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## High plasma adrenomedullin concentrations in patients with high-renin essential hypertension

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### Abstract

Adrenomedullin (AM) is a novel peptide, first isolated from human pheochromocytoma, which elicits a long-lasting vasorelaxant activity. Recently, it has been reported that endothelial cells produce AM and that immunoreactive AM plasma levels may be elevated in human arterial hypertension, although the exact pathophysiological role of AM remains to be established.

The aim of our study was to determine the relationship between the components of the renin-angiotensin-aldosterone system (RAAS) and plasma AM levels in patients with low-, medium- or high-renin essential hypertension.

The study groups included 10 patients with low-renin essential hypertension (average age  $42 \pm 15$  years), nine patients with medium-renin essential hypertension ( $46 \pm 13$  years), 11 patients with high-renin essential hypertension ( $42 \pm 14$  years) and 12 healthy subjects ( $43 \pm 11$  years).

Our results demonstrated that the mean AM values of all patients with essential hypertension were  $10.85 \pm 3.14$  pg/ml; there was a statistical correlation ( $r=0.705$ ;  $p<0.001$ ) between plasma renin activity (PRA) and AM levels in hypertensives.

In patients with high-renin essential hypertension, plasma AM levels ( $14.2 \pm 2.2$  pg/ml) were significantly higher ( $p<0.001$ ) than those of healthy subjects ( $8.7 \pm 2.1$  pg/ml), patients with medium-renin essential hypertension ( $8.5 \pm 1.4$  pg/ml), and patients with low-renin essential hypertension ( $9.1 \pm 1.5$  pg/ml). There was no statistical difference in AM concentrations between medium- and low-renin hypertensive patients. In conclusion, we have found that, in hypertensive patients, plasma AM levels were increased only in high-renin individuals, suggesting a role of AM in this particular form of human essential hypertension.

### Introduction

Adrenomedullin (AM) is a novel peptide first isolated from human pheochromocytoma.<sup>1</sup>

AM has been detected in plasma<sup>2,3</sup> and other biological fluids<sup>3,4</sup> from healthy humans and may behave as a circulating hormone. When injected intravenously into rats and humans, AM elicits intense, long-lasting hypotension, a consequence of vasodilatation of resistance arteries.<sup>5,6</sup> Subsequent studies have demonstrated that AM has various physiological actions, including diuresis,<sup>7</sup> natriuresis<sup>8</sup> and increased cardiac output.<sup>9</sup>

Recently, it was reported that patients with hypertension show high levels of plasma AM compared with normotensive control subjects.<sup>10-13</sup> Nevertheless, the exact physiopathological role of AM in human hypertension remain to be established.

The renin-angiotensin-aldosterone system (RAAS) is known as an essential modulator of cardiovascular disease and the hypothesis of a relationship between AM and RAAS has been reported in some experimental and clinical studies.<sup>14-16</sup>

The purpose of the study was to determine the relationship between plasma renin activity (PRA), plasma aldosterone concentrations (PAC) and plasma AM levels in patients with low-, medium- and high-renin essential hypertension.

### Material and methods

#### Study population

This study was conducted under the guidelines of the Department of Clinical Science Review Committee. Mild hypertensive and normotensive individuals participated. The hypertensive patients were recruited as part of an ongoing study in which hypertensive factors were evaluated. An initial evaluation of each patient included the determination of the plasma renin/urinary sodium intake. Renin/sodium profiles were developed according to the procedure described by Laragh and colleagues.<sup>17</sup> Using this procedure, we enrolled 30 hypertensive patients subdivided into: a) 9 patients with medium-renin levels (5M, 4F; mean age  $46 \pm 13$  years); b) 10 patients with low-renin levels (5M, 5F; mean age  $43 \pm 15$  years); c) 11 patients with high-renin levels (7M, 4F; mean age  $42 \pm 14$  years).

Twelve normal subjects (7M, 5F; mean age  $43 \pm 11$  years) were enrolled as controls.

Normal blood pressure (BP) was defined as systolic BP (SBP)  $\leq 140$  mmHg and diastolic BP (DBP)  $\leq 90$  mmHg. Hypertension was defined as SBP  $\geq 140$  mmHg and DBP  $\geq 95$  mmHg. Borderline hypertensive subjects (SBP 140-160 mmHg and DBP 85-90 mmHg) were not included in this study.

The diagnosis of essential hypertension was established before the study by the absence of clinical evidence of secondary hypertension: normal serum electrolytes, creatinine and urine analysis, normal abdominal sonogram, normal 12-

**Table 1** Demographic and laboratory data in the studied groups.

	Healthy subjects	Essential hypertensives		
		Low-renin	Medium-renin	High-renin
Age (years)	43±11	43±15	46±13	42±14
Systolic blood pressure (mmHg)	120±6	156±9*	150±7*	151±7*
Diastolic blood pressure (mmHg)	75±7	99±3*	99±4*	97±3*
Heart rate (b/min)	67±4	74±5*	71±5	72±6
Plasma renin activity (ng/ml/h)	1.2±0.3 <sup>†</sup>	0.38±0.1	1.33±0.21	4.5±1.7* <sup>†</sup>
Plasma aldosterone concentration (ng/dl)	11.9±7.5	14.2±5.1*	12.3±8	13.9±9.8
Urinary sodium excretion (mEq/day)	138.5±10.6	146±17	151±12	159±11.3*

\* = p<0.05 vs. healthy subjects; † = p<0.05 vs. essential hypertensives with low-renin

lead ECG and a normal fundoscopic examination. No subjects were overweight (body mass index [BMI] <27 kg/m<sup>2</sup>).

Subjects and patients were excluded from the study if any of the following criteria were found: heart failure, pregnancy and pre-eclampsia, diabetes, liver disease, sepsis, hyperthyroidism, acute asthma and chronic renal failure. All recruited subjects adhered to a diet of 120–140 mmol Na<sup>+</sup>/day and 40–60 mmol K<sup>+</sup>/day for at least two weeks before testing, and 24-hour Na<sup>+</sup> and K<sup>+</sup> urine collection evaluation confirmed the adherence to the diet. None of the participants showed signs of renal impairment and none were on antihypertensive or any other medical treatment.

On each protocol day, subjects were kept in the supine position for at least 60 minutes. BP was measured four times at three minute intervals, by means of a standard Riva-Rocci manometer with a cuff of appropriate size and a stethoscope located over the brachial artery was used. The first measurement was discarded and the mean of the last three pressures was calculated.

10 ml of venous blood samples were collected from the antecubital vein between 8 and 9 a.m. after overnight fasting. Five ml blood samples were collected in polystyrene tubes containing EDTA (1 mg/ml) and aprotinin (500 kIU/ml). Blood samples were then centrifuged at 3000 g at 0°C for 15 minutes. The plasma was immediately stored in glass tubes at -70°C, until assayed.

### Assay procedures

AM was measured with a specific radioimmunoassay (RIA, Phoenix Pharm, Inc., Mountain View, CA, USA) as previously described.<sup>12,13</sup> The anti-adrenomedullin antibody cross-reacts at 100% with human adrenomedullin (1–52) but not with rat adrenomedullin-(1–50), human amylin, human calcitonin-gene-related peptide, endothelin-1,  $\alpha$ -atrial natriuretic peptide-(1–28),  $\beta$ -atrial natriuretic peptide-(32) or c-atrial natriuretic peptide-(22).

Inter-assay variation was 12% and intra-assay variation was 5%. All assays were performed in duplicate. Concentrations of AM were expressed as pg/ml. Both PRA and PAC were determined by means of RIA kits supplied by Sorin (Sorin, Saluggia, Italy). The intra- and inter-assay coefficients of variations were 5.5% and 16.8% for PRA and 10.7% and 8% for PAC.

### Statistical analysis

All results are expressed as mean±standard deviation (SD). The statistical calculation was performed using 'Primer' software (Primer of Biostatistics, S.A. Glantz, McGraw-Hill, S. Francisco, USA). The individual values were inserted by group on the spread sheet and were evaluated by non-parametric ANOVA test and Bonferroni's *t*-test, where appropriate.

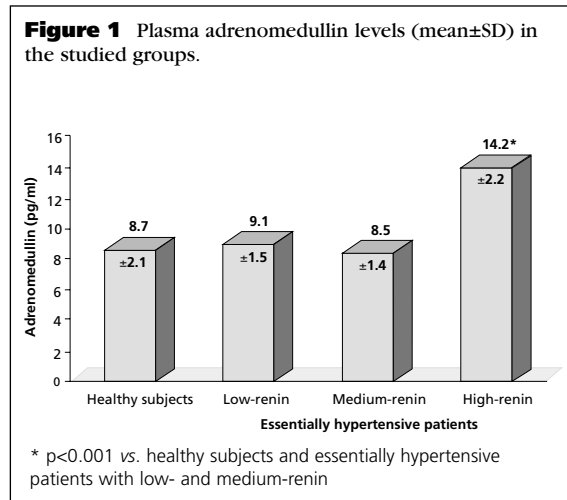
Correlation between AM values and other variables were determined by means of linear regression analysis. Statistical significance was set at p<0.05.

### Results

Table 1 shows the demographic characteristics and biochemical parameters of the study groups. As expected, the mean ( $\pm$ SD) values of arterial BP (systolic and diastolic) were significantly higher (p<0.05) in hypertensive patients than in controls. No differences (p>0.05) were found in DBP or SBP between patients with low-, medium- and high-renin essential hypertension.

Only patients with low-renin essential hypertension showed higher mean heart rate values and PAC than controls (p<0.05). In patients with high-renin hypertension, 24-hour urinary sodium excretion was higher (p<0.05) than in healthy subjects (Table 1).

Figure 1 shows the behaviour of plasma AM in the matched groups. In all hypertensive patients, mean ( $\pm$ SD) plasma AM values were significantly higher (10.85±3.14 pg/ml) than in normal



subjects ( $8.7 \pm 2.1$  pg/ml;  $p < 0.05$ ) and in these patients, there was a statistical correlation ( $r = 0.705$ ;  $p < 0.001$ ) between PRA and AM levels (Figure 2).

Mean plasma AM values in patients with high-renin essential hypertension ( $14.2 \pm 2.2$  pg/ml) were significantly higher ( $p < 0.05$ ) than those of healthy subjects ( $8.7 \pm 2.1$  pg/ml), patients with low-renin essential hypertension ( $9.1 \pm 1.5$  pg/ml) and patients with medium-renin essential hypertension ( $8.5 \pm 1.4$  pg/ml). No significant difference was found between plasma AM concentration in patients with medium- and low-renin essential hypertension (Figure 1).

Plasma AM levels did not correlate with BP and heart rate in either group.

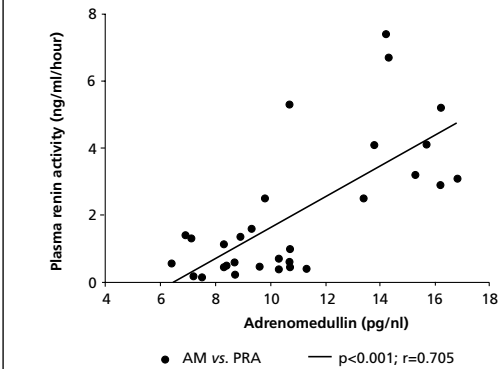
## Discussion

Adrenomedullin is a novel, 52 amino acid peptide hormone, originally isolated from human pheochromocytoma.<sup>1</sup> AM acts as a local autocrine and/or paracrine vasoactive hormone and has vasodilatory and BP-lowering properties.<sup>1</sup> AM is considered to play an important endocrine role in various tissues in maintaining electrolyte and fluid homeostasis.<sup>18</sup>

The present study demonstrated that in essential hypertensive patients plasma AM levels were only increased in high-renin patients, compared with normal humans. Moreover, we observed a significant correlation between AM and PRA in the group of patients with essential hypertension. These data were very similar to those reported by previous authors<sup>19,20</sup> and suggest that the RAAS and AM may influence each other, independently of BP. In fact, plasma AM levels did not correlate with BP in our three groups of essential hypertensive patients, as previously reported in hypertension.<sup>10</sup>

The relationship between the RAAS and AM has been well demonstrated. *In vitro* studies, using mouse juxtaglomerular cells, indicate a direct renin-stimulating action of AM<sup>21</sup> and AM has also been shown to inhibit angiotensin II (Ang II)-induced secretion of aldosterone from dispersed rat adrenal zona glomerulosa cells.<sup>14,22</sup> The short-term infusion of AM decreased plasma aldosterone

**Figure 2** Correlation between plasma renin activity (PRA) and adrenomedullin (AM) levels in hypertensive patients.



in patients with congestive heart failure<sup>23</sup> and PRA levels rose during high-dose AM infusion in essential hypertensive patients.<sup>24</sup> Thus, it is interesting to speculate that AM may play a compensatory role in the pathophysiology of high-renin hypertension, in part through antagonism of the vasopressor action of the RAAS. In addition, Charles and co-workers<sup>25</sup> reported that infusion of AM in conscious sheep inhibited the pressor effects of infused Ang II, with no such effect on noradrenaline. These data support the possibility that there is a specific interaction between AM and the RAAS.

Another possible explanation for elevation of plasma AM in patients with high-renin essential hypertension is the increased production of AM by an alteration in body fluid volume. In fact, in these hypertensive patients we revealed high urinary sodium excretion compared with other hypertensive groups. AM has a diuretic effect<sup>7</sup> that helps to control fluid and electrolyte balance. Petrie *et al.*<sup>26</sup> have demonstrated, in nine healthy subjects, that systemic administration of AM (with small increments in plasma AM concentration) augments the increase in plasma atrial natriuretic peptide (ANP) (an index of body fluid volume) concentration induced by intravenous infusion of Ang II. Nagaya *et al.*<sup>23</sup> showed that intravenous infusion of AM caused significant increases of urinary sodium excretion in seven patients with congestive heart failure.

In conclusion, we have found that, in essential hypertensive patients, plasma AM levels were increased only in high-renin patients, suggesting a role of AM in this particular form of human hypertension. Additional studies are required to further explore the potential interaction between the RAAS system and AM in hypertension.

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