Original Article

Pulmonary Vasodilator Response to Adrenomedullin in Patients with Pulmonary Hypertension

Noritoshi NAGAYA, Kunio MIYATAKE, Shingo KYOTANI, Toshio NISHIKIMI*, Norifumi NAKANISHI, and Kenji KANGAWA**

This study sought to investigate pulmonary vasodilator responses to intrapulmonary and intravenous infusion of adrenomedullin (AM) in patients with pulmonary hypertension. In 10 patients with pulmonary hypertension, blood flow velocity in a segmental pulmonary artery was measured using a Doppler flow wire during intrapulmonary infusion of AM, acetylcholine (ACh), and adenosine triphosphate (ATP). The hemodynamic effects of intravenously administered AM (0.05 μg/kg/min) were examined in another 5 patients with primary pulmonary hypertension. Intrapulmonary infusion of AM, ACh or ATP caused a significant dose-dependent increase in blood flow velocity in a segmental pulmonary artery, respectively. The increase in flow velocity with AM at 10⁻⁸ mol/l (41 ± 6% of the baseline value) was comparable to that with ACh at 10⁻⁴ mol/l (39 ± 11%) and that with ATP at 10⁻⁵ mol/l (36 ± 14%), suggesting a strong pulmonary vasodilator activity of AM. Intravenous infusion of AM produced a 41% increase in cardiac index (p < 0.05) and a 30% decrease in pulmonary vascular resistance (p < 0.05) with a 3% reduction in mean pulmonary arterial pressure (p = NS). These results suggest that, on a molar basis, AM may have much more potent pulmonary vasodilator activity than ACh and ATP, and thus may have beneficial hemodynamic effects in patients with pulmonary hypertension. (Hypertens Res 2003; 26 (Suppl): S141–S146)

Key Words: adrenomedullin, pulmonary hypertension, hemodynamics

Introduction

Adrenomedullin (AM) is a potent vasodilator peptide that was originally isolated from human pheochromocytoma (1). Immunoreactive AM has subsequently been detected in plasma and a variety of tissues, including vascular smooth muscle cells, endothelial cells, and lungs (2, 3). It has been reported that there are many specific receptors for AM in the lungs (4). We have shown that plasma AM level increases in proportion to the severity of pulmonary hypertension, and that circulating AM is partially metabolized in the lungs of such patients (5, 6). These findings suggest that AM plays an important role in the regulation of pulmonary vascular tone.

Indeed, experimental studies have recently demonstrated that exogenously administered AM induces pulmonary vasodilation and increases pulmonary blood flow in rats and cats (7–9). Moreover, we have shown that short-term infusion of AM ameliorates pulmonary hypertension secondary to congestive heart failure in rats (10), and that long-term infusion of AM attenuates progressive pulmonary hypertension and medial thickening of the pulmonary arteries in rats treated with monocrotaline (11). In humans, however, little information is available regarding the effects of AM on the pulmonary vasculature in pulmonary hypertension.

Recently, acetylcholine (ACh), which stimulates nitric ox-
ide release in endothelial cells, has been used for assessment of endothelium-dependent pulmonary vasodilator reserve (12). Alternatively, adenosine triphosphate (ATP), which can be degraded to adenosine, has been shown to cause vasodilation partly via activation of adenosine receptor in smooth muscle cells (13, 14). Although AM has been shown to regulate pulmonary vascular tone in part through an endothelium-derived nitric oxide-dependent mechanism in rats (7), the mechanism and potency of the vasorelaxant effects of AM in human pulmonary arteries remain unknown.

Thus, the purposes of this study were 1) to compare the pulmonary vasodilator potency of AM with that of ACh and ATP, by assessing changes in pulmonary blood flow in response to intrapulmonary infusion of each agent using a Doppler flow wire, and 2) to investigate the hemodynamic effects of intravenous infusion of AM in patients with primary pulmonary hypertension.

### Methods

**Study Patients**

The intrapulmonary infusion study included 10 patients with pulmonary hypertension (mean pulmonary arterial pressure ≥25 mmHg, all women; age, 45 ± 5 years). The cause of pulmonary hypertension was primary pulmonary hypertension in 4 patients, chronic thromboembolic pulmonary hypertension in 4, atrial septal defect in 1, and Eisenmenger syndrome with atrial septal defect in 1. Incremental doses of ACh, ATP, and AM were infused into a segmental pulmonary artery to compare the pulmonary vasodilator potency of AM with that of ACh and ATP. Demographic and hemodynamic data of the patients are summarized in Table 1. Another 5 patients with primary pulmonary hypertension (2 men and 3 women; age, 46 ± 7 years) received intravenous infusion of AM. All patients had a thorough evaluation to identify the cause of their pulmonary hypertension according to the protocol of the National Institutes of Health registry on primary pulmonary hypertension (15). Patients with one or both of the following conditions were excluded: 1) chronic renal impairment (serum creatinine level ≥2.0 mg/dl) and 2) systolic blood pressure < 100 mmHg. All cardiovascular drugs were withdrawn at least 24 hours before beginning the study protocol. The study was approved by the ethical committee of the National Cardiovascular Center, and all patients gave written informed consent.

**Preparation of Human AM, ACh, and ATP**

Human AM was obtained from the Peptide Institute Inc. (Minoh, Japan). The homogeneity of human AM was confirmed by reverse-phase, high-performance liquid chromatography and amino acid analysis. Human AM was dissolved in saline with 4% d-mannitol and was sterilized by passage through a 0.22-µm filter (Millipore Co., Bedford, USA). At the time of dispensing, randomly selected vials were submitted for sterility and pyrogen testing. The chemical nature and content of the human AM in vials were verified by high-performance liquid chromatography and radioimmunoassay. All vials were stored frozen at -80°C from the time of dispensing until the time of preparation for administration. ACh (Dai-ichi Pharmaceutical Co., Ltd., Tokyo, Japan) and ATP (Kowa Co., Ltd., Nagoya, Japan) were prepared for each experiment by dilution of a vial.

### Table 1. Patient Characteristics and Maximum Flow Changes in the Intrapulmonary Infusion Study

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Etiology (year)</th>
<th>mPAP (mmHg)</th>
<th>CI (l/min)</th>
<th>PVR (units)</th>
<th>RAP (mmHg)</th>
<th>PCWP (mmHg)</th>
<th>Max flow velocity increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64</td>
<td>CTEPH</td>
<td>37</td>
<td>2.3</td>
<td>9</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>ASD</td>
<td>26</td>
<td>3.6</td>
<td>3</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>CTEPH</td>
<td>51</td>
<td>1.5</td>
<td>17</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>Eisen</td>
<td>42</td>
<td>2.2</td>
<td>12</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>PPH</td>
<td>48</td>
<td>1.7</td>
<td>18</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>CTEPH</td>
<td>26</td>
<td>3.1</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>29</td>
<td>PPH</td>
<td>60</td>
<td>1.6</td>
<td>21</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>56</td>
<td>CTEPH</td>
<td>46</td>
<td>1.8</td>
<td>13</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>PPH</td>
<td>62</td>
<td>1.6</td>
<td>23</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>17</td>
<td>PPH</td>
<td>42</td>
<td>2.0</td>
<td>15</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mean</td>
<td>45</td>
<td></td>
<td>44</td>
<td>2.1</td>
<td>14</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>SEM</td>
<td>5</td>
<td></td>
<td>4</td>
<td>0.2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

mPAP, mean pulmonary arterial pressure; CI, cardiac index; PVR, pulmonary vascular resistance; RAP, right arterial pressure; PCWP, pulmonary capillary wedge pressure; ACh, acetylcholine; ATP, adenosine triphosphate; AM, adrenomedullin; CTEPH, thromboembolic pulmonary hypertension; ASD, atrial septal defect; Eisen, Eisenmenger syndrome with atrial septal defect; PPH, primary pulmonary hypertension.
Intrapulmonary Infusion

A 7.5 French Swan-Ganz catheter (TOO21H-7.5F; Baxter Co., Irvine, USA) was positioned in a pulmonary artery through a jugular vein. A 22-gauge cannula was inserted into a radial artery for hemodynamic measurements and blood sampling. After completion of hemodynamic measurements at baseline, an 8 French guiding catheter was positioned in a segmental pulmonary artery through a jugular vein. To determine the diameter of the pulmonary artery, an intravascular ultrasound catheter (Sonicate-8F 20 MHz; Boston Scientific Co., Boston, USA) was advanced through the guiding catheter. Then, a Doppler flow wire (1400-0.014 inch; EndoSonics Co., Rancho Cordova, USA) connected to a 5500 Flowmap (EndoSonics Co.) was positioned in a segmental pulmonary artery with a diameter of 3 to 5 mm as determined by intravascular ultrasound. Each drug was infused through an end-hole catheter using a Y-connector, which allowed the simultaneous measurement of pulmonary arterial pressure. Serial intrapulmonary infusions were performed according to the following protocol: 1) 5-min control infusion of 0.9% saline; 2) serial 3-min infusions of ACh with final estimated intrapulmonary concentrations of $10^{-6}$, $10^{-5}$, and $10^{-4}$ mol/l; 3) 5-min control infusion of 0.9% saline; 4) serial 3-min infusions of ATP with concentrations of $10^{-6}$, $10^{-5}$, and $10^{-4}$ mol/l; 5) 5-min control infusion of 0.9% saline; and 6) serial 3-min infusions of AM with concentrations of $10^{-9}$, and $10^{-8}$ mol/l. Blood flow velocity in the segmental pulmonary artery was continuously measured with a Doppler flow wire. The flow velocity was determined by averaging the average peak velocity of 10 consecutive cardiac cycles. The diameter of the segmental pulmonary artery was determined by subselective angiography before and after each infusion. Systemic arterial pressure, pulmonary arterial pressure, and heart rate were continuously monitored during each infusion.

Intravenous Administration

A 7.5 French Swan-Ganz catheter was positioned in a pulmonary artery through a jugular vein. A 22-gauge cannula was inserted into a radial artery for hemodynamic measurements and blood sampling. Another 22-gauge cannula was inserted into a forearm vein for infusion of 0.9% saline, with or without AM. After an equilibration period of 60 min, saline was infused at a rate of 0.5 ml/min for 30 min. Baseline measurements were obtained during this period. Then, AM (0.05 $\mu$g/kg/min) was intravenously administered at a rate of 0.5 ml/min for 30 min, followed by 30-min saline infusion. Hemodynamic parameters including mean pulmonary arterial pressure, mean right atrial pressure, pulmonary capillary wedge pressure, and mean arterial pressure were measured at 15-min intervals starting 15 min before AM infusion until 30 min post-infusion. Cardiac output was measured by Fick’s method (16). Pulmonary vascular resistance was calculated using standard formulas (17). Blood samples were taken immediately before AM infusion and at the end of infusion.

Radioimmunoassay

Blood samples for measurement of total AM, cyclic adenosine 3',5'-monophosphate (cAMP), and cyclic guanosine 3',5'-monophosphate (cGMP) were immediately transferred into chilled glass tubes containing disodium EDTA (1 mg/ml) and aprotinin (500 U/ml) and centrifuged at 4°C. The plasma was then frozen and stored at -80°C until assayed. Plasma AM level was measured by immunoradiometric assay using a specific kit, developed by Diagnostic Science Department, Shionogi Pharmaceutical Co., Ltd., Osaka, Japan (18). Plasma cAMP and cGMP were determined with specific radioimmunoassay kits (a cAMP assay kit and a cGMP assay kit; Yamasa Shoyu, Chiba, Japan) (19).

Statistical Analysis

All data were expressed as the mean ± SEM unless otherwise indicated. Comparisons of parameters between two groups were made by Fisher’s exact test or Student’s unpaired t-test. Changes in pulmonary flow velocity and diameter of a segmental pulmonary artery during infusion of each agent were analyzed by multiple regression analysis with dummy variables (20). Comparisons of the time course of parameters between the AM group and placebo group were made by two-way analysis of variance (ANOVA) for repeated measures, followed by Newman-Keuls test. Values of p < 0.05 were considered to indicate statistical significance.

Results

Intrapulmonary Infusion

All study patients tolerated this study protocol without any adverse effects. There was no significant change in diameter of the segmental pulmonary artery before and after infusion of each agent (ACh, 3.5 ± 0.2 to 3.6 ± 0.2 mm; ATP, 3.6 ± 0.2 to 3.9 ± 0.2 mm; AM, 3.5 ± 0.2 to 3.8 ± 0.2 mm; p = NS, respectively). Thus, changes in flow velocity determined by means of a Doppler flow wire were considered to reflect proportional changes in blood flow in a segmental pulmonary artery. Systemic arterial pressure tended to decrease during infusion of each agent, but not to a statistically significant extent. Heart rate and pulmonary arterial pressure remained unchanged throughout the procedure.

Intrapulmonary infusion of ACh caused a dose-dependent increase in blood flow velocity in a segmental pulmonary artery (Fig. 1). At the maximal dose of ACh ($10^{-4}$ mol/l), a more than 20% increase in flow velocity (mean, 59 ± 13% of the baseline value) was observed in 6 patients (with thromboembolic pulmonary hypertension in 4, primary pulmonary
hypertension in 1, and atrial septal defect in 1). In contrast, the increase in flow velocity in response to ACh was attenuated (mean, 9 ± 4%) in 3 patients with primary pulmonary hypertension and 1 patient with Eisenmenger syndrome, suggesting impaired endothelial function in pulmonary vascular beds.

Intrapulmonary infusion of ATP dose-dependently increased blood flow velocity in a segmental pulmonary artery (Fig. 1). At the maximal dose of ATP (10⁻⁴ mol/l), a more than 20% increase in flow velocity (mean, 66 ± 14% of the baseline value) was observed in 9 of the 10 patients.

Intrapulmonary infusion of AM caused a significant increase in flow velocity at a dose of 10⁻⁷ mol/l (Fig. 1), associated with an increase in plasma cAMP level (11.3 ± 1.8 to 12.9 ± 2.0 pmol/ml, p < 0.05). At this dose, a more than 20% increase in flow velocity was observed in 9 of the 10 patients. The increase in flow velocity with AM at 10⁻⁸ mol/l (41 ± 6% of the baseline value) was comparable to that with ACh at 10⁻⁴ mol/l (39 ± 11%) and that with ATP at 10⁻⁵ mol/l (36 ± 14%).

**Intravenous Administration**

All study patients tolerated this study protocol. No arrhythmias were noted during AM infusion. Intravenous administration of AM did not significantly decrease mean pulmonary arterial pressure (Fig. 2). However, AM markedly increased the cardiac index by 41%. Thus, AM resulted in a 30% decrease in pulmonary vascular resistance. These hemodynamic effects of AM lasted at least 15 min after the end of infusion. AM significantly decreased mean systemic arterial pressure (78 ± 2 to 69 ± 2 mmHg, p < 0.05) and increased heart rate (71 ± 4 to 78 ± 5 bpm, p < 0.05) at the end of infusion. There was a significant increase in pulmonary arterial oxygen saturation (55 ± 6 to 66 ± 5%, p < 0.05), but no significant change in systemic arterial oxygen saturation (90 ± 5 to 92 ± 4%, p = NS).

The plasma AM level at baseline was significantly in-
creased in patients with primary pulmonary hypertension compared with that in age-matched healthy subjects (16.3 ± 1.3 vs. 10.7 ± 0.5 pmol/ml, \( p < 0.05 \)). The plasma AM level increased about 3-fold compared with the baseline value at the end of AM infusion. The plasma cAMP level significantly increased during AM infusion (12.7 ± 3.1 to 15.6 ± 3.2 pmol/ml), whereas the plasma cGMP level was not significantly altered (4.9 ± 0.8 to 4.3 ± 0.6 pmol/ml).

**Discussion**

A variety of vasodilators, including calcium-channel blockers, adenosine, and prostacyclin, have been proposed as potential therapeutic agents for pulmonary hypertension (21–24). In humans, however, whether AM, a novel vasodilator peptide, has beneficial effects in pulmonary hypertension has remained unclear. In this study, we demonstrated that 1) intrapulmonary infusion of AM caused a significant increase in pulmonary flow velocity even in patients who showed impaired endothelium-dependent vasorelaxation in response to ACh, and that 2) the vasodilator effect of AM at 10 \(^{-4} \) mol/l was comparable to that of ACh at 10 \(^{-4} \) mol/l and that of ATP at 10 \(^{-5} \) mol/l. We also demonstrated that 3) intravenous infusion of AM significantly increased the cardiac index and decreased pulmonary vascular resistance in patients with primary pulmonary hypertension, and that 4) intravenous AM increased plasma cAMP, but not cGMP, in association with its hemodynamic effects. These results suggest that AM produces marked pulmonary vasodilation in part through a cAMP-dependent, endothelium-independent mechanism.

Earlier studies have shown that AM regulates pulmonary vascular tone in rats, but not in cats, through an endothelium-derived nitric oxide-dependent mechanism (7). Nakamura et al. have shown that AM exerts a potent vasodilatory effect in the human peripheral vasculature through the involvement of a nitric oxide-dependent mechanism (25). However, the mechanisms responsible for the vasodilator activity of AM in the human pulmonary vasculature remain unclear. In the present study, ACh-induced pulmonary endothelium-dependent vasorelaxation was impaired in 3 patients with primary pulmonary hypertension and 1 patient with Eisenmenger syndrome, which findings are consistent with the results of an earlier study (12). ATP has been shown to cause endothelium-independent vasorelaxation via adenosine as well as endothelium-dependent vasorelaxation via \( P_2 \) receptors (13, 14). Like ATP, AM significantly increased blood flow velocity in association with an increase in plasma cAMP, even in patients who showed impaired endothelium-dependent vasorelaxation. It is therefore possible that AM may directly relax pulmonary vascular smooth muscle at least in part by increasing the level of intracellular cAMP. Interestingly, the vasodilator effect of AM at 10 \(^{-3} \) mol/l was comparable to that of ACh at 10 \(^{-4} \) mol/l and that of ATP at 10 \(^{-5} \) mol/l, indicating that on a molar basis, AM may have much more potent pulmonary vasodilator activity than ACh and ATP. These results suggest that the pulmonary vasodilator response to AM persists despite chronic pulmonary hypertension and that AM may cause marked pulmonary vasorelaxation in part through a cAMP-dependent, endothelium-independent mechanism. Adenosine is more specific in terms of endothelium-independent vasorelaxation than ATP, and has been commonly used to test the reversibility of pulmonary vasoconstriction (26). In the present study, however, we used ATP as a substitute of adenosine because adenosine is not available in Japan.

Experimental studies have shown that intralobar arterial infusion of AM causes a dose-related decreases in pulmonary vascular resistance under conditions of high pulmonary vascular tone (7–9). Shirai et al. have shown that AM dilates small pulmonary arteries in cats (100–500 \( \mu \)m), which are the main site of hypoxic pulmonary vasoconstriction (27). In the present study, intravenous infusion of AM significantly decreased pulmonary vascular resistance in patients with primary pulmonary hypertension. Pulmonary blood flow determined by means of a Doppler flow wire increased significantly during AM infusion, although there was no significant change in the diameter of the segmental pulmonary artery. These findings raise the possibility that infusion of AM may attenuate increased pulmonary vascular tone mainly by dilating small pulmonary vessels in patients with pulmonary hypertension. The hemodynamic effects of AM lasted at least 15 min after the end of infusion, suggesting that AM has relatively long-lasting vasodilator activity in patients with pulmonary hypertension.

Intravenous infusion of AM markedly increased the cardiac index in patients with pulmonary hypertension. Considering the strong vasodilator activity of AM in the systemic and pulmonary vasculature, the significant decrease in cardiac afterload may be responsible for the increased cardiac index during infusion. On the other hand, a recent binding study has shown abundant, specific binding sites for AM in the ventricular myocardium (4). AM has been shown to increase cardiac cAMP (28), which is known to mediate the positive inotropic action of \( \beta \)-adrenergic stimulants. Alternatively, AM has been shown to produce a positive inotropic action through cAMP-independent mechanisms (29). These findings suggest that the increase in cardiac index may be attributable not only to a fall in cardiac afterload but also to the direct positive inotropic action of AM.

The baseline plasma AM level was significantly higher in patients with pulmonary hypertension than in control subjects, implying that endogenous AM may play an important role as a counter-regulatory hormone in states where there is pathological pulmonary vasoconstriction. Nevertheless, exogenously administered AM increased plasma cAMP in association with hemodynamic effects. Thus, additional administration of AM may be effective in patients with pulmonary hypertension.

In conclusion, on a molar basis, AM may have much more
potent pulmonary vasodilator activity than ACh and ATP. Intravenous infusion of AM exerts beneficial hemodynamic effects primarily by increasing cardiac output and decreasing pulmonary vascular resistance in patients with primary pulmonary hypertension. These results suggest that infusion of AM may be a new therapeutic approach for treatment of severe pulmonary hypertension.

Acknowledgements

We thank Nobuo Shirahashi for his helpful advice regarding statistical analysis. We also thank Masahiko Shibakawa for preparing the adrenomedullin injection.

References