Cardiovascular and renal effects of adrenomedullin in rats with heart failure

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Adrenomedullin (AM) is a novel hypotensive peptide originally isolated from human pheochromocytoma (8). AM peptide and mRNA have been reported to be expressed not only in adrenal medulla but also in the heart, kidney, lung, and endothelium (5, 23). AM has been shown to promote vasodilatation (1), diuresis, and natriuresis (6, 10, 11) and to inhibit aldosterone production in normal animals (27). Recently, AM has been shown to have a positive inotropic action in the perfused rat heart (25, 26). We and others have shown that plasma AM levels are increased in patients with heart failure (HF) (7, 15). Tissue levels of AM peptide and mRNA have been shown to be increased in the heart, kidney, and lungs of HF rats (13). These findings suggest that AM may play a role in the regulation of volume and pressure homeostasis in HF via its renal, vascular, cardiac, and endocrine effects. However, the potential effects of AM as a therapeutic agent for HF are not fully understood. Recently, Rademaker et al. (22) showed that intravenous infusion of high-dose AM exerted diuresis and natriuresis without inducing hypotension and, in the higher dose, produced beneficial effects on pulmonary hemodynamics in a rat model of HF produced by left coronary ligation.

METHODS

Model of HF. Male Wistar rats weighing 180–220 g were used in this study. Myocardial infarction was produced by a previously described method (16). In brief, after rats were anesthetized by intraperitoneal injection of pentobarbital sodium (30 mg/kg body wt), they were intubated with a polyethylene tube (PE-240) and artificially ventilated using a volume-regulated respirator. The heart was exposed via a left thoracotomy, and the left coronary artery was ligated 2–3 mm from its origin between the pulmonary artery conus and the left atrium using a 6–0 Prolene suture. To reduce deaths due to arrhythmia, we pretreated the rats with an intraperitoneal injection of xylocaine (1–2 mg/kg) before coronary artery ligation and observed the electrocardiogram on the monitor. If ventricular tachycardia or fibrillation was observed, we injected additional xylocaine intraperitoneally or tapped the heart gently using a cotton applicator (17). These procedures were effective for converting the fatal arrhythmia to sinus rhythm. Finally, the heart was restored to its normal position and the lungs were hyperinflated to help evacuate air from the thoracic cavity before the chest was closed. The control rats received a sham operation involving thoracotomy and cardiac exposure but without coronary artery ligation. The surviving rats were maintained on standard rat chow.

The HF and control rats were randomly assigned to five groups. Low-dose AM (0.01 µg·kg body wt⁻¹·min⁻¹; HF, n = 8; control, n = 9), high-dose AM (0.05 µg·kg body wt⁻¹·min⁻¹; HF, n = 10; control, n = 9), or saline (HF, n = 10) was intravenously administered in each group. The saline group was prepared to rule out a time-course effect during the intravenous infusion protocol.

Study protocol. Approximately 4 wk after surgery, subsequent experimental procedures were performed. Rats were anesthetized by intraperitoneal injection of Inactin (100 mg/kg body wt) and placed on a heating pad to maintain body temperature at 37–38°C throughout the study. Tracheostomy was performed with a PE-240 tube. A polyethylene catheter was inserted into the right atrium before the chest was closed. The catheter was advanced into the right ventricle and connected to a pressure transducer (Statham P231B). Heart rate was measured from the electrocardiogram recorded on a monitoring device (Nihon Kohden NCC-1000, Tokyo, Japan).

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(PE-10) was inserted into the right femoral artery to measure the heart rate (HR) and mean arterial pressure (MAP). A polyethylene catheter (PE-50) was inserted into the right jugular vein and was advanced through the right atrium into the right ventricle for measurement of mean right atrial pressure (RAP) and right ventricular systolic pressure (RVSP). These hemodynamic variables were measured using a pressure transducer (model P23 ID; Gould, Glen Burnie, MD) connected to a polygraph and were recorded by a thermal recorder (7758 B System; Hewlett-Packard, Palo Alto, CA). A thermocouple probe was advanced into the ascending aorta via the right carotid artery and connected to a cardiac output (CO) computer (Cardiotherm-500; Columbus Instruments). For measurement of CO, 0.1 ml of 0.9% saline at room temperature was injected as a bolus via the jugular vein catheter. A second PE-50 catheter was inserted into the left jugular vein for the infusion of saline containing 5% inulin and 1.8% p-aminohippuric acid (PAH) at a rate of 0.2 ml·100 g body wt \(-1 \cdot \text{min}^{-1}\). A third PE-50 catheter was inserted into the left femoral vein for the continuous infusion of AM or saline at a rate of 0.3 ml·100 g body wt \(-1 \cdot \text{min}^{-1}\). Finally, a PE-100 catheter was inserted into the bladder to collect urine. After an equilibration period of 60 min, we measured RAP at baseline. Then the catheter was advanced into the right ventricle by monitoring the pressure. Urine was collected over two periods of 20 min each, and 150-µl blood samples were drawn at the midpoint of each urine collection for measurement of PAH and inulin. Simultaneously, we recorded hemodynamic variables, including HR, MAP, RVSP, and CO. The averaged values in the two periods were used as the values before AM infusion. After the control measurements and samples were obtained, AM (0.01 µg·kg body wt \(-1 \cdot \text{min}^{-1}\) or 0.05 µg·kg body wt \(-1 \cdot \text{min}^{-1}\)) was intravenously administered. Thirty minutes later, all measurements were repeated over two periods of 20 min and the averaged values in the two periods were used as the values after AM infusion.

After completion of these measurements, 4 ml of blood were drawn from the femoral artery for measurements of plasma AM and atrial natriuretic peptide (ANP). Then the heart was arrested by the injection of 2 mmol of KCl through the femoral artery. The ventricles and lungs were excised, dissected free, and weighed. Infarct size was determined as a percentage of left ventricular area, as previously reported (1). Only rats with infarct size \(\geq 30\%\) of the left ventricle were enrolled in this study as a model of HF (21).

Analysis. Urinary concentration of sodium was measured by flame photometry. Inulin was measured by the anthrone method. PAH was measured by Marshall’s method. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined from inulin and PAH clearance, respectively. Effective renal blood flow (ERBF) was calculated as ERPF/(1 - hematocrit); renal vascular resistance (RVR) was determined as (MAP - RAP)/ERBF. The filtration fraction was calculated as GFR/ERPF.

Measurement of AM, ANP, cAMP, and cGMP. The plasma concentrations of AM and ANP were determined by radioimmunoassay after extraction with Sep-Pak C18 cartridges (Millipore; Waters, Milford, MA), as previously reported (12, 23). The urinary concentrations of cAMP and cGMP were determined with radioimmunoassay kits (cAMP assay kit and cGMP assay kit; Yamasa Shoyu, Chiba, Japan), as previously reported (4). A normal value of plasma AM in rats was established from nine sham-operated rats.

Statistical analysis. Numerical values were expressed as means ± SD unless otherwise indicated. Comparisons of variables between two groups were made by Student’s unpaired t-test. Comparisons of values among three or more groups were made using one-way ANOVA followed by Scheffé’s multiple comparison test. Changes in hemodynamic and renal variables during AM infusion were analyzed with Student’s paired t-test. A P value <0.05 was considered significant.

RESULTS

Physiological profiles. The physiological profiles of the five experimental groups are summarized in Table 1. Moderate to large infarcts were observed in three HF groups, and the mean infarct size was similar in the three groups. HF rats showed significantly increased weights of the right ventricle and the lungs compared with control rats. Plasma ANP levels were markedly increased in HF rats compared with control rats. MAP and CO at baseline were significantly decreased in HF rats compared with control rats (Figs. 1 and 2). RVSP and RAP at baseline were significantly increased in HF rats. These results suggest the presence of chronic HF in this experimental model.

Effects of AM on systemic and pulmonary circulation. Infusion of saline did not significantly change MAP, CO, RVSP, or RAP in HF rats (Table 2). The infusion of low-dose AM (0.01 µg·kg body wt \(-1 \cdot \text{min}^{-1}\)) did not significantly alter any hemodynamic variables in either HF or control rats (Fig. 1). In contrast, high-dose AM (0.05 µg·kg body wt \(-1 \cdot \text{min}^{-1}\)) produced a slight decrease in MAP and a marked increase in CO in both HF and control rats (Fig. 2). High-dose AM significantly reduced RVSP and RAP in HF rats, although it did not significantly alter these variables in control rats. HR was not significantly changed during infusion of low-dose AM (HF, 383 ± 31 to 386 ± 30 beats/min; control,

Table 1. Baseline characteristics of control and HF rats

<table>
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<tr>
<th></th>
<th>Con Low AM</th>
<th>Con High AM</th>
<th>HF Saline</th>
<th>HF Low AM</th>
<th>HF High AM</th>
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<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>8</td>
<td>10</td>
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<tr>
<td>Body weight, g</td>
<td>280 ± 17</td>
<td>278 ± 13</td>
<td>250 ± 33*†</td>
<td>248 ± 21†</td>
<td>242 ± 17†</td>
</tr>
<tr>
<td>Infarct size, %</td>
<td>1.9 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>42 ± 6</td>
<td>41 ± 8</td>
<td>40 ± 6</td>
</tr>
<tr>
<td>LV wt/body wt</td>
<td>2.1 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>2.2 ± 0.3</td>
<td>2.2 ± 0.3</td>
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<tr>
<td>RV wt/body wt</td>
<td>0.55 ± 0.1</td>
<td>0.55 ± 0.1</td>
<td>1.1 ± 0.3*†</td>
<td>1.0 ± 0.3*†</td>
<td>1.1 ± 0.3*†</td>
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<tr>
<td>Lung wt/body wt</td>
<td>4.7 ± 0.4</td>
<td>4.4 ± 0.6</td>
<td>8.5 ± 2.5*†</td>
<td>8.1 ± 1.8*†</td>
<td>8.5 ± 2.2*†</td>
</tr>
<tr>
<td>Plasma ANP, fmol/ml</td>
<td>132 ± 36</td>
<td>133 ± 52</td>
<td>743 ± 403*†</td>
<td>599 ± 421*†</td>
<td>736 ± 426*†</td>
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</table>

Values are means ± SD. Con, sham-operated control rats; HF, heart failure rats; low adrenomedullin (AM), AM infusion at a rate of 0.01 µg·kg body wt \(-1 \cdot \text{min}^{-1}\); high AM, AM infusion at a rate of 0.05 µg·kg body wt \(-1 \cdot \text{min}^{-1}\); LV, left ventricle; RV, right ventricle; ANP, atrial natriuretic peptide. *P < 0.05 vs. Con low AM; †P < 0.05 vs. Con high AM.
Effects of AM on renal function. Infusion of saline did not change any renal variables in HF rats (Table 2). Infusion of low-dose AM (0.01 µg·kg body wt·min⁻¹) significantly increased urine volume (UV) and urinary sodium excretion (UNaV) in both HF and control rats, but it did not change GFR or ERPF (Fig. 3). RVR was not significantly changed by low-dose AM (HF, 2.7 ± 0.4 to 2.6 ± 0.4 mmHg·ml⁻¹·min⁻¹·kg; control, 2.7 ± 0.4 to 2.6 ± 0.5 mmHg·ml⁻¹·min⁻¹·kg). In contrast, high-dose AM (0.05 µg·kg body wt·min⁻¹) resulted in slight,
but significant, increases in GFR and ERPF as well as increases in UV and UNaV in both HF and control rats (Fig. 4). RVR in both groups was significantly decreased by high-dose AM (HF, 2.8 ± 0.8 to 2.4 ± 1.0 mmHg·ml⁻¹·min⁻¹·kg⁻¹; control, 2.6 ± 0.7 to 1.9 ± 0.7 mmHg·ml⁻¹·min⁻¹·kg⁻¹, P < 0.05, respectively).

Neurohumoral measurements. Plasma AM levels in HF rats treated with saline were significantly increased compared with a normal value of plasma AM in rats (3.5 fmol/ml). Plasma AM levels in HF rats treated with low-dose AM tended to be increased (1.4-fold) compared with those in HF rats treated with saline, but not to a statistically significant extent (Table 3). In contrast, plasma AM levels in HF rats treated with high-dose AM were significantly elevated (sevenfold) compared with those in HF rats treated with saline.

Interestingly, the same dose of AM (0.05 µg·kg body wt⁻¹·min⁻¹) resulted in different changes in plasma AM levels between HF rats (56 ± 29 fmol/ml) and control rats (30 ± 8 fmol/ml).

Urinary cGMP excretion at baseline was markedly increased in HF rats compared with control rats. During the infusion of low- or high-dose AM, urinary cGMP excretion remained unchanged in HF and control rats. Urinary cAMP excretion at baseline did not significantly differ between HF and control rats. The infusion of low- or high-dose AM did not significantly change urinary cAMP excretion in either group.

DISCUSSION

In this study, we examined the cardiovascular and renal effects of intravenous infusion of AM in HF rats and sham-operated control rats using two doses of AM that did not induce a marked decrease in arterial pressure. We demonstrated for the first time 1) that intravenous infusion of low-dose AM significantly increased UV and UNaV without changes in GFR, ERPF, or any hemodynamic variables in HF and control rats. We also demonstrated 2) that high-dose AM infusion significantly decreased MAP and increased CO in both HF and control rats, whereas high-dose AM significantly reduced RVSP and RAP only in HF rats with pulmonary hypertension, and 3) that high-dose AM significantly increased GFR and ERPF as well as UV and UNaV in both groups. These results suggest that AM plays a role in the regulation of pressure and volume homeostasis via renal and cardiovascular effects in HF.

To date, the biological actions of AM have been examined in a variety of normal animals, whereas the therapeutic effects of AM on HF are not fully understood. Intravenous infusion of AM has been shown to produce a marked decrease in arterial pressure via its strong vasorelaxant effect mediated by the accumulation of cAMP (22, 24). However, the AM-induced hypotension may cause adverse effects in HF such as decreased renal perfusion pressure. Thus, in a preliminary study, we determined two doses of AM that would not induce a marked decrease in arterial pressure. In this study, infusion of low-dose AM (0.01 µg·kg body wt⁻¹·min⁻¹) did not significantly decrease MAP, whereas high-dose AM (0.05 µg·kg body wt⁻¹·min⁻¹) produced a slight but significant decrease in MAP from baseline.

Table 3. Plasma levels of AM and urinary excretion of cAMP and cGMP in control and heart failure rats

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<th>Con Low AM</th>
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<tr>
<td>Plasma AM, fmol/ml</td>
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<tr>
<td>After</td>
<td>8 ± 2</td>
<td>30 ± 8‡</td>
<td>8 ± 1</td>
<td>11 ± 2</td>
<td>56 ± 29‡*§</td>
</tr>
<tr>
<td>Urinary cAMP, pmol/min</td>
<td></td>
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<tr>
<td>Before</td>
<td>16 ± 8</td>
<td>15 ± 5</td>
<td>17 ± 3</td>
<td>15 ± 3</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>After</td>
<td>19 ± 7</td>
<td>16 ± 3</td>
<td>16 ± 2</td>
<td>15 ± 3</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>Urinary cGMP, pmol/min</td>
<td></td>
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<tr>
<td>Before</td>
<td>1.3 ± 0.3</td>
<td>1.1 ± 0.4</td>
<td>3.4 ± 2.0‡</td>
<td>3.3 ± 1.3‡</td>
<td>3.7 ± 1.3‡</td>
</tr>
<tr>
<td>After</td>
<td>1.3 ± 0.4</td>
<td>1.0 ± 0.3</td>
<td>3.3 ± 1.9‡</td>
<td>3.1 ± 2.3‡</td>
<td>3.2 ± 1.2‡</td>
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</tbody>
</table>

Values are means ± SD. After, after infusion of AM or saline; before, before infusion of AM or saline. *P < 0.05 vs. Con low AM; †P < 0.05 vs. Con high AM; ‡P < 0.05 vs. HF saline; §P < 0.05 vs. HF low AM.
In this study, we showed that high-dose AM increased CO in HF and control rats. This is consistent with results from a previous study using conscious sheep (22). One of the mechanisms underlying this increase in CO may be a fall of cardiac afterload by decreasing MAP. Recently, Szokodi et al. (26) showed that AM produced a direct positive inotropic action via Ca\(^{2+}\) release from intracellular ryanodine- and thapsigargin-sensitive Ca\(^{2+}\) stores, activation of protein kinase C, and Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels. These findings suggest that the increase in CO may be attributed not only to the fall of cardiac afterload but also to the positive inotropic action of AM.

Interestingly, in this study, AM significantly decreased RVSP only in HF rats with pulmonary hypertension, not in control rats. It has been reported that there are many binding sites of AM in the lung (19) and that AM preferentially dilates pulmonary arterial resistance vessels (3, 18). Circulating AM has been shown to be increased and to be partially metabolized in the lungs of patients with pulmonary hypertension (14, 28). These findings raise the possibility that AM is involved in the pathophysiology of pulmonary hypertension. Recently, Lippton et al. (9) showed that AM does not alter pulmonary arterial pressure under conditions of resting pulmonary vascular tone, whereas it produces a dose-dependent reduction in pulmonary arterial pressure under conditions of increased pulmonary vascular tone, consistent with our results. Thus it may be interesting to speculate that AM is more effective in rats with increased pulmonary vascular resistance secondary to severe left HF.

AM has been shown to have diuretic and natriuretic activities in normal animals (6, 10, 11). In this study, we demonstrated that low-dose AM significantly increased UV and UNaV without increases in GFR or ERPF in both HF and control rats. Edwards et al. (2) have reported that AM dose dependently increases intracellular cAMP levels in the cortical thick ascending limb and distal convoluted tubule dissected from rat kidney. These findings suggest that AM has a direct action on the renal tubules via the accumulation of cyclic AMP, and thereby AM may be involved in the regulation of urine flow and UNaV. However, in the present study, intravenous infusion of AM did not increase urinary cAMP level, consistent with a previous report (11). It is possible that AM stimulates intracellular cAMP at the tissue level sufficiently to induce a biological response but insufficiently to induce a measurable rise in urinary level. In addition, AM did not significantly increase urinary cGMP level. This result suggests that the renal action of AM might not be mediated mainly by the nitric oxide-cGMP pathway in rats. Moreover, in this study we showed that intravenous administration of high-dose AM significantly increased GFR and RPF in both HF and control rats, supporting the earlier findings that intrarenal infusion of AM increases GFR and RPF (6, 11). These results suggest that AM dilates renal arteries as well as peripheral vessels. Recently, Rademaker et al. (22) showed that intravenous infusion of AM increases UNaV without an increase in urine flow or creatinine clearance in pacing-induced HF sheep. In their study, MAP at baseline was considerably low (72 mmHg) in HF sheep, and AM infusion induced a further reduction in MAP to 63 mmHg. Thus the discrepancy between their results and ours with regard to changes in UV and GFR may be explained, in part, by the difference in renal perfusion pressure.

It remains unclear whether exogenous AM functions at pathophysiological or pharmacological levels. In this study, plasma AM levels in HF rats treated with low-dose AM tended to be increased (1.4-fold) compared with those in HF rats treated with saline, but not to a statistically significant extent. In contrast, plasma AM levels in HF rats treated with high-dose AM were significantly elevated compared with those in HF rats treated with saline (sevenfold). Accordingly, intravenous infusion of low-dose AM may cause natriuresis and diuresis at pathophysiological levels, whereas high-dose infusion may induce hemodynamic effects as well as natriuresis and diuresis at pharmacological levels. In this study, the same dose of AM (0.05 µg·kg body wt\(^{-1}\)·min\(^{-1}\)) resulted in different increases in plasma AM levels between HF rats (56 ± 29 fmol/ml) and control rats (30 ± 8 fmol/ml). These results raise the possibility that the clearance of AM may be decreased in HF rats.

Limitations. First, we could not assess the effect of AM on other vasoactive peptides such as renin and aldosterone because their measurements require a large volume of blood. Second, we could only measure RVSP as an index of pulmonary arterial systolic pressure. A more detailed analysis of pulmonary arterial pressure is needed to examine effects of AM on pulmonary circulation. Finally, this study was conducted in rats with chronic HF that was relatively well compensated. The effects of AM in the acute phase, or in the development of HF, need further study.

In conclusion, intravenous infusion of AM exerted diuresis and natriuresis without inducing hypotension and in the higher dose produced beneficial hemodynamic and renal vasodilator effects in rats with compensated HF.

Perspectives

The natriuretic and diuretic effects of AM infusion without a marked decrease in arterial pressure suggest that AM may be beneficial in the treatment of HF. Moreover, intravenous infusion of AM at pharmacological levels produced vasodilatation and increased CO in association with diuresis and natriuresis. These findings, together with the direct inotropic effect of AM (26), imply that AM may have a place among inotropic and vasodilator agents such as phosphodiesterase inhibitors. Thus intravenous infusion of AM may be a new therapeutic approach to the treatment of HF. Because AM has the great advantage of being an endogenous substance, large-scale human studies are necessary to confirm a potential therapeutic benefit of AM in patients with HF.
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