Effects of Adrenomedullin Inhalation on Hemodynamics and Exercise Capacity in Patients With Idiopathic Pulmonary Arterial Hypertension

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Background—Adrenomedullin (AM) is a potent pulmonary vasodilator peptide. However, whether intratracheal delivery of aerosolized AM has beneficial effects in patients with idiopathic pulmonary arterial hypertension remains unknown. Accordingly, we investigated the effects of AM inhalation on pulmonary hemodynamics and exercise capacity in patients with idiopathic pulmonary arterial hypertension.

Methods and Results—Acute hemodynamic responses to inhalation of aerosolized AM (10 μg/kg body wt) were examined in 11 patients with idiopathic pulmonary arterial hypertension during cardiac catheterization. Cardiopulmonary exercise testing was performed immediately after inhalation of aerosolized AM or placebo. The work rate was increased by 15 W/min until the symptom-limited maximum, with breath-by-breath gas analysis. Inhalation of AM produced a 13% decrease in mean pulmonary arterial pressure (54±3 to 47±3 mm Hg, P<0.05) and a 22% decrease in pulmonary vascular resistance (12.6±1.5 to 9.8±1.3 Wood units, P<0.05). However, neither systemic arterial pressure nor heart rate was altered. Inhalation of AM significantly increased peak oxygen consumption during exercise (peak VO₂, 14.6±0.6 to 15.7±0.6 mL · kg⁻¹ · min⁻¹, P<0.05) and the ratio of change in oxygen uptake to that in work rate (ΔVO₂/ΔW ratio, 6.3±0.4 to 7.0±0.5 mL · min⁻¹ · W⁻¹, P<0.05). These parameters remained unchanged during placebo inhalation.

Conclusions—Inhalation of AM may have beneficial effects on pulmonary hemodynamics and exercise capacity in patients with idiopathic pulmonary arterial hypertension. (Circulation. 2004;109:351-356.)

Key Words: peptides • hypertension, pulmonary • respiration • exercise • hemodynamics
in the alveoli causes pulmonary vasodilation matched to ventilated areas.\textsuperscript{20} In clinical settings, inhalation therapy may be more simple, noninvasive, and comfortable than continuous intravenous infusion therapy. Thus, the purpose of the present study was to investigate the effects of AM inhalation on hemodynamics and exercise capacity in patients with idiopathic pulmonary arterial hypertension.

### Methods

#### Study Subjects

Eleven patients with idiopathic pulmonary arterial hypertension (9 women and 2 men; age, 39±3 years) were included in this study. Idiopathic pulmonary arterial hypertension was defined as pulmonary hypertension unexplained by any secondary cause, on the basis of the criteria of the National Institutes of Health registry.\textsuperscript{1} Ten patients were classified as New York Heart Association (NYHA) functional class III and 1 as class IV (Table 1). Two of the 11 patients (18%) were acute responders who showed a significant decrease in mean pulmonary arterial pressure of ≥20% with a decrease in mean pulmonary arterial pressure to <35 mm Hg and no change or an increase in cardiac index during short-term infusion of epoprostenol. Long-term medication, including anticoagulant agents, digitalis, and diuretics, was kept constant. Vasodilator agents, such as oral prostacyclin analogue and calcium antagonists, were stopped ≥12 hours before the study procedure was begun. The ethics committee of the National Cardiovascular Center approved the study, and all patients gave written informed consent.

#### Preparation of Human AM

Human AM was dissolved in saline with 4% d-mannitol and sterilized by passage through a 0.22-µm filter (Millipore Co). 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.

#### Hemodynamic Studies

Acute hemodynamic responses to AM inhalation were assessed in all patients while they were in a stable condition during hospitalization. Hemodynamic variables, including pulmonary arterial pressure, right atrial pressure, pulmonary capillary wedge pressure, and cardiac output (in triplicate), were determined with a thermodilution catheter (TOO21H-7.5F, Baxter Co).\textsuperscript{22} A 22-gauge cannula was inserted into a radial artery for hemodynamic measurements and blood sampling. After an equilibration period of 30 minutes, baseline hemodynamics were measured. Then, AM (10 µg/kg body wt) was inhaled as an aerosol with a jet nebulizer (Porta-Nebu, MEDIC-AID) for 15 minutes, which resulted in a cumulative dose of 400 to 600 µg AM. Hemodynamic parameters were measured at 15-minute intervals starting 15 minutes before AM inhalation until 60 minutes after inhalation. Blood samples for AM measurement were taken at 15-minute intervals from 15 minutes before inhalation until 60 minutes after the end of inhalation.

#### Cardiopulmonary Exercise Testing

The effects of AM inhalation on exercise capacity were examined in 10 of 11 patients; 1 patient with NYHA class IV underwent the 6-minute walk test according to decision of attending physicians. Cardiopulmonary exercise testing was performed immediately after inhalation of aerosolized AM (10 µg/kg body wt) or saline in a double-blind, randomized, crossover design. This study was performed on 2 separate days, 1 week apart. The first cardiopulmonary exercise testing was performed within 10 days after the cardiac catheterization. The patients performed exercise seated on a cycle ergometer. They first pedaled at 55 rpm without any added load for 1 minute. The work rate was then increased by 15 W/min up to the symptom-limited maximum. Breath-by-breath gas analysis was performed with an AE280 (Minato Medical Science) connected to a personal computer running analyzing software.\textsuperscript{23} The ratio of change in oxygen uptake to that in work rate (ΔVO₂/ΔW ratio) was calculated as the slope of oxygen consumption per unit workload from 1 minute after the start of load addition until 85% maximal VO₂.

Exercise capacity was evaluated by peak oxygen consumption (peak VO₂), which was defined as the value of averaged data during the final 15 seconds of exercise. Ventilatory efficiency during exercise was represented by the VE-VCO₂ slope, which was determined as the linear regression slope of VE and VCO₂ from the start of exercise until the RC point (the time until which ventilation is stimulated by CO₂ output and end-tidal CO₂ tension begins to decrease).

#### Measurement of Plasma AM, cAMP, and cGMP

Blood samples were immediately transferred into chilled glass tubes containing disodium EDTA (1 mg/mL) and aprotinin (500 U/mL) and centrifuged immediately at 4°C, and the plasma was frozen and stored at −80°C until assayed. Plasma AM level was measured by a specific immunoradiometric assay kit (Shionogi Pharmaceutical Co Ltd).\textsuperscript{24} Plasma cAMP and cGMP were determined with radioimmunoassay kits (cAMP assay kit, cGMP assay kit, Yamasa Shoyu).\textsuperscript{18}

### Statistical Analysis

All data were expressed as mean±SEM unless otherwise indicated. Changes in hemodynamic and hormonal parameters by AM inhalation were analyzed by 1-way ANOVA for repeated measures,
followed by Newman-Keuls test. Comparisons of exercise parameters between the 2 groups were analyzed with paired Student's t test. A probability value of $P<0.05$ was considered statistically significant.

**Results**

All patients tolerated this study protocol. One patient developed a headache, and another patient had mild arterial hypoxemia during AM inhalation. None of them experienced other adverse effects, such as systemic hypotension, infection, or arrhythmia.

**Plasma AM Level After Inhalation**

Baseline plasma AM level in patients with idiopathic pulmonary arterial hypertension was significantly higher than the normal value, which was determined from pooled data of 15 age-matched healthy subjects (11.9±0.8 versus 9.3±0.1 fmol/mL, $P<0.05$). Inhalation of AM significantly increased the plasma AM level to 22.9±2.1 fmol/mL immediately after inhalation (Figure 1). The half-life of plasma AM after inhalation was approximately 20 minutes, and the elevation of AM lasted for >45 minutes. Plasma cAMP level increased significantly 30 minutes after the initiation of AM inhalation (10.8±0.7 to 12.0±0.6 pmol/mL, $P<0.05$), although plasma cGMP level was not significantly altered (6.5±1.0 to 6.8±1.0 pmol/mL, $P=NS$).

**Hemodynamic Effects of AM Inhalation**

Inhalation of AM significantly decreased mean pulmonary arterial pressure in patients with idiopathic pulmonary arterial hypertension (54±3 to 47±3 mm Hg, $P<0.05$) without a significant decrease in mean arterial pressure (85±4 to 83±4 mm Hg, $P=NS$) (Figure 2). AM inhalation slightly but significantly increased cardiac index by 12% (2.4±0.1 to 2.7±0.2 L·min⁻¹·m⁻², $P<0.05$). Thus, AM inhalation resulted in a 22% decrease in pulmonary vascular resistance (12.6±1.5 to 9.8±1.3 Wood units, $P<0.05$) (Figure 3). Inhaled AM did not significantly alter systemic vascular resistance. The ratio of pulmonary vascular resistance to systemic vascular resistance was decreased significantly at the end of inhalation (0.63±0.08 to 0.55±0.07, $P<0.05$). These hemodynamic effects of AM lasted for >45 minutes.

**Effects of AM Inhalation on Exercise Capacity and Ventilatory Efficiency**

As the limiting symptom at the end of exercise, 6 patients reported muscle weakness and 4 reported dyspnea. There was no difference in these symptoms when exercise testing was performed with or without inhalation of AM. Inhalation of AM altered neither heart rate nor blood pressure either at rest or at peak exercise (Table 2). Inhalation of AM significantly increased peak workload (86±5 to 93±6 W, $P<0.05$) (Table 2). AM also significantly increased peak VO₂ (14.6±0.6 to 15.7±0.6 mL·kg⁻¹·min⁻¹, $P<0.05$) (Figure 4). Inhalation of AM significantly increased $\Delta$VO₂/$\Delta$W ratio (6.3±0.4 to
Discussion

In the present study, we demonstrated that inhalation of AM improved hemodynamics with pulmonary selectivity and exercise capacity in patients with idiopathic pulmonary arterial hypertension.

AM is one of the most potent endogenous vasodilators in the pulmonary vascular bed. The vasodilatory effect is mediated by cAMP-dependent and nitric oxide–dependent mechanisms. Endogenous AM production is enhanced in a variety of cardiovascular diseases through a compensatory mechanism. Nonetheless, additional supplementation of AM has beneficial effects in these diseases. These results suggest that endogenous AM level is not sufficient to improve deteriorated conditions despite the increased AM production. Interestingly, Champion et al have shown that intratracheal gene transfer of calcitonin gene–related peptide, a member of the same peptide family as AM, to bronchial epithelial cells attenuates chronic hypoxia-induced pulmonary hypertension in the mouse. These results raise the possibility that intratracheal delivery of a vasodilator peptide may be sufficient to alter pulmonary vascular function. In fact, in the present study, inhalation of AM significantly decreased pulmonary vascular resistance, whereas it did not alter systemic arterial pressure or systemic vascular resistance. The ratio of pulmonary vascular resistance to systemic vascular resistance was reduced significantly by AM inhalation. These results suggest that inhaled AM improves hemodynamics with pulmonary selectivity. This is consistent with earlier findings that inhaled prostacyclin or its analogue iloprost acts transepithelially with pulmonary selectivity and improves pulmonary hypertension. Inhalation of AM slightly but significantly increased cardiac index in patients with idiopathic pulmonary arterial hypertension. Considering the strong vasodilator activity of AM in the pulmonary vasculature, the significant decrease in cardiac afterload may be responsible for increased cardiac index with

<table>
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<th>Variables</th>
<th>Placebo</th>
<th>AM</th>
<th>P</th>
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<tr>
<td>Peak workload, W</td>
<td>86±5</td>
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<tr>
<td>Rest</td>
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<td>75±3</td>
<td>NS</td>
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<tr>
<td>Peak</td>
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<td>148±6</td>
<td>NS</td>
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<tr>
<td>MAP, mm Hg</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
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<td>87±5</td>
<td>NS</td>
</tr>
<tr>
<td>Peak</td>
<td>108±5</td>
<td>110±6</td>
<td>NS</td>
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<tr>
<td>Peak Borg score (D/L)</td>
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<td>NS</td>
</tr>
<tr>
<td>Peak VO₂, mL · kg⁻¹ · min⁻¹</td>
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<td>15.7±0.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ΔVO₂/ΔW ratio, mL · min⁻¹ · W⁻¹</td>
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<tr>
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<tr>
<td>Peak</td>
<td>95±1</td>
<td>95±1</td>
<td>NS</td>
</tr>
</tbody>
</table>

HR indicates heart rate; MAP, mean arterial pressure; Peak Borg score (D/L), Borg score at peak exercise (dyspnea/leg fatigue); Peak VO₂, peak oxygen consumption; ΔVO₂/ΔW ratio, VO₂ increase per unit workload; Ve-VCO₂ slope, slope of regression line of relation between Ve and VCO₂ and SaO₂, arterial oxygen saturation. Data are mean±SEM.

Figure 3. Changes in pulmonary vascular resistance (PVR), systemic vascular resistance (SVR), and ratio of pulmonary vascular resistance to systemic vascular resistance (Rp/Rs) by inhalation of aerosolized AM in patients with idiopathic pulmonary arterial hypertension. Data are mean±SEM, *P<0.05 vs value at time 0.

7.0±0.5 mL · min⁻¹ · W⁻¹, P<0.05). AM did not significantly alter the Ve-VCO₂ slope (Table 2). No significant changes in arterial oxygen saturation were observed either at rest or at peak exercise. In 1 patient with NYHA class IV who did not undergo cardiopulmonary exercise testing, the distance walked in 6 minutes increased from 150 to 180 m by inhalation of AM.

Figure 4. Changes in peak oxygen consumption (peak VO₂) and ratio of change in oxygen uptake to that in work rate (ΔVO₂/ΔW ratio) by inhalation of aerosolized AM or placebo in patients with idiopathic pulmonary arterial hypertension. Data are mean±SEM, *P<0.05 vs placebo.
AM. Interestingly, the hemodynamic effects of inhaled AM lasted for >45 minutes. A previous study demonstrated that intravenous injection of AM produces a long-lasting vasodilator response because of its long half-life (≈15 minutes). The half-life of plasma AM after inhalation was longer (20 minutes). Thus, inhalation of AM may cause relatively long-lasting pulmonary vasodilator activity in patients with idiopathic pulmonary arterial hypertension. In the present study, plasma cAMP level increased after AM inhalation, suggesting that the hemodynamic effects of AM may be mediated by activation of cAMP.

Earlier studies have shown that peak VO₂ during exercise is markedly lower in patients with idiopathic pulmonary arterial hypertension than in healthy subjects. Peak VO₂ is determined primarily by the maximal cardiac output during exercise and the potential for O₂ extraction by the exercising muscle. Thus, the decreased peak VO₂ may reflect insufficient oxygen delivery to the body during exercise, at least in part because of an inadequate increase in cardiac output under conditions of severe pulmonary hypertension. In the present study, inhalation of AM significantly increased peak VO₂ in patients with pulmonary hypertension. AM also increased the ΔVO₂/ΔW ratio, which indicates oxygen transport per unit workload to the exercising legs. These results suggest that inhalation of AM improves exercise capacity in patients with idiopathic pulmonary arterial hypertension. It is possible that an increase in cardiac output during exercise may contribute to increases in peak VO₂ and the ΔVO₂/ΔW ratio.

The major limitation of this pilot trial relates to the lack of a randomized, placebo-controlled group in acute hemodynamic studies, which was as result not only of invasive assessment of hemodynamics but also of the limited number of patients available. Nevertheless, cardiopulmonary exercise testing was performed in a double-blind, randomized, crossover design. Thus, it is unlikely that the hemodynamic effects of inhaled AM are attributable to the placebo effect.

Inhalation therapy may be more simple, noninvasive, and comfortable than continuous intravenous infusion therapy. An experimental study demonstrated that repeated inhalation of AM (for 30 minutes, 4 times a day) inhibited monocrotaline-induced pulmonary hypertension and markedly improved survival in rats. Recently, pulmonary delivery of a dry-powder insulin has been shown to improve glycemic control without adverse pulmonary effects. Although further studies are necessary to maximize the efficiency and reproducibility of pulmonary AM delivery, combining AM inhalation therapy with other modalities that have a different mode of action may have beneficial effects in patients with idiopathic pulmonary arterial hypertension.

Conclusions
These preliminary results suggest that inhalation of AM may have beneficial effects on pulmonary hemodynamics and exercise capacity in patients with idiopathic pulmonary arterial hypertension.

Acknowledgments
This work was supported by grants from NEDO, the Mochida Memorial Foundation for Medical and Pharmaceutical Research, the Japan Cardiovascular Research Foundation, Health and Labor Sciences Research Grant genome 005, and the Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research of Japan. We thank Masahiko Shibakawa for preparing AM.

References


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_Circulation_. 2004;109:351-356; originally published online January 12, 2004;
doi: 10.1161/01.CIR.0000109493.05849.14
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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