatography. No measurable endotoxin was detected ($<0.01563 \text{ EU ml}^{-1}$), and the material was determined to be pyrogen-free by the Japan Food Research Laboratories (Tokyo, Japan).

Study protocol

Fixed time points (0800 and 1700 hours) were assigned for comprehensive hemodynamic examination and blood sampling. Vehicle or AM administration was started at 1400 hours, as indicated in Figure 1. The comprehensive hemodynamic examination included blood pressure, heart rate (by ECG) and arteriosclerosis-related markers, such as pulse wave velocity (form PWV/ABI, BP-203RPE; Omron Colin, Komaki, Japan), augmentation index (HEM9010AI tonometer; Omron Healthcare, Kyoto, Japan) and elastic property of the carotid artery.¹³ A 22-gauge cannula was inserted into the forearm vein for infusion of AM ($2.5 \text{ pmol min}^{-1} \text{ kg}^{-1}$) or vehicle diluted by 0.9% saline. Saline was administered at the rate of 5 ml h^{-1} for 27 h, followed by 15 h of recovery time. Continuous ECG monitoring and blood pressure measurements at 60-min intervals were performed throughout the experiment (Figure 1). The first urine sample was collected at around 0700 hours, before, during and after AM or vehicle administration. Plasma total and mature AM were measured using a specific immunoradiometric assay kit (Shionogi, Osaka, Japan). Plasma concentrations of other hormones, high-sensitivity C-reactive protein (CRP) and cytokines were measured using a commercially available laboratory testing service (SRL, Hachioji, Japan). Urinary concentrations of 8-isoprostane and 8-hydroxydeoxyguanosine were also measured by the laboratory testing service provided by SRL and normalized by the concentration of urinary creatinine.

Statistical analysis

All data were expressed as means $\pm$ s.e.m. Comparisons between the two groups (control vs. PA) were performed using the unpaired Student's *t*-test. The significance of differences was evaluated by one-factor analysis of variance

Table 1 Basal characteristics

	Control	PA	P-value
Age (years)	65.3 $\pm$ 3.1	63.6 $\pm$ 2.5	NS
Sex (M/F)	7/0	3/2	
SBP (mm Hg)	154.4 $\pm$ 7.0	155.4 $\pm$ 5.8	NS
DBP (mm Hg)	93.7 $\pm$ 3.6	93.2 $\pm$ 4.5	NS
Heart rate (b.p.m.)	63.3 $\pm$ 2.8	63.6 $\pm$ 3.8	NS
PWV (cm s^{-1})	1943 $\pm$ 117	1939 $\pm$ 221	NS
Augmentation index (%)	83.7 $\pm$ 6.2	97.2 $\pm$ 4.4	NS
Elastic property (kPa)	109.7 $\pm$ 11.0	93.1 $\pm$ 7.9	NS
Aldosterone (pg ml^{-1})	81.7 $\pm$ 9.1	300 $\pm$ 58.4	0.001
Renin activity ($\text{ng ml}^{-1} \text{ h}^{-1}$)	0.90 $\pm$ 0.40	0.16 $\pm$ 0.04	NS
Adrenomedullin (fmol ml^{-1})	11.8 $\pm$ 0.6	19.2 $\pm$ 1.7	0.001
ANP (pg ml^{-1})	15.6 $\pm$ 2.8	23.4 $\pm$ 9.8	NS
BNP (pg ml^{-1})	16.6 $\pm$ 3.3	55.8 $\pm$ 36.2	NS
Noradrenaline (pg ml^{-1})	297 $\pm$ 31	384 $\pm$ 81	NS
High-sensitivity CRP (mg l^{-1})	0.78 $\pm$ 0.18	1.80 $\pm$ 0.83	0.06

Abbreviations: ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CRP, C-reactive protein; DBP, diastolic blood pressure; NS, non-significant; PA, primary aldosteronism; PWV, pulse wave velocity; SBP, systolic blood pressure.

with repeated measures on a time course of variables, followed by Bonferroni–Dunn *post hoc* comparison tests. A value of $P < 0.05$ was the criterion for statistical significance.

RESULTS

To avoid extreme rises in blood pressure, the minimal amount of Ca^{2+} -channel blocker (amlodipine $5\text{--}10 \text{ mg day}^{-1}$) was administered to three of seven patients in the control group and to all five patients with PA. The baseline characteristics of the control group and PA group were fairly matched, particularly with regard to age and blood pressure (Table 1). The plasma concentration of aldosterone and, interestingly, the plasma concentration of AM were increased in the PA group (Table 1).

Prolonged AM administration caused a strong and steady decrease in blood pressure; this effect was quite similar in both control and PA groups (Figure 2). Heart rate was increased by almost the same magnitude in both groups during AM administration (approximately +26% in control and +31% in PA). In addition, similar decreases in arteriosclerotic markers, such as pulse wave velocity, augmentation index and elastic property of the carotid artery, were accompanied by reductions in blood pressure in both groups (Figure 2). These effects returned to baseline after a 15-h interval. Harmful symptoms or reactions were not observed in any of the participants.

As indicated in Figure 3, AM administration caused significant increases in total AM (approximately 3.1-fold in the control group and 2.1-fold in the PA group). Peak concentrations of AM were comparable in both groups. Plasma concentration of mature AM also increased: approximately 6.7-fold in both groups (Table 2). Most interestingly, AM administration caused strong and significant suppression of aldosterone release in the PA group; levels reached the normal range ($111.6 \pm 13.5 \text{ pg ml}^{-1}$) at the end of AM infusion (Figure 3). Significant but moderate suppression of aldosterone release was also observed in the control group during AM administration. AM infusion stimulated renin release in the control group, but this change was not significant. A very small but significant increase in renin was observed in the PA group (Figure 3). Standard renin-secretion stimulating tests (captopril loading and furosemide loading+walking) did not increase renin levels in the PA group (data not shown).

Figure 2 Changes in blood pressure, pulse wave velocity (PWV), augmentation index (AI) and elastic property (Ep) of the carotid artery during infusion of adrenomedullin (●: control group, ▲: PA group) or vehicle (○: control group). Data are means $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, each vs. baseline.

Other hormonal changes are summarized in Table 2. Levels of cAMP, the second messenger of AM, were unchanged in both groups. An increase in atrial natriuretic peptide (ANP) level, accompanied by a cGMP increase, was only observed in the PA group during AM administration. Brain natriuretic peptide (BNP) level was increased

Figure 3 Changes in the plasma concentration of adrenomedullin, aldosterone and plasma renin activity (PRA) during infusion of adrenomedullin (●: control group, ▲: PA group) or vehicle (○: control group). Data are means $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, each vs. baseline.

in both groups in a late phase of the experiment, but this alteration was not associated with the cGMP increase. It is noteworthy that AM did not affect the adrenocorticotrophic hormone–cortisol system in either group.

To evaluate the acute effects of AM on oxidative stress and the immune system, we assessed oxidative stress markers, cytokines and high-sensitivity CRP. Basal levels of CRP were below the normal range ($< 0.3 \text{ mg dl}^{-1}$) in all participants, and the average value was $0.12 \pm 0.04 \text{ mg dl}^{-1}$. AM administration did not affect the oxidative stress markers (8-isoprostane and 8-hydroxydeoxyguanosine) in either group (Table 2). Surprisingly, AM administration induced expression of interleukin-6 (IL-6), which was followed by an increase in CRP. This reaction was confirmed in every participant, without exception. All results are summarized in Figure 4. (The unit for CRP is mg l^{-1} .)

Table 2 Changes of parameters in blood and urine tests

	Before	3 h	18 h	27 h	Recovery
Total protein, g dL⁻¹					
Vehicle	6.7 ± 0.1		6.7 ± 0.1		6.7 ± 0.1
Cnt-AM	7.1 ± 0.2		6.8 ± 0.1		7.2 ± 0.2
PA-AM	7.2 ± 0.1		6.6 ± 0.2*		7.1 ± 0.3
Mature adrenomedullin, fmol mL⁻¹					
Vehicle	2.4 ± 0.1	3.2 ± 0.9	2.7 ± 0.6	3.1 ± 0.7	2.3 ± 0.2
Cnt-AM	2.0 ± 0.4	8.1 ± 1.1**	13.4 ± 1.8**	11.8 ± 2.6**	1.6 ± 0.3
PA-AM	2.8 ± 0.4	9.6 ± 2.0*	18.8 ± 3.5**	12.2 ± 2.4*	2.8 ± 0.8
Noradrenaline, pg mL⁻¹					
Vehicle	226 ± 35	290 ± 52	219 ± 29	242 ± 27	219 ± 25
Cnt-AM	297 ± 31	336 ± 21	374 ± 30	316 ± 25	341 ± 55
PA-AM	384 ± 81	428 ± 110	487 ± 146	469 ± 122	417 ± 99
cAMP, pmol mL⁻¹					
Vehicle	11.7 ± 0.7	11.3 ± 0.7	12.5 ± 1.0	12.4 ± 0.4	12.4 ± 0.8
Cnt-AM	14.4 ± 0.8	15.1 ± 0.6	15.6 ± 0.8	14.9 ± 0.9	14.0 ± 0.6
PA-AM	13.2 ± 1.0	14.6 ± 1.1	13.8 ± 1.1	13.6 ± 1.0	12.5 ± 1.4
cGMP, pmol mL⁻¹					
Vehicle	4.5 ± 0.7	3.5 ± 0.8	3.2 ± 0.4	3.9 ± 0.5	3.3 ± 0.3
Cnt-AM	3.4 ± 0.3	3.9 ± 0.5	4.5 ± 0.8	3.6 ± 0.4	3.3 ± 0.2
PA-AM	2.6 ± 0.4	4.7 ± 0.5**	3.6 ± 0.3*	4.4 ± 0.6**	2.7 ± 0.3
ANP, pg mL⁻¹					
Vehicle	23.6 ± 4.1	19.6 ± 2.0	17.0 ± 1.6	20.9 ± 1.5	17.1 ± 1.6
Cnt-AM	15.6 ± 2.8	23.9 ± 6.8	29.6 ± 7.4	29.6 ± 9.1	18.6 ± 4.4
PA-AM	23.4 ± 9.8	40.0 ± 12.0*	41.0 ± 7.6*	49.8 ± 9.2**	29.0 ± 10.4
BNP, pg mL⁻¹					
Vehicle	18.9 ± 5.0	17.7 ± 4.4	15.1 ± 3.3	13.2 ± 2.9	10.6 ± 2.1
Cnt-AM	16.6 ± 3.3	12.7 ± 2.8	31.1 ± 7.4	61.1 ± 15.2**	53.0 ± 12.4**
PA-AM	55.8 ± 36.2	42.9 ± 24.8	79.8 ± 37.6	131.3 ± 53.5*	115.7 ± 56.3*
ACTH, pg mL⁻¹					
Vehicle	26.0 ± 5.9		21.7 ± 3.6		22.3 ± 3.8
Cnt-AM	20.6 ± 1.9		18.7 ± 5.3		22.1 ± 3.2
PA-AM	18.5 ± 2.8		18.2 ± 3.9		20.6 ± 5.9
Cortisol, µg dL⁻¹					
Vehicle	8.4 ± 0.5		11.2 ± 1.6		11.9 ± 1.3
Cnt-AM	10.7 ± 1.2		14.9 ± 2.5		13.2 ± 1.6
PA-AM	16.7 ± 3.6		17.8 ± 4.6		15.6 ± 1.9
8-Isoprostane, pg per mg creatinine					
Vehicle	256 ± 62		169 ± 23		237 ± 60
Cnt-AM	197 ± 25		206 ± 25		168 ± 19
PA-AM	207 ± 41		252 ± 46		253 ± 54
8-OHdG, ng per mg creatinine					
Vehicle	10.1 ± 1.3		10.1 ± 1.3		9.6 ± 1.1
Cnt-AM	7.8 ± 1.6		7.6 ± 0.9		7.7 ± 0.5
PA-AM	11.9 ± 5.1		9.4 ± 2.0		12.5 ± 2.9

Abbreviations: ACTH, adrenocorticotrophic hormone; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; Cnt-AM, control group with adrenomedullin infusion; 8-OHdG, 8-hydroxydeoxyguanosine; PA, primary aldosteronism group.

* $P < 0.05$, ** $P < 0.01$ vs. before.

Figure 4 Changes in the serum concentration of cytokines and high-sensitivity CRP during infusion of adrenomedullin or vehicle. Data are summarized for all participants (control+PA group, $n=12$) and expressed as means $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, each vs. baseline. Abbreviations: IL-1 β , interleukin-1 β (high sensitivity); IL-6, interleukin-6; TNF- α , tumor necrosis factor- α (high sensitivity).

DISCUSSION

This is the only study to investigate long-term administration (over 24 h) of AM in humans.¹⁴ The study put considerable strain on the participants, and hence the minimum number of participants required to achieve statistical significance was considered. Prolonged infusion of AM caused a hypotensive reaction, accompanied by improvements in arteriosclerotic markers (pulse wave velocity, augmentation index and elastic property of the carotid artery) in both the control group, with normal aldosterone levels, and in patients with PA. More importantly, we confirmed for the first time that AM infusion suppressed aldosterone release, producing a normal range in patients with PA.

AM is located in the zona glomerulosa of the adrenal cortex and Conn's adenoma.²⁻⁴ AM has a direct inhibitory effect on aldosterone release from adrenocortical cells and Conn's adenoma cells.¹⁵ Previous human experiments have produced varied results on the effects of intravenous infusion of AM on aldosterone release.⁸⁻¹² Our study showed that suppression of aldosterone release by AM in the control group was significant but quite limited (Figure 3). A hypotensive reaction due to AM infusion was observed over a wide dosage range, but the suppressive effect of AM on aldosterone release was time and dose dependent, suggesting that there may be relatively high thresholds for suppression.^{8,16} We used a 'moderate' amount of AM ($2.5 \text{ pmol kg}^{-1} \text{ min}^{-1}$ or $15 \text{ ng kg}^{-1} \text{ min}^{-1}$), which led to a significant decrease in aldosterone release after 3 h of infusion (Figure 3). In a previous report, a similar amount of AM ($16 \text{ ng kg}^{-1} \text{ min}^{-1}$), infused for 2 h, did not change renin or aldosterone release in healthy volunteers.⁹ The dose of AM was increased to $32 \text{ ng kg}^{-1} \text{ min}^{-1}$ and infusion was continued for another 2 h: a vigorous increase in renin release was reported, but there were no changes in aldosterone.⁹ A large dose of AM infusion caused strong hypotension and strongly stimulated renin release as well as sympathetic nervous activity.⁸ This may have interfered with the suppressive effect on aldosterone release. AM administration stimulates renin release.⁸⁻¹² On the other hand, AM can partially, but not completely, suppress increases in aldosterone induced by angiotensin II in humans.¹⁷ In PA patients, renin activity is extremely suppressed, suggesting that AM could mediate essential aldosterone suppression. Under the conditions studied here

($2.5 \text{ pmol kg}^{-1} \text{ min}^{-1}$ for 3 h), AM may be useful as an alternative renin-stimulating (and aldosterone-suppressing) test for PA detection. In addition, it is important to elucidate the suppressive mechanism of AM because the lower aldosterone concentration in blood that results could benefit the cardiovascular system.

In this study, the plasma concentration of AM in PA patients was increased (Table 1). We and one other research group have previously reported this phenomenon.^{18,19} The underlying mechanism remains to be elucidated: there may be a mutual relationship between aldosterone and AM. Aldosterone stimulates AM production in rat aortic adventitia or cardiac fibroblasts; increased AM, in turn, regulates the proliferative action of aldosterone in those cells.^{5,6} Increased levels of AM were able to suppress blood pressure and aldosterone release in PA patients (Figures 2 and 3). AM may counteract or buffer the impact of hyperaldosteronism; however, AM stimulates or maintains cell proliferation in the adrenal zona glomerulosa as well as Conn's adenoma cells.^{4,20,21} Letizia *et al.*¹⁹ reported that the plasma level of AM was positively related to the tumor size of the adenoma in PA. It is highly probable that AM modulates the pathophysiological condition of PA, but further study is required to elucidate the participation of AM in PA.

AM administration caused several alterations in the humoral factors measured (Table 2). In particular, ANP was significantly increased, accompanied by continuous increases in cGMP, but only in PA patients. AM did not affect ANP or BNP levels in normal subjects or in patients with hypertension or heart failure.^{9,10,12} The ANP-stimulating effect in PA patients is interesting, although the mechanism is unclear. ANP inhibits aldosterone secretion and is considered to be a key factor in aldosterone escape (or aldosterone breakthrough).^{22,23} Thus, increased ANP may participate in the suppression of aldosterone release in PA. However, aldosterone-producing adenomas do not have a receptor for ANP and ANP did not suppress aldosterone release from the adenoma.²⁴ In addition, ANP infusion did not suppress aldosterone release in patients with PA.²⁵ The AM-induced ANP increase does not seem to be related to aldosterone suppression in PA by AM. ANP increased during AM infusion, whereas BNP was increased in both groups during the late phase of the experiment (Table 2). AM has a cAMP-dependent and -independent positive inotropic effect on myocardium.^{26,27} In addition to the decrease in cardiac afterload induced by vasodilation, cardiac output was markedly increased by AM administration.^{9–12} The cumulative increase in BNP may reflect cardiac overload induced by prolonged infusion of AM. Although none of the participants experienced adverse events, cardiac overload must be carefully avoided during longer term application of AM. AM has diuretic and natriuretic effects,^{10–12} and AM administration increased ANP and BNP levels in this study; however, we did not study the diuretic and natriuretic effects of these factors. Participants would not agree to use an additional balloon catheter for accurate urine collection. Total saline infusion was only 135 ml over 27 h, so it should not have influenced urine samples in this experiment. Because total protein was decreased after AM administration (Table 2), significant decreases were measured in PA patients, while serum levels of sodium were not altered (control: 141.1 ± 1.1 , 141.4 ± 0.8 and $140.9 \pm 0.8 \text{ mEq l}^{-1}$; PA: 143.6 ± 0.5 , 144.4 ± 0.5 and $143.4 \pm 0.4 \text{ mEq l}^{-1}$ before, during and after AM administration, respectively). The decreases in total protein may be due to vasodilation induced by AM. Decreases in hematocrit have been previously reported,²⁸ suggesting some hemodilution.

This is the first report suggesting that prolonged administration of AM can induce CRP production through IL-6 in humans. AM is known to inhibit strong inflammation, such as sepsis.²⁹ However, AM

can exert both pro-inflammatory and anti-inflammatory effects, and it stimulates IL-6 production in macrophages.³⁰ As yet, there are no data on the effect of AM on CRP production in humans who do not exhibit accelerated inflammation. The time course of IL-6 and CRP changes (Figure 4) and the close relationship between both factors (relationship between maximum changes of CRP and IL-6; $r=0.64$, $P=0.034$) in the present study strongly suggest an interaction of IL-6 with CRP. Isumi *et al.*³¹ reported that the stimulatory effect of AM on IL-6 gene transcription took place immediately, reached a plateau within 30 min, and then decreased gradually. The short-term increase in IL-6 in the present study is compatible with the acute-phase stimulant nature of AM. However, IL-6 is a key factor in the regulation of CRP production in the liver, the main source of serum CRP.³¹ As CRP has a higher rate of increase and longer half-life (about 19 h) in comparison with IL-6 or AM,³² the extended increase in CRP observed in this study was not unexpected.

In addition to modulating the vascular tonus, AM influences the progression of atherosclerosis¹ and stimulates production of IL-6.³⁰ Moreover, AM and inflammatory markers, such as IL-6 and CRP, are elevated in patients with hypertension, CHD and peripheral artery disease: positive correlations between AM and IL-6 or CRP^{1,33} have been reported. AM production is most likely stimulated in the vasculature, as a reaction to a variety of stress-related factors, including hormones, mechanical stresses, metabolic factors and cytokines.³⁴ In addition to inflammation, many kinds of stimuli to blood vessels would influence AM levels and consequently CRP. Although AM is merely one factor regulating CRP production, this intimate relationship of AM with CRP via IL-6 may represent a pivotal role for AM in a mechanism linking serum CRP and vascular alterations. However, prolonged elevation of AM in PA patients did not completely correlate with CRP elevation in PA patients (Table 1). Further studies to confirm the roles of AM in the regulation of CRP levels under various conditions are required.

In conclusion, we have shown that prolonged administration of AM can normalize blood pressure and aldosterone release in PA. The ability of AM to suppress autonomous release of aldosterone in PA seems to be substantial when compared with the suppressive properties of ANP in PA that are unrelated to aldosterone. AM may be an important modulator in PA, and AM seems to be a unique tool and potential target for research into aldosterone release in PA. In addition, AM mildly stimulates CRP production at baseline, through IL-6 or non-stimulated inflammatory conditions in humans. This pathway might participate in CRP elevation in cardiovascular disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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