Elevated plasma levels of adrenomedullin in congenital cyanotic heart disease

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ABSTRACT

Adrenomedullin is a novel hypotensive peptide originally isolated from human pheochromocytoma. Accumulating evidence suggests the possible involvement of adrenomedullin in the physiology of the pulmonary circulation and the pathophysiology of hypoxaemia. The aim of the present study was to investigate the pathophysiological significance of adrenomedullin in hypoxaemia caused by congenital cyanotic heart disease. Subjects were 16 patients with congenital cyanotic heart disease aged 0.8–10 years (Group C) and 12 age-matched control subjects (patients with coronary artery dilatation after Kawasaki disease; Group N). Plasma adrenomedullin concentrations were measured, using radioimmunoassay, in femoral venous, pulmonary arterial and pulmonary venous blood obtained during cardiac catheterization. Plasma adrenomedullin concentrations in Group C were significantly (3-fold) higher than those in Group N at all sampling sites. In Group C, plasma adrenomedullin concentrations in pulmonary venous blood were significantly lower than those in pulmonary arterial blood. Pulmonary uptake of adrenomedullin in Group C was significantly greater than that in Group N. Patients with congenital cyanotic heart disease showed elevated plasma adrenomedullin concentrations and an increased uptake of adrenomedullin in the pulmonary circulation, which may act to dilate pulmonary vessels and increase pulmonary blood flow to alleviate hypoxaemia. Intrinsically increased adrenomedullin levels may function as a compensatory mechanism for hypoxaemia in congenital cyanotic heart disease.

INTRODUCTION

Adrenomedullin is a novel hypotensive peptide originally isolated from human pheochromocytoma [1]. Adrenomedullin mRNA is strongly expressed in vascular walls (endothelium [2] and vascular smooth muscle cells [3]) and in human lung tissue [4]. Binding sites for adrenomedullin are abundant in rat lung [5], and mRNA for the adrenomedullin receptor is strongly expressed in lung tissue [6]. Several reports have shown that adrenomedullin decreases the pulmonary vascular resistance in a dose-dependent manner [7–9]. Plasma levels of adrenomedullin are elevated in patients with pulmonary hypertension [10–12] and in patients with hypoxaemia due to lung disease [13,14]. In addition, a recent study showed that hypoxia induces adrenomedullin gene expression in human umbilical vein endothelial cells [15]. These observations raise the possibility that adrenomedullin is involved in the physiology of the pulmonary circulation and in the pathophysiology of hypoxaemia.

Patients with congenital cyanotic heart disease show various degrees of hypoxaemia, the severity of which is dependent on the extent of pulmonary blood flow reduction due to right-to-left shunt. In the present study,

Key words: adrenomedullin, congenital cyanotic heart disease, hypoxaemia.

Abbreviations: FV, femoral vein; PA, pulmonary artery; PV, pulmonary vein or left ventricle (pulmonary venous).

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in order to investigate the pathophysiological significance of adrenomedullin in hypoxaemia due to congenital cyanotic heart disease, plasma adrenomedullin concentrations were measured by radioimmunoassay in patients with congenital cyanotic heart disease, and compared with those in control subjects. The blood samples were obtained from various sites during clinically indicated cardiac catheterization.

### METHODS

#### Subjects

We studied 16 consecutive patients with congenital cyanotic heart disease [Group C; nine males and seven females; age 0.8–10 years (mean ± S.D. 3.7 ± 2.5 years)]. Their diagnoses were as follows: tricuspid atresia with pulmonary stenosis in four patients, tetralogy of Fallot in four patients, univentricular heart with pulmonary stenosis in four patients, common atrioventricular canal with pulmonary stenosis in two patients, and double outlet right ventricle with pulmonary stenosis in two patients. We also studied 12 consecutive patients with coronary artery dilatation after Kawasaki disease, who had neither cyanosis nor heart failure, as control subjects [Group N; seven males and five females; age 0.8–12 years (mean ± S.D. 3.3 ± 3.6 years)]. The mean oxygen saturation of the femoral arterial blood was 79.2 ± 6.7% in Group C and 97.9 ± 1.0% in Group N. All of the patients in the two groups had normal left ventricular systolic function and normal renal function. None of the patients had lung disease or signs or symptoms of heart failure. None of the patients had systemic or pulmonary hypertension. The clinical parameters of the two groups are shown in Table 1.

Informed consent was obtained from the parents of each child for the blood sampling. The protocol of this study was in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and with the guidelines of our institution’s Committee on Human Research.

#### Cardiac catheterization and blood sampling

Cardiac catheterization was performed under heparinization and routine sedation. Pressure measurement was carried out using fluid-filled catheters connected to pressure transducers. Pulmonary and systemic blood flow volumes were determined according to the Fick principle and indexed for body surface area. Oxygen consumption was estimated based on age, sex and heart rate by the method of LaFarge and Miettinen [16]. Blood samples of 3 ml were obtained at the following sites during cardiac catheterization: the femoral vein (FV), the pulmonary artery (PA) and the pulmonary vein or the left ventricle (PV; pulmonary venous blood).

Blood samples were transferred to chilled, siliconized disposable glass tubes containing aprotinin (500 kallikrein inactivator units/ml) and EDTA (1 mg/ml), placed immediately on ice, and centrifuged promptly (1882 g, 15 min, 4 °C). An aliquot of plasma was frozen immediately at −80 °C and thawed only at the time of extraction.

#### Measurement of plasma adrenomedullin concentration

Plasma adrenomedullin concentrations were measured by radioimmunoassay, as described previously [17]. In this assay, the IC$_{50}$ value was 4 fmol/tube and the minimal detectable amount was 0.5 fmol/tube. This assay showed 100% cross-reactivity with the Met sulphoxide form of adrenomedullin. Cross-reactivity with adrenomedullin-(13–52), adrenomedullin-(40–52), adrenomedullin-(1–12) and adrenomedullin-(13–31) was 2%, 0.5%, < 0.01% and < 0.01% respectively. The intra- and inter-assay variations were 5.0% (n = 10) and 8.0% (n = 10) respectively. The extraction of adrenomedullin from plasma (1.5 ml) was performed using Sep-pak C18 cartridges (Millipore Corp.), as described previously [17].

The pulmonary uptake of adrenomedullin was calculated as follows:

\[
\text{Pulmonary uptake of adrenomedullin} = \frac{\text{plasma adrenomedullin concentration in PA} - \text{plasma adrenomedullin concentration in PV}}{\text{pulmonary blood flow index}}
\]

Because pulmonary adrenomedullin release cannot be measured, we cannot determine the precise amount of

#### Table 1 Clinical parameters of the two groups

<table>
<thead>
<tr>
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<th>Group C</th>
<th>Group N</th>
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<tr>
<td>n</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>3.7 ± 2.5</td>
<td>3.3 ± 3.6</td>
</tr>
<tr>
<td>Sex (male:female)</td>
<td>9:7</td>
<td>7:5</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td>90.9 ± 19.5</td>
<td>105.9 ± 14.9</td>
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<tr>
<td>Haematocrit (%)</td>
<td>49.7 ± 5.6</td>
<td>37.5 ± 2.7</td>
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<tr>
<td>Blood urea nitrogen level (mg/dl)</td>
<td>15.6 ± 4.7</td>
<td>14.7 ± 3.9</td>
</tr>
<tr>
<td>Serum creatinine level (mg/dl)</td>
<td>0.33 ± 0.06</td>
<td>0.33 ± 0.13</td>
</tr>
<tr>
<td>Oxygen saturation at femoral artery (%)</td>
<td>79.2 ± 6.7</td>
<td>97.9 ± 1.0*</td>
</tr>
<tr>
<td>Pulmonary artery systolic pressure (mmHg)</td>
<td>16.6 ± 6.7</td>
<td>20.0 ± 4.0</td>
</tr>
<tr>
<td>Aorta systolic pressure (mmHg)</td>
<td>98.3 ± 11.9</td>
<td>93.3 ± 10.1</td>
</tr>
<tr>
<td>Pulmonary blood flow (l·min$^{-1}$·m$^{-2}$)</td>
<td>2.92 ± 0.99</td>
<td>4.24 ± 0.57*</td>
</tr>
<tr>
<td>Systemic blood flow (l·min$^{-1}$·m$^{-2}$)</td>
<td>4.52 ± 1.12</td>
<td>4.24 ± 0.57</td>
</tr>
</tbody>
</table>

In accordance with the Declaration of Helsinki (1989) of the World Medical Association and with the guidelines of our institution’s Committee on Human Research.
adrenomedullin taken up into pulmonary vessels. Therefore the results calculated as described above represent the net pulmonary uptake of adrenomedullin.

**Statistical analysis**

Values are expressed as means ± S.D. Welch’s *t*-test was used to compare mean values of plasma adrenomedullin concentrations and of pulmonary adrenomedullin uptake between the two groups. Student’s *t* test was used to compare mean values of the clinical parameters between the two groups. To analyse the differences in plasma adrenomedullin concentrations among the sampling sites in each group, a paired *t* test with the Bonferroni correction was used. Linear regression analysis was used to determine correlations between the results. Probability values of < 0.05 were considered statistically significant.

**RESULTS**

As shown in Figure 1, plasma adrenomedullin concentrations in Group C were significantly higher than those in Group N at all sampling sites. In Group C, the plasma adrenomedullin concentration at PV was lower than that at PA, though there were no significant differences between FV and PA or between PV and FV. In Group N, the plasma adrenomedullin concentration at PV was lower than that at PA, but there were no significant differences among sampling sites. The plasma adrenomedullin concentrations (fmol/ml) at the sampling sites were as follows: Group C: PV, 7.8 ± 3.2; PA, 8.1 ± 2.6; PV, 6.7 ± 2.7; Group N: FV, 2.4 ± 0.4; PA, 2.4 ± 0.4; PV, 2.2 ± 0.5. The decrement in the plasma adrenomedullin concentration from PA to PV in Group C (1.3 ± 1.2 fmol/ml) was significantly (*P* < 0.001) greater than that in Group N (0.2 ± 0.3 fmol/ml).

Plasma adrenomedullin concentrations showed significant inverse correlations with femoral arterial oxygen saturation (FV, *r* = −0.72, *P* < 0.001; PA, *r* = −0.78, *P* < 0.001; PV, *r* = −0.74, *P* < 0.001) and with pulmonary blood flow index (FV, *r* = −0.64, *P* < 0.005; PA, *r* = −0.57, *P* < 0.005; PV, *r* = −0.56, *P* < 0.005). There was a significant correlation between plasma adrenomedullin concentrations and the haematocrit (FV, *r* = 0.69, *P* < 0.001; PA, *r* = 0.78, *P* < 0.001; PV, *r* = 0.73, *P* < 0.001). None of the other parameters showed significant correlations with plasma adrenomedullin concentrations.

The pulmonary uptake of adrenomedullin was calculated as described in the Methods section. That in Group C (4.2 ± 3.7 pmol·min⁻¹·m⁻²) was significantly (*P* < 0.005) greater than that in Group N (0.7 ± 1.1 pmol·min⁻¹·m⁻²). The pulmonary uptake of adrenomedullin showed a significant correlation with femoral arterial oxygen saturation (*r* = −0.61, *P* < 0.005) and with the haematocrit (*r* = 0.53, *P* < 0.01).

**DISCUSSION**

In the present study, we have demonstrated that plasma adrenomedullin concentrations at FV, PA and PV were elevated 3-fold in patients with congenital cyanotic heart disease, compared with those in control subjects. We also demonstrated a significantly increased uptake of plasma adrenomedullin in the pulmonary circulation in patients with congenital cyanotic heart disease.

Elevated plasma adrenomedullin concentrations have been reported in patients with heart failure [18] or renal failure [19]. In patients with such cardiovascular or renal diseases, an increased circulatory blood volume is thought to be a stimulus for the synthesis or secretion of adrenomedullin. In the present study, it should be noted that the patients with congenital cyanotic heart disease showed an elevation of plasma adrenomedullin concentrations without heart failure, renal failure or an increase in the circulatory blood volume. In addition, the extent of the elevation was comparable with that in patients with severe heart failure [18] or severe renal failure [19]. Elevated plasma adrenomedullin concentrations have been observed in patients with hypoxaemia due to bronchial asthma [13] or chronic lung disease [14]. A recent study showed that the low oxygen tension at physiological levels augments the expression of the adrenomedullin gene in cultured human umbilical vein endothelial cells [15]. These observations suggest that hypoxaemia itself may be a stimulus for the synthesis or the secretion of adrenomedullin, and that vascular endothelial cells may be a major site involved in mediating the increased plasma adrenomedullin levels found in...
patients with congenital cyanotic heart disease. Further studies are necessary to verify this hypothesis.

In the present study, plasma adrenomedullin concentrations decreased significantly on passing through the pulmonary circulation in patients with congenital cyanotic heart disease. In addition, the net pulmonary uptake of adrenomedullin was significantly greater in patients with congenital cyanotic heart disease than in control subjects. It has been shown that adrenomedullin stimulates cAMP formation in vascular smooth muscle cells [20] via its specific receptors [21], and that binding sites for adrenomedullin are abundant in the lung [5]. Adrenomedullin receptors on pulmonary vessels may be more abundant in patients with congenital cyanotic heart disease than in control subjects.

The exact roles of the elevated adrenomedullin levels observed in congenital cyanotic heart disease are unknown. Several reports have shown that adrenomedullin decreases the pulmonary vascular resistance in a dose-dependent manner [7–9]. Recently we found that chronic adrenomedullin infusion into rats significantly reduced pulmonary hypertension and attenuated the medial thickening of the pulmonary artery, with an increase in plasma adrenomedullin at pathophysiological levels [22]. Therefore it can be speculated that elevated plasma adrenomedullin and its increased uptake in the pulmonary circulation dilate pulmonary vessels and increase pulmonary blood flow to alleviate hypoxaemia in patients with congenital cyanotic heart disease. Recently Vijay et al. [23] have suggested that, in patients with congenital heart disease, adrenomedullin may function as a pulmonary circulatory regulator in a peri-operative period. Thus adrenomedullin, either in isolation or in conjunction with other vasoactive substances, may play significant roles in homoeostasis of the pulmonary circulation in congenital heart disease.

In conclusion, patients with congenital cyanotic heart disease showed elevated plasma adrenomedullin levels and an increased uptake of adrenomedullin in the pulmonary circulation, which may act as compensation for hypoxaemia. The intrinsically increased adrenomedullin concentrations may have beneficial roles in patients with hypoxaemia caused by congenital cyanotic heart disease. Further studies are necessary in order to elucidate the actual roles of adrenomedullin in hypoxaemia.

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