Elevated plasma levels of human adrenomedullin in cardiovascular, respiratory, hepatic and renal disorders

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INTRODUCTION

Human adrenomedullin (AM) is a 52-amino acid peptide which was first isolated from phaeochromocytoma and adrenal medulla [1]. AM shows partial sequence similarity with the potent vasodilator, calcitonin gene-related peptide (CGRP) [1, 2]. AM-like immunoreactivity is also found in the heart, lungs and kidneys, as well as in plasma [3], which suggests that AM may be a circulating hormone. The physiological role of AM is unclear at present. Intravenous injection of AM in anaesthetized rats lowered systemic vascular resistance and blood pressure [4], AM induces vasorelaxation by at least two mechanisms: a direct action on vascular smooth muscle to increase intracellular cAMP [5] and stimulation of the release of nitric oxide from endothelial cells [6, 7]. The latter pathway is thought to involve the activation of phospholipase C and inositol 1,4,5-trisphosphate, and an increase in intracellular Ca2+ [7].

Plasma AM levels are elevated in certain disease states. In patients with essential hypertension, plasma AM is increased compared with normotensive control subjects and the degree of elevation correlates with the WHO stage of hypertension [8]. In congestive heart failure, plasma AM is raised and there is a weak inverse correlation with ejection fraction [9–11]. In renal failure, plasma AM has also been reported to be increased, and plasma AM correlates with plasma creatinine [8].

Apart from its probable systemic role, AM may also be a paracrine factor because endothelial and smooth muscle cells synthesize AM as well as express the receptors [5, 12]. AM immunoreactivity is present in the brain, consistent with a role as a neurotransmitter [13]. Central effects of AM include raising systemic blood pressure [14], inhibiting water drinking [15] and inhibiting adrenocorticotropic hormone secretion [16].

The objective of the present study is to investigate the pathophysiological role of AM as a circulating hormone, in particular, to confirm reports of elevated plasma AM in hypertension, congestive heart failure and renal failure, and to investigate the plasma level of AM in ascites due to hepatic cirrhosis, and in hypoxia due to chronic obstructive pulmonary disease.

METHODS

Fifty-eight male subjects (age ± SD, 60.3 ± 15.8 years) were recruited into the study. All subjects were ethnically Chinese. They were inpatients with the following conditions: essential hypertension uncomplicated by cardiac or renal failure (n = 8; mean age 63.9 years, range 41–77 years; mean blood pressure ± SD 195/103 ± 29/13 mmHg), congestive heart failure (n = 12; mean age...
67.5 years, range 42–85 years; New York Heart Association Class 3–4), hepatic cirrhosis with ascites (n = 10; mean age 59.5 years, range 40–80 years; mean albumin ± SD 30 ± 4.8 g/l), chronic renal failure (n = 12; mean age 58.5 years, range 45–76 years; mean plasma creatinine ± SD 1031 ± 373 mol/l), chronic obstructive pulmonary disease with hypoxia but without clinical signs of pulmonary hypertension (n = 4; mean age 76.8 years, range 68–92 years; mean PaO$_2$ 9.0 kPa, range 8.4–9.4 kPa; mean PaCO$_2$ 5.8 kPa, range 4.9–7.1 kPa) and 12 normal control subjects (mean age 48.7 years, range 22–84 years). Venous blood (10 ml) was obtained, with consent, from an antecubital vein and placed in EDTA tubes on ice. Samples were promptly centrifuged and the plasma stored at -40°C until the time of assay.

Plasma immunoreactivity of AM was measured with a radioimmunoassay kit (Peninsula Laboratories, Belmont, CA, U.S.A.). Briefly, 3 ml plasma samples were acidified with 0.75 ml of 2 mol/l HCl and centrifuged for 10 min at 1500 g. The supernatants were loaded onto Sep-Pak C18 cartridges (Waters Associate, Milford, MA, U.S.A.) which had been activated with 100% methanol and double-distilled deionized water. The cartridges were then washed twice with 5 ml of 0.1% trifluoroacetic acid (TFA) and eluted with 60% acetonitrile in 0.1% TFA. The eluates were dried under vacuum overnight and resuspended in 250 µl of radioimmunoassay buffer. One-hundred microlitres of standard AM or assay sample were incubated overnight at 4°C with 100 µl of rabbit anti-AM antiserum (0% crossreactivity with AM[13-52], rat AM, amylin, CGRP, endothelin-1, atrial natriuretic peptide[1-28], brain natriuretic peptide-32 or C-natriuretic peptide-22). One-hundred microlitres of ¹²⁵I-AM (18 000 c.p.m.) was added to each tube and incubated for a further 24 h. Antibody-bound AM was precipitated using a goat anti-rabbit antiserum and counted in a Gamma counter. A standard curve was constructed using serial dilutions of freshly reconstituted synthetic human AM (Fig. 1). The detection limit (2 SD from zero) was 1 pg/tube or 0.14 pmol/l AM in plasma. All samples, including those from control subjects, were well above the limit of detection. The IC$_{50}$ was 28.7 pg/tube. The intra-assay coefficient of variation was 7%. All samples were assayed together to avoid interassay variability. The recovery of AM after extraction was determined by adding labelled AM to plasma before extraction and was 72%. All values are quoted without correction for extraction efficiency.

Data were analysed using a statistical software programme (SPSS for Windows; SPSS Inc., Chicago, U.S.A.). Plasma levels of AM in disease states were compared with normal controls using analysis of variance to determine if there were any significant differences among the groups, and the least-significant difference (protected t) tests to determine if the AM level in each disease state differed significantly from control. The latter test was used instead of unpaired t-test to avoid type 1 error arising from multiple comparisons. P < 0.05 was considered significant. Non-parametric statistics were also applied because no assumption of normal distribution needed to be made. Hence, Kruskal-Wallis test was applied to AM levels of the groups. However, there is no commonly adopted non-parametric procedure for multiple comparisons, so the plasma AM level in each disease state was compared with normal control using Mann–Whitney U-test. For each test, P < 0.01 was considered significant because there were five comparisons.

RESULTS

In normal subjects, the mean plasma immunoreactivity of AM was 7.8 pmol/l [confidence interval (CI): 4.6–10.9] (Fig. 2).

In patients, the mean plasma levels of AM were 16.3 pmol/l (CI: 11.9–20.7) (essential hypertension), 17.5 pmol/l (CI: 11.2–23.7) (congestive heart failure), 15.5 pmol/l (CI: 11.1–19.8) (cirrhosis with ascites), 17.7 pmol/l (CI: 12.2–23.1) (renal failure) and 20.0 pmol/l (CI: 15.2–24.9) (chronic obstructive pulmonary disease (COPD) with hypoxia. Columns and error bars represent mean values and standard errors respectively. Statistical significance: *P < 0.05 compared with control, least-significant difference (protected t) test and P < 0.01 compared with control, Mann–Whitney U-test.
pulmonary disease with hypoxia). All were raised compared with controls ($P<0.05$, least-significant difference test; $P<0.01$, Mann–Whitney U-test).

The plasma levels of AM in patients with cirrhosis did not correlate significantly with serum albumin ($r=-0.12$, not significant) or bilirubin ($r=-0.22$, not significant). In patients with renal failure, there was no correlation between plasma AM and creatinine ($r=0.24$, not significant). Plasma AM levels did not correlate significantly with age in normal subjects ($r=-0.04$, not significant) nor overall ($r=0.10$, not significant).

**DISCUSSION**

Our radioimmunoassay for AM was sensitive and sufficiently precise to demonstrate changes in plasma levels of this peptide in various disease states, confirming previous reports [8–11]. The approximately two-fold rises in the mean plasma AM level in essential hypertension, heart failure and renal failure compared with controls observed in our study were similar to the changes in AM reported by other groups [8–11] although the absolute values differed, probably due to differences in extraction efficiency and antisera.

The physiological and pathophysiological effects of AM in man are not yet known. In animals, AM lowers systemic vascular resistance, and is natriuretic and diuretic [17]. Such properties resemble those of atrial natriuretic peptide. Interestingly, plasma concentrations of AM and atrial natriuretic peptide are correlated [8]. It is tempting to speculate that AM may also be part of the neuroendocrine response in congestive heart failure, renal failure and other volume overload states. The present study confirmed previous reports of elevation of plasma AM levels in congestive heart failure and renal failure [8–11]. We report for the first time, in another condition characterized by oedema, hepatic cirrhosis, that AM levels are raised. However, the rise in AM did not appear to be related to serum bilirubin or albumin. AM immunoreactivity is present in the liver which also contains AM binding sites [18]. This suggests that AM may play a role in controlling hepatic circulation. Further research is needed to clarify the role of AM in the liver and its relationship to portal hypertension.

The present study confirmed the results of a previous study which showed that plasma AM levels are higher in hypertensive patients [8]. Since the patients have been treated for hypertension, we did not attempt to correlate blood pressure with AM levels. In our study, the patients were uncomplicated by clinically overt heart failure or renal failure. Although mild degrees of heart failure or renal failure cannot be excluded without echocardiography or measurement of creatinine clearance, the patients who were classified as having congestive heart failure and renal failure in our study and had elevated AM levels had clinically overt disease. As AM lowers peripheral vascular resistance and blood pressure, the increase in plasma levels of AM may be a homoeostatic response to raised blood pressure. On the other hand, if the production, secretion or signal transduction of AM is defective, it is conceivable that hypertension may be aggravated. AM and its receptors may therefore be candidate genes for essential hypertension.

AM is secreted in stress; exercise [19] and glucocorticoids [20] both stimulate the secretion of AM. Indeed, AM may be co-secreted with catecholamines from adrenal medullary cells [21]. While this may be a useful pathophysiological response, in endotoxic shock, interleukin-1, tumour necrosis factor and lipopolysaccharide stimulate the production of AM and may contribute to cause hypotension [22]. The development of a pharmacological agonist of AM may be useful as an antihypertensive agent, while an antagonist may be beneficial in hypotensive shock.

Surprisingly, the source of AM in the circulation has not been conclusively shown [23]. The adrenal medulla is almost certainly not the major source of AM in the bloodstream and contributions from a variety of sites are likely. The ubiquitous production of AM suggests that it may have a local vasodilatory role. It is noteworthy that the vasodilatory effects of AM are most pronounced in organs where it is expressed, e.g. lung, heart, kidney and adrenal glands, but not skin or skeletal muscle [24].

AM may have a local role in the kidneys. AM is produced in renal glomerular and tubular cells [25]. At the same time, AM causes renal vasodilatation, natriuresis and diuresis when infused intrarenally in the dog [25]. Thus, AM found in the urine may come from plasma or be produced within the kidney. AM levels do not change with haemodialysis, suggesting that it may be cleared by non-renal mechanisms [26]. Hence, the high levels observed in renal failure may not be simply due to decreased renal clearance [5].

AM may have a special role in the lungs. Binding studies showed the presence of high-density specific binding sites in the lungs which differed from the binding sites in the heart [27]. AM dilates the pulmonary vascular bed, seemingly more potently than CGRP [28]. Surprisingly, AM-mediated pulmonary vasodilatation appears to be independent of CGRP and nitric oxide [29]. This may be explained by the recent discovery of a novel G-protein-coupled AM receptor, 395 amino acid residues in size, in the lung [30]. Plasma AM levels are raised in experimental pulmonary hypertension [31]; its secretion may be augmented to ameliorate the effects of pulmonary hypertension. Although our patients with chronic obstructive pulmonary disease did not have clinical signs of pulmonary hypertension (e.g. parasternal heave, loud $P_2$, right-sided cardiac murmurs, raised jugular venous pressure, pulsatile hepatomegaly or peripheral oedema),
pulmonary arterial pressure could still have been raised, which would be detected with invasive pressure measurements or echocardiography if these had been performed.

AM is a potent bronchodilator [32]. Since it also inhibits the secretion of the interleukin cytokine-induced neutrophil chemoattractant (CINC) from alveolar macrophages [33], AM may have a role in suppressing inflammation of the airways, as in asthma or bronchitis. Thus, high levels of AM in hypoxia may be a pathophysiological response to counter hypoxic vasoconstriction and dilate the airways. Our finding of elevated AM in hypoxic patients with chronic airway obstruction, although preliminary, merits further investigation.

Drugs could have altered the levels of AM in the patient groups. However, the drug regimes patients received were extremely heterogeneous. For instance, hypertensive patients received calcium channel blockers and β-blockers. Patients with cardiac or respiratory failure did not receive β-blockers. Patients with heart failure or ascites had been given loop diuretics. The former received angiotensin-convertase enzyme inhibitors in addition. Patients with chronic obstructive pulmonary disease received inhaled β-agonists for bronchodilatation.

In conclusion, plasma levels of AM are elevated in conditions characterized by fluid overload, congestive heart failure, chronic renal failure and cirrhosis with ascites; and also in essential hypertension and chronic airway obstruction with hypoxia. Further studies are needed to define the role of AM in these diseases.

ACKNOWLEDGMENTS


REFERENCES


