Increased plasma levels of adrenomedullin in patients with pulmonary hypertension

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ABSTRACT

Adrenomedullin, a potent hypotensive peptide, reduces blood pressure and pulmonary vascular resistance, and increases pulmonary blood flow. The mRNA for adrenomedullin and its receptor is highly expressed in the lung, suggesting a regulatory role for adrenomedullin in the pulmonary circulation. To investigate the clinical significance of adrenomedullin in patients with pulmonary hypertension, we studied the relationship between plasma levels of adrenomedullin and pulmonary haemodynamics. Venous, arterial and pulmonary arterial blood samples were obtained during cardiac catheterization and plasma levels of adrenomedullin were measured by specific radioimmunoassay in 33 consecutive patients with severe pulmonary hypertension (12 cases of primary pulmonary hypertension, 21 with chronic thromboembolic pulmonary hypertension; age 49±16 years, mean pulmonary arterial pressure 50±15 mmHg). In addition, plasma levels of adrenomedullin were measured before and after acute nitric oxide inhalation. The changes in plasma adrenomedullin during the follow-up period of 10.3±4.3 months were also evaluated (n=5). Sixty-two healthy subjects served as the control group. Adrenomedullin was measured in an antecubital vein in the controls. Plasma levels of adrenomedullin were significantly higher in the patients with pulmonary hypertension than in the control subjects (10.1±8.7 versus 4.9±1.1 pmol/l, P<0.01). Plasma levels of adrenomedullin, expressed as their natural logarithm, were significantly correlated with mean right atrial pressure (r=0.71, P<0.01), stroke volume (r=−0.63, P<0.01), total pulmonary resistance (r=0.60, P<0.01), mean pulmonary arterial pressure (r=0.37, P<0.05), and the natural logarithm of plasma atrial natriuretic peptide (r=0.63, P<0.01). Plasma levels of adrenomedullin did not change significantly after nitric oxide inhalation, but significantly increased in association with the elevation of the total pulmonary resistance during the long-term follow-up period. These results suggest that plasma levels of adrenomedullin increase in proportion to the extent of pulmonary hypertension.

INTRODUCTION

Adrenomedullin is a hypotensive peptide that was discovered in human phaeochromocytoma by monitoring platelet cAMP activity in the rat. Adrenomedullin consists of 52 amino acids with an intramolecular disulphide bond, and has some similarity to calcitonin gene-related peptide [1]. It has been demonstrated that...
vascular smooth muscle cells possess specific adrenomedullin receptors that are functionally coupled to adenylate cyclase [2,3]. It has also been demonstrated that adrenomedullin is actively produced and secreted by vascular endothelial and smooth muscle cells [4–6]. More recently, it has been demonstrated that in human peripheral vessels, an infusion of adrenomedullin causes the rapid and long-lasting vasoconstriction of skeletal muscle arteries [7]. Moreover, Lainchbury et al. [8] have demonstrated that an intravenous infusion of adrenomedullin significantly reduces blood pressure with an approximate 1.5-fold increase of venous adrenomedullin levels in normal subjects. These findings indicate that adrenomedullin may be involved in the regulation of vascular tone.

In experimental studies, adrenomedullin preferentially reduces the pulmonary arterial pressure through the decrease of pulmonary vascular resistance [9–12]. Owji et al. [13] reported that numerous adrenomedullin specific binding sites exist in the lung. A recent report has confirmed that finding by showing that adrenomedullin receptor mRNA is highly expressed in the rat lung [14]. Adrenomedullin mRNA is known to be highly expressed in the lung [15]. These observations indicate that adrenomedullin may regulate the pulmonary vascular tone in a paracrine and/or autocrine manner.

Although plasma levels of adrenomedullin are elevated in patients with pulmonary hypertension (PH) [16] and in animal models with experimental PH induced by monocrotaline [17], the pathophysiological significance of adrenomedullin in PH remains unknown. To investigate the clinical significance of adrenomedullin in PH, we studied the relationship between plasma levels of adrenomedullin and pulmonary haemodynamics in patients with severe PH.

**METHODS**

**Subjects**

We studied 33 consecutive patients with PH (15 men and 18 women; 49 ± 16 years of age (mean ± S.D.)). Of these patients, 12 had primary pulmonary hypertension (PPH) and 21 had chronic pulmonary thromboembolism (CPTE). PH was defined as a mean pulmonary arterial pressure (mPAP) in excess of 25 mmHg at rest [18]. PPH was defined as PH unexplained by any secondary cause, based on the criteria set by the National Institutes of Health registry [18]. CPTE was identified by radio-nuclide perfusion lung scans and pulmonary angiography [19,20]. All of the patients were in a clinically stable condition during this study protocol. Patients with renal failure (serum creatinine level > 195 μmol/l) or a history of renal disease were excluded. Sixty-two healthy subjects (35 men and 27 women; 51 ± 10 years of age) served as the control group. Informed consent was obtained from all

| Table 1 Clinical characteristics of the patients with pulmonary hypertension |
|-----------------------------|-----------------------------|
| Patients with PH            | (n = 33)                    |
| Age (years)                 | 49 ± 16                     |
| Sex (M/F)                   | 15/18                       |
| NYHA class (n, %)           |                             |
| I                          | 14 (42)                     |
| II                         | 17 (52)                     |
| III                        | 2 (6)                       |
| Serum creatinine (μmol/l)   | 80 ± 29                     |
| Body surface area (m²)      | 1.56 ± 0.16                 |
| Medication (n, %)           |                             |
| warfarin                    | 28 (85)                     |
| diuretics                   | 16 (48)                     |
| digitalis                   | 10 (30)                     |
| vasodilators                | 17 (51)                     |

Data are presented as number (%) of patients or as means ± S.D. Abbreviations: NYHA, New York Heart Association; PH, pulmonary hypertension.

patients and control subjects. The study protocol was approved by the ethics committee of the National Cardiovascular Center. Table 1 summarizes the clinical characteristics of the study patients. All of the patients were being treated for their disease; the agents they were taking are listed in Table 1. Additional therapy for PH and right ventricular failure was prescribed and administered by the attending physician.

**Haemodynamic study**

All patients underwent Swan–Ganz catheterization, and the following haemodynamic indices were measured: heart rate (beats/min), mPAP (mmHg) and mean right atrial pressure (mRAP; mmHg). Cardiac output (l/min) was determined by the Fick method. Stroke volume (SV; ml) was calculated by dividing cardiac output by heart rate. Total pulmonary resistance (TPR; dyn s · cm⁻⁵) was calculated by dividing mPAP by cardiac output. The mean haemodynamic variables of all subjects are presented in Table 2.

**Blood sampling**

Blood samples were withdrawn from the pulmonary artery, the femoral artery and the femoral vein during cardiac catheterization which was performed with the patient in the supine position after an overnight fast. The blood samples were immediately transferred into chilled glass tubes containing disodium–EDTA (1 mg/ml) and aprotinin (500 units/ml). The samples were promptly centrifuged at 4 °C and the plasma frozen and stored at −80 °C until assayed. Data analyses were carried out on the samples from the main pulmonary artery unless otherwise stated. Blood samples from the control subjects were obtained through the antecubital vein.
Effects of acute and chronic haemodynamic changes on plasma levels of adrenomedullin

NO was inhaled by 26 of the 33 patients with PH. NO gas (2000 p.p.m. in nitrogen gas) was mixed with air using an originally assembled apparatus that controlled the gas flow with computerized mass flow meters [21]. The mixture was then introduced into a circuit consisting of large-bore tubing and a continuous positive airway pressure mask. After a complete baseline haemodynamic evaluation and blood sampling for the measurement of plasma adrenomedullin and atrial natriuretic peptide (ANP), NO at 80 p.p.m. was inhaled by the patient for 10 min. Subsequently, the haemodynamic evaluation and blood sampling were repeated. Of these patients, we defined eight patients as responders, in whom TPR decreased by more than 20% compared with the basal level [22].

To assess the effects of chronic haemodynamic changes on plasma levels of adrenomedullin, we measured plasma adrenomedullin levels in five patients who had progressive PH, ultimately producing right ventricular failure. The mean follow-up period was 10.3 ± 4.3 months. Right heart catheterization was also performed.

### Assay for plasma adrenomedullin concentration

The stored plasma samples were extracted before the radioimmunoassay. Sep-Pak C18 cartridges (Millipore-Waters, Milford, MA, U.S.A.) were prewashed sequentially with 5 ml each of chloroform, methanol, 50% acetonitrile containing 0.1% trifluoroacetic acid (TFA), 0.1% TFA and saline. Plasma (2 ml) was acidified with 24 µl of 1 mol/l HCl, diluted with 2 ml of saline, and then loaded on to a Sep-Pak C18 cartridge. After being washed with 5 ml each of saline, 0.1% TFA and 20% acetonitrile containing 0.1% TFA, the absorbed materials were eluted with 4 ml of 50% acetonitrile containing 0.1% TFA. The eluate was then lyophilized. The lyophilized material was dissolved in radioimmunoassay buffer, and the clear solution was radioimmunoassayed. The radioimmunoassay for adrenomedullin was performed with anti-(human adrenomedullin) rabbit polyclonal antibody (AM-M-2), using a method reported previously [23–25].

### Assay for ANP

A radioimunoassay for ANP (pmol/l) was also performed using plasma from the pulmonary artery (Shiono RIA ANP assay kit, Shionogi Co., Osaka, Japan).

### Statistics

Because of a skewed distribution of data, hormone plasma levels were logarithmically transformed for statistical analysis. However, data are expressed as non-transformed mean values ± S.D. unless otherwise indicated. Comparisons of haemodynamic data between PPH and CPTE were performed with the Student t-test for unpaired values. Comparisons of the mean values among the three groups were performed by one-way ANOVA. Correlation coefficients were calculated by linear regression analysis. The effects of acute and chronic haemodynamic changes on plasma levels of adrenomedullin and ANP were analysed with the Student paired t-test. A value of $P < 0.05$ was considered significant.

### RESULTS

#### Plasma levels of adrenomedullin

Plasma levels of adrenomedullin measured from the pulmonary artery of the patients with PH ($n = 33$) were significantly greater than those from the antecubital vein of the control subjects ($n = 62$) (10.1 ± 8.7 versus 4.9 ± 1.1 pmol/l, $P < 0.01$). Plasma levels of adrenomedullin did not significantly differ between the 12 patients with PPH (10.7 ± 10.6 pmol/l) and the 21 patients with CPTE (9.7 ± 7.7 pmol/l). Within the entire group of patients with PH, plasma levels of adrenomedullin measured from the femoral vein and pulmonary artery did not differ significantly, whereas those from the femoral artery were significantly lower than those from the pulmonary artery ($P < 0.05$) (femoral vein, 9.7 ± 10.1; pulmonary vein, 10.7 ± 10.6 pmol/l).

## Table 2 Haemodynamics of patients with pulmonary hypertension

Data are presented as means ± S.D. Abbreviations: CPTE, chronic pulmonary thromboembolism; mPAP, mean pulmonary arterial pressure; mRAP, mean right atrial pressure; mSAP, mean systemic arterial pressure; PH, pulmonary hypertension; PPH, primary pulmonary hypertension; SV, stroke volume; TPR, total pulmonary resistance. Statistical significance: * $P < 0.01$ and † $P < 0.05$ compared with CPTE.

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (beats/min)</th>
<th>mSAP (mmHg)</th>
<th>mPAP (mmHg)</th>
<th>mRAP (mmHg)</th>
<th>SV (ml)</th>
<th>TPR (dyn · s · cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH</td>
<td>74 ± 14</td>
<td>87 ± 12</td>
<td>50 ± 15</td>
<td>7.4 ± 5.2</td>
<td>47 ± 18</td>
<td>1359 ± 701</td>
</tr>
<tr>
<td>PPH</td>
<td>77 ± 15</td>
<td>82 ± 10</td>
<td>60 ± 10*</td>
<td>9.2 ± 5.0</td>
<td>41 ± 17</td>
<td>1783 ± 725†</td>
</tr>
<tr>
<td>CPTE</td>
<td>73 ± 13</td>
<td>89 ± 13</td>
<td>45 ± 14</td>
<td>6.4 ± 5.1</td>
<td>51 ± 18</td>
<td>1116 ± 573</td>
</tr>
</tbody>
</table>

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Figure 1  Correlations between plasma levels of adrenomedullin measured from pulmonary artery and mean right atrial pressure (mRAP), stroke volume (SV), total pulmonary resistance (TPR), mean pulmonary atrial pressure (mPAP) and plasma levels of atrial natriuretic peptide (ANP) in 12 patients with primary pulmonary hypertension (○) and 21 patients with chronic pulmonary thromboembolism (●). Plasma adrenomedullin and ANP concentrations were logarithmically transformed (ln).

Correlations of plasma levels of adrenomedullin with cardiac catheterization data and ANP
The natural logarithm of plasma levels of adrenomedullin measured from the pulmonary artery of the 33 patients with PH significantly correlated with mRAP ($r = 0.71, P < 0.01$), SV ($r = -0.63, P < 0.01$), TPR ($r = 0.60, P < 0.01$), mPAP ($r = 0.37, P < 0.05$), and with the natural logarithm of plasma ANP ($r = 0.63, P < 0.01$) (Figure 1).

Effects of acute and chronic haemodynamic changes on plasma levels of adrenomedullin
After NO inhalation, TPR and plasma levels of ANP significantly decreased in the eight responders [TPR, SV, TPR, mPAP].
1416 ± 760 to 984 ± 384 dyn·s·cm⁻² (P < 0.05), ANP, 36 ± 31 to 18 ± 17 pmol/l (P < 0.05), but plasma levels of adrenomedullin did not change markedly (6.3 ± 1.5 to 6.1 ± 1.5 pmol/l, P not significant). In the 18 non-responders, plasma ANP and adrenomedullin did not change significantly with NO inhalation [ANP, 62 ± 56 to 53 ± 42 pmol/l (P not significant); adrenomedullin, 10.6 ± 10.1 to 12.1 ± 12.6 pmol/l (P not significant)]. In the long-term follow-up study, plasma levels of adrenomedullin increased (7.1 ± 1.3 to 16.8 ± 6.9 pmol/l, P < 0.05) in association with the elevation of TPR (1280 ± 424 to 2072 ± 504 dyn·s·cm⁻², P < 0.05). Plasma levels of ANP and mRAP tended to increase [ANP, 42 ± 37 to 129 ± 124 pmol/l (P not significant); mRAP, 5.2 ± 3.3 to 12.8 ± 5.1 mmHg (P = 0.06)].

**DISCUSSION**

In this study, we demonstrated that (i) plasma levels of adrenomedullin were elevated in patients with severe PH and (ii) the circulating adrenomedullin in those patients was partly metabolized in the pulmonary circulation. We also demonstrated that (iii) there were significant correlations between plasma levels of adrenomedullin and mRAP, SV, TPR, mPAP and plasma levels of ANP. Finally, we demonstrated that (iv) plasma levels of adrenomedullin increased in association with the elevation of TPR during the long-term follow-up period.

Plasma levels of adrenomedullin have been reported to be increased in adult patients with essential hypertension, chronic renal failure, acute myocardial infarction and congestive heart failure in association with the clinical severity [25–29]. Plasma levels of adrenomedullin are also increased in rats with monocrotaline-induced PH [17]. In humans, plasma levels of adrenomedullin have recently been shown to be increased in infants with PH associated with congenital heart defects [16]. In the present study, we first showed increased plasma levels of adrenomedullin in patients with severe PH and significant correlations between plasma levels of adrenomedullin and pulmonary haemodynamics in adult patients with PH.

There is a debate as to which organs produce circulating adrenomedullin. Human adrenomedullin mRNA has been shown to be highly expressed not only in the adrenal medulla of patients with pheochromocytoma, but also in the adrenal medulla, kidney, lung, atrium and ventricle of normal subjects [15]. It has been shown that adrenomedullin is actively produced and secreted by the vascular endothelial and smooth muscle cells, suggesting that the vascular wall may be a major site for the production of circulating adrenomedullin [4–6]. These findings suggest that the production and secretion of adrenomedullin may be promoted in these organs during severe PH.

Previous studies have shown that adrenomedullin mRNA and its receptor mRNA are highly expressed in the lung [14,15], suggesting that elevated adrenomedullin may participate in a paracrine and/or autocrine manner as one of the regulators of vascular tone in the pulmonary circulation. Our present finding that plasma levels of adrenomedullin in the pulmonary artery significantly exceeded those in the femoral artery is consistent with the presence of abundant and specific binding sites for adrenomedullin in the lung and with the finding that the pulmonary circulation is the site of adrenomedullin clearance [30].

The stimulating factors for adrenomedullin secretion during severe PH remain unclear. It has been reported that plasma levels of adrenomedullin are significantly correlated with those of noradrenaline and ANP [27], which suggests that an increased plasma volume and an activated sympathetic nervous system may be related to the synthesis or secretion of adrenomedullin. The present findings of significant relationships between plasma adrenomedullin levels and plasma ANP and mRAP in patients with PH are consistent with the idea that an increased plasma volume is a stimulus for adrenomedullin secretion. Moreover, the increase in plasma adrenomedullin and ANP concomitant with the progression of PH during the long-term follow-up period raises the possibility that adrenomedullin may reflect the reduction in right ventricular function.

The functional roles of elevated plasma levels of adrenomedullin in PH remain unknown. Synthetic human adrenomedullin exhibits significant vasodilator activity in the pulmonary vascular bed of the cat and rat when the vascular tone is elevated under constant flow conditions [9–12]. Recently, Nakamura et al. [7] demonstrated that the vasodilator potency of adrenomedullin was approximately 10- and 200-fold greater than sodium nitroprusside and acetylcholine respectively when infused into the brachial artery in humans. In addition, Lainchbury et al. [8] recently reported that an intravenous infusion of adrenomedullin at 8 ng·min⁻¹·kg⁻¹ significantly decreased blood pressure with a 50% increase in plasma adrenomedullin levels in human subjects, suggesting that the threshold for the biological activity of adrenomedullin in humans is lower for dilating vessels, and is evident in plasma concentrations seen in cardiovascular diseases. In the present study, we observed a significant correlation between plasma levels of adrenomedullin and TPR. In view of the strong vasodilating effect of adrenomedullin, the marked elevation of adrenomedullin in patients with PH may represent a compensatory mechanism under conditions of increased pulmonary vascular resistance.

Recent studies have shown that adrenomedullin inhibits the proliferation of cultured rat vascular smooth muscle cells stimulated with fetal calf serum and platelet-derived growth factor, suggesting that adrenomedullin
acts as a local antiproliferation factor to inhibit the development of atherosclerosis [31]. PH is characterized by increased vascular tone and an abnormal proliferation of smooth muscle cells in the pulmonary vasculature. Therefore, it is suggested that increased plasma adrenomedullin observed during PH may be involved in its pathophysiology by also serving as an antiproliferation factor.

In this study, the adrenomedullin concentration did not change significantly after NO inhalation. In contrast, the ANP concentration decreased significantly, in association with the decrease in TPR. This may be attributed to the different modes of synthesis and secretion of adrenomedullin and ANP. Adrenomedullin is secreted mainly from the vascular wall without being stored, and the amount of secretion depends on its genomic expression [6], whereas ANP is secreted mainly from stored granules in atrial tissue via a regulated pathway. During the long-term follow-up period, adrenomedullin concentration increased concomitantly with the progression of PH. Thus, adrenomedullin may respond to the changes in haemodynamics as a slow regulating factor in the pulmonary circulation.

**Study limitations**

The primary limitation of the present study is the small number of follow-up study patients. Further studies are required to clarify the definite role of adrenomedullin in PH in each basal disease and the effects of medication on plasma adrenomedullin concentration in such patients.

A second limitation is that adrenomedullin was measured in the antecubital vein in the controls, but in the pulmonary artery in the patients with PH. However, we have reported that there was no significant difference in plasma adrenomedullin concentration between the peripheral vein and the pulmonary artery in various types of patients [16,30,32].

**CONCLUSIONS**

The present study demonstrated that plasma levels of adrenomedullin were elevated in patients with severe PH. These patients showed a significant reduction of adrenomedullin concentration in the pulmonary circulation, suggesting that this substance is partly metabolized in the pulmonary circulation. We also observed that plasma levels of adrenomedullin were significantly correlated with right ventricular haemodynamics and those of ANP, and that plasma levels of adrenomedullin increased with the elevation of TPR (and ANP and mRAP). These findings suggest that plasma levels of adrenomedullin increase in proportion to the extent of PH.

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