Ventricular Adrenomedullin Levels Correlate With the Extent of Cardiac Hypertrophy in Rats

Atsushi Morimoto, Toshio Nishikimi, Fumiki Yoshihara, Takeshi Horio, Noritoshi Nagaya, Hisayuki Matsuo, Kazuhiro Dohi, Kenji Kangawa

Abstract—We investigated the pathophysiological significance of adrenomedullin (AM) in the development of left ventricular hypertrophy (LVH). LVH was produced by aortic banding (AB) in rats. The left ventricular weight/body weight (LV/BW) ratio, ventricular AM peptide and mRNA levels, and hemodynamics were measured at 1, 3, 7, and 21 days after the operation. Both LV/BW ratio and ventricular AM levels showed a significant increase from 1 day after the operation in the AB rats versus the sham-operated rats. Both increased in a time-dependent manner. The ventricular AM levels correlated with the LV/BW ratio ($r=0.76$, $P<0.01$). The AM mRNA levels were highly expressed at 1 day after the operation in the AB rats but showed no difference from 3 to 21 days after the operation between the AB and sham groups. The plasma AM levels showed a peak at 1 day after the operation in both groups. Then, we treated AB rats with an angiotensin-converting enzyme inhibitor (quinapril) in 2 doses (1 and 10 mg·kg$^{-1}$·d$^{-1}$) for 21 days. The quinapril treatment attenuated similarly both the LV/BW ratio and the ventricular AM levels. We also assessed the effects of AM and hydralazine administration for 7 days on the LV/BW ratio and hemodynamics of AB rats. Both AM and hydralazine administration reduced the blood pressure by $\approx10\%$ compared with the nontreated AB rats, but a reduction of the LV/BW ratio was observed only in the AM-treated group ($P<0.05$). These results suggest that ventricular AM levels are elevated by chronic pressure overload in a time-dependent manner concomitant with the extent of LVH and that AM may play a pathophysiological role in the development of LVH in chronic pressure overload. (Hypertension. 1999;33:1146-1152.)

Key Words: adrenomedullin ■ gene expression ■ hypertrophy, left ventricular ■ pressure overload

Adrenomedullin (AM) is widely distributed in various tissues including the heart. Cloning of the cDNA encoding the AM precursor showed that this gene is highly expressed in the heart, adrenal glands, kidneys, and lungs of both rats and humans. Binding studies demonstrated that abundant and specific receptors for this peptide are highly present in the heart, lung, spleen, and kidneys. The presence of AM peptide, mRNA, and the receptors of AM in the heart indicates that this peptide may be involved in the regulation of cardiac hypertrophy. Indeed, previous studies revealed that ventricular AM levels are increased in several hypertensive models with cardiac hypertrophy. Furthermore, a recent study revealed that rats subjected to a chronic pressure overload by administration of angiotensin II have higher left ventricular weight and left ventricular AM mRNA levels. However, the pathophysiological significance and time course of AM peptide and mRNA levels in the development of pressure-overload hypertrophy remain unknown.

In the present study, to investigate the pathophysiological significance of AM in the development of left ventricular hypertrophy (LVH), we measured the hemodynamics and tissue peptide and mRNA levels of AM and plasma AM, hormone levels, and the degree of LVH at 1, 3, 7, and 21 days after an aortic banding operation in a LVH rat model. We also assessed the effect of an angiotensin-converting enzyme inhibitor (ACEI) on the hemodynamics, the ventricular peptide and mRNA levels of AM, and the degree of hypertrophy to elucidate the relationship between the regression of hypertrophy and ventricular AM levels. In addition, to investigate the effect of increased plasma AM levels on LVH, we assessed the effects of a chronic AM infusion on the hemodynamics and ventricular hypertrophy in this LVH model. We compared the effect of AM with hydralazine to examine the effect of AM (excluding the reduction of blood pressure).

Methods

Animals and Surgical Procedures

Study 1

Seven-week-old male Wistar rats (Oriental Bio Service Co, Kyoto, Japan) weighing from 250 to 300 g and given free access to standard rat chow and tap water were used. We randomly divided the rats into 2 groups: (1) a sham-operated group (n=24) and (2) an aortic banded...
(AB) group (n=28). The animals were anesthetized with sodium pentobarbital (30 mg/kg IP). The aortic banding was performed between the right and left renal arteries with a blunt 26-gauge needle to establish the diameter of the ligature. The sham procedure involved the same amount of anesthesia, approximately the same size of incision, and the placement of a loosely tied ligature in the identical position at the abdominal aorta. At 1, 3, 7, and 21 days after these operations, sham-operated and AB rats were anesthetized with sodium pentobarbital (30 mg/kg IP) and placed on a warming pad to maintain body temperature at 37°C. To measure arterial pressure and heart rate, the right carotid artery was cannulated with a PE-50 polyethylene catheter and the right femoral artery was cannulated with a PE-10 catheter fused to a PE-50 catheter. The catheters were connected to a transducer, and carotid and femoral arterial pressures were recorded on a polygraph (7758B System, Hewlett-Packard). The catheter inserted from the carotid artery was then advanced into the left ventricle (LV) to measure the left ventricular end-diastolic pressure (LVEDP). Blood samples were drawn from the catheter after hemodynamics were measured. The rats were then euthanized by an injection of 2 mmol of KCl and various tissues were weighed. The tissue specimens were immediately frozen in liquid nitrogen.

**Study 2**

To examine the effect of ACEI on the hemodynamics, tissue peptide and mRNA levels of AM, and the degree of hypertrophy, we produced LVH by an injection of 2 mmol of KCl and various tissues were weighed. The rats were divided into nontreated (n=11), low-dose (15 mg/L in drinking water, n=6), and high-dose (150 mg/L in drinking water, n=5) quinapril-administered groups. Quinapril was dissolved in the drinking water. The rats drank ~20 mL of drinking water per day. Thus, the low-dose ACEI treated group was administered 1 mg kg⁻¹ d⁻¹ of quinapril and high-dose treated group, 10 mg kg⁻¹ d⁻¹. On day 21 of quinapril treatment, the rats were anesthetized and the hemodynamics and blood and tissue samples were measured in the same manner as in Study 1.

**Study 3**

To investigate the effect of a chronic infusion of AM on hemodynamics and pressure overload–induced LVH, we administered the ACEI quinapril. We made AB rats (n=22) and sham rats (n=6) in the same manner as in Study 1. Quinapril was administered for 21 days from the day of the banding operation. AB rats were divided into nontreated (n=11), low-dose (15 mg/L in drinking water, n=6), and high-dose (150 mg/L in drinking water, n=5) quinapril-administered groups. Quinapril was dissolved in the drinking water. The rats drank ~20 mL of drinking water per day. Thus, the low-dose ACEI treated group was administered 1 mg kg⁻¹ d⁻¹ of quinapril and high-dose treated group, 10 mg kg⁻¹ d⁻¹. On day 21 of quinapril treatment, the rats were anesthetized and the hemodynamics and blood and tissue samples were measured in the same manner as in Study 1.

**AM mRNA Determination**

Total RNA was extracted from the left ventricle by the acid guanidinium thiocyanate–phenol-chloroform method, according to the method previously described. The total RNA pellet was dissolved in 0.1% diethyl pyrocarbonate–treated water and stored at -80°C until use. The RNA concentration was determined on the basis of absorbance at 260 nm. The transfer, cross-link, and hybridization were performed as reported previously with a 32P-labeled cDNA probe for rat AM. The band intensity was estimated by a radioimage analyzer (BAS-5000, Fuji Film). To normalize the rat AM signal to the loaded amounts and transfer efficiencies, the same membrane was rehybridized with an 18S oligonucleotide probe.
were highly expressed at 1 day after banding in the AB group compared with those in the sham-operated group, but at 3, 7, and 21 days after the banding, the mRNA levels of AM were not significantly different between the AB and sham-operated groups (Figures 1E and 2). The LV AM levels correlated with the LV/BW ratio \( r = 0.76, P < 0.01; n = 52 \); in contrast, the plasma AM levels did not correlate with the LV weight.

**Study 2: Effects of Quinapril on Hemodynamics, LV Weight, and the Peptide and mRNA Levels of AM**

As shown in Table 2, the nontreated and quinapril-treated AB rats had lower BW compared with the sham-operated rats. The heart rate was not significantly different among these groups. Quinapril administration decreased both the mean carotid and femoral arterial pressures in a dose-dependent manner. The LVEDP was normalized by the administration of both low- and high-dose quinapril. The weight of the right kidney was increased and that of the left kidney was decreased in the AB rats versus the sham-operated rats, and quinapril administration tended to decrease the bilateral renal weights in a dose-dependent manner. The plasma renin concentration was \( \approx 10 \)-fold higher in the low-dose quinapril group and \( \approx 20 \)-fold higher in the high-dose quinapril group than in the sham-operated group. In contrast, the elevated plasma aldoste-
Norepinephrine concentration due to AB was markedly decreased by administering quinapril.

Administration of quinapril for 21 days inhibited ventricular hypertrophy and similarly decreased the LV AM levels in a dose-dependent manner (Figure 3A and 3B). The LV AM levels correlated with the LV/BW ratio ($r=0.59$, $P<0.01$; $n=28$). The AM mRNA levels of the LV tended to decrease only in the high-dose quinapril group but not significantly (data not shown).

**Study 3: Effects of AM on Hemodynamics, LV Weight, and AM Levels**

As shown in Table 3, the nontreated, AM-infused, and hydralazine-administered AB rats had lower BW versus the sham-operated rats. The heart rate was not significantly different among these groups. The AM infusion and the hydralazine administration reduced the mean carotid arterial pressure by $\approx10\%$ compared with the nontreated AB group. The mean femoral artery pressure was not significantly different between the AB and AB with AM infusion groups; in contrast, it was significantly lower in the AB with hydralazine group. LVEDP was normalized by the AM infusion and by the hydralazine administration. The right kidney weight was increased and the left kidney weight was decreased in the nontreated AB group versus the sham-operated group. The AM infusion and hydralazine did not affect the renal weights. The plasma renin concentration tended to be higher in the hydralazine-treated group. In contrast, plasma aldosterone concentration tended to be lower in the AM infusion group and higher in the hydralazine group compared with the nontreated AB group, and it was significantly decreased in the AM infusion group versus the hydralazine-treated group.

The LV/BW ratio was attenuated only in the AB with AM infusion group compared with the nontreated AB group (Figure 4A). Hydralazine did not affect either the LV/BW ratio or the LV AM level. LV AM levels were 22% greater in the AM infusion group than in the nontreated AB group, and the plasma AM levels were about 5-fold greater in the AM infusion group compared with the nontreated AB group (Figure 4B and 4C).

**Immunohistochemistry**

The immunohistochemical analysis revealed that AM immunoreactivity was more intense in hypertrophied ventricular myocytes than normal ventricular myocytes. The hypertrophied ventricles showed perivascular fibrosis, and there was no evidence of immunoreactivity in such connective tissues (Figure 5).

**Discussion**

In the present study, we demonstrated that the ventricular AM levels increase with the development of ventricular hypertrophy induced by AB and that the LV AM levels correlated with the extent of LVH. The plasma AM levels showed a peak at 1 day after the aortic banding operation; higher AM gene expression was observed only 1 day after this operation.
The ACEI treatment decreased the LV AM levels with a concomitant reduction of LVH. In addition, a chronic AM infusion to AB rats significantly decreased the arterial pressure and LVH. These results suggest that the mechanisms underlying regulation of plasma and ventricular AM during the development of LVH by pressure overload differ from each other and that increased ventricular AM levels correlate with the extent of LVH induced by chronic pressure overload caused by aortic banding in rats.

In the present study, the LV AM levels were higher in the AB group than in the sham-operated group; levels gradually increased in a time-dependent manner with a concomitant increase of LV weight. The LV AM levels correlated with the extent of LVH. Previous studies showed that ventricular AM levels are also increased in several rat hypertension models.6–9 These results suggest that tissue AM levels in the ventricle become increasingly elevated with the development of pressure-overload cardiac hypertrophy. These findings may support a potential role for AM in cardiac hypertrophy.

Although the LV AM levels gradually increased in a time-dependent manner, the AM mRNA levels of LV were not elevated in the present AB group compared with the sham-operated group, except at 1 day after the operation. Shimokubo et al7 reported the similar finding that cardiac AM levels were higher in spontaneous hypertensive rats versus normotensive Wistar-Kyoto rats, although the AM mRNA levels in the spontaneously hypertensive rats were not significantly different from those of the control rats. These findings in chronic pressure-overloaded rats are consistent with our present results. In contrast, Kaiser et al14 recently reported that pressure overload by AB did not stimulate AM mRNA levels from soon after the operation to 28 days later. Although the reason for the discrepancy between their results and ours is unknown at present, the LV/BW ratio was not increased at 1 day after banding in their study, whereas it was significantly increased at 1 day after banding in the present study. A difference in the severity of the banding in the 2 studies may account for the discrepancy. The exact cellular mechanism of the elevation of LV AM levels without enhanced gene expression in pressure overloaded rats remains to be identified.

In the present study, plasma AM levels showed a peak at 1 day after the operation in the present AB group and a small peak in the sham-operated group. The small peak in the plasma AM levels at 1 day after the operation in the present sham-operated group may be due to the effect of invasive

<table>
<thead>
<tr>
<th>Variable</th>
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<th>AB + Quinapril, low Dose (n=6)</th>
<th>AB + Quinapril, High Dose (n=5)</th>
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<tr>
<td>BW, g</td>
<td>340±10*†</td>
<td>259±52*†</td>
<td>267±32*†</td>
<td>251±62*†</td>
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<td>HR, bpm</td>
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<td>443±15</td>
<td>438±21</td>
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<td>Mean CAP, mm Hg</td>
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<td>177±26†</td>
<td>142±14†</td>
<td>121±16†</td>
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<td>Mean FAP, mm Hg</td>
<td>126±4†</td>
<td>86±20*†</td>
<td>77±13*†</td>
<td>56±5*†</td>
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<td>LVEDP, mm Hg</td>
<td>6.0±1.8†</td>
<td>10.4±2.9*†</td>
<td>5.4±2.6†</td>
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<td>Right Kidney, g/kg</td>
<td>3.62±0.18†</td>
<td>5.66±1.07*†</td>
<td>5.33±0.39*†</td>
<td>5.17±0.27*†</td>
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<td>Left Kidney, g/kg</td>
<td>3.52±0.23†</td>
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<tr>
<td>PRC, ng Ang/mL per h</td>
<td>4.9±1.5</td>
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<td>83.3±29.6*†</td>
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<td>PAC, ng/L</td>
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<td>1793±1302*†</td>
<td>391±154†</td>
<td>327±130†</td>
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Abbreviations as in Table 1. Values are mean±SD.

*P<0.05 vs sham; †P<0.05 vs nontreated AB rats.

Figure 3. Effects of quinapril on LV/BW ratio (A) and LV AM level (B), AB indicates aortic banding. *P<0.05 vs sham-operated group, †P<0.05 vs AB group. Data are mean±SD.

Figure 4. Effects of AM and hydralazine (H) treatment on LV/BW ratio(A), ventricular AM level (B), and plasma AM level (C). AB indicates aortic banding. *P<0.05 vs sham-operated group, †P<0.05 vs AB group, ‡P<0.05 vs AB treated with AM group. Data are mean±SD.
operation. The higher peak of the AB group at 1 day after the operation compared with the sham-operated rats may be due to mechanical, stress-stimulated AM production at the vascular wall in addition to the effect of invasive operation. It has been reported that mechanical stress stimulates the AM production in vascular endothelial walls. The elevated plasma AM levels in the chronic phase in the present AB group may also reflect mechanical, stress-stimulated AM production from systemic vascular walls with pressure overload. It has been found that shear stress augments expression of AM from the vascular endothelial wall in a time-dependent manner. The exact mechanism of increased plasma AM levels in the chronic phase of this model needs further study.

In this study, AB rats treated with quinapril showed a similar decrease in both LV AM levels and LV/BW ratio. In study 1, we demonstrated that the ventricular AM levels increase with the development of ventricular hypertrophy in a time-dependent manner. In addition, LV AM levels decreased according to the regression of LVH by quinapril treatment. These results suggest that AM and the renin-angiotensin-aldosterone system may affect each other and regulate the cardiac hypertrophy. However, the exact mechanism of AM in cardiac hypertrophy is unknown at present. Further research is necessary to reveal the exact role of tissue AM of LV in cardiac hypertrophy.

The infusion of AM and the administration of hydralazine to AB rats produced a similar reduction in the mean carotid arterial pressure of ~10%. However, only the AM infusion reduced ventricular weight. It is well known that the reduction of blood pressure by hydralazine induces the activation of the sympa-

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**TABLE 3. Body Weight, Hemodynamics, Kidney Weights, and Plasma Renin and Aldosterone Concentrations in Sham-Operated Rats and Nontreated and Adrenomedullin and Hydralazine-Treated AB Rats**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham (n=5)</th>
<th>Nontreated AB (n=11)</th>
<th>AB+AM (n=10)</th>
<th>AB+Hydralazine (n=10)</th>
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</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>301±10†</td>
<td>260±52*</td>
<td>279±57*</td>
<td>266±16*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>442±12</td>
<td>436±22</td>
<td>440±20</td>
<td>441±18</td>
</tr>
<tr>
<td>Mean CAP, mm Hg</td>
<td>122±4†</td>
<td>176±6*</td>
<td>159±11†</td>
<td>159±8†</td>
</tr>
<tr>
<td>Mean FAP, mm Hg</td>
<td>120±4†</td>
<td>68±16*</td>
<td>70±18*</td>
<td>48±12†</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>4.6±1.7†</td>
<td>10.9±2.5*</td>
<td>5.6±1.9†</td>
<td>6.4±2.4†</td>
</tr>
<tr>
<td>Right Kidney, g/kg</td>
<td>3.78±0.33†</td>
<td>6.15±0.59*</td>
<td>5.71±0.63*</td>
<td>6.17±0.55*</td>
</tr>
<tr>
<td>Left Kidney, g/kg</td>
<td>3.62±0.24†</td>
<td>2.87±0.31*</td>
<td>2.84±0.45*</td>
<td>2.91±0.21*</td>
</tr>
<tr>
<td>PRC, ng Ang l/mL per h</td>
<td>4.7±1.0†</td>
<td>25.6±18.1*</td>
<td>23.4±11.0*</td>
<td>32.5±7.5*</td>
</tr>
<tr>
<td>PAC, ng/L</td>
<td>814±92††</td>
<td>5587±4499*</td>
<td>4357±2853</td>
<td>6978±3507‡</td>
</tr>
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</table>

Abbreviations as in Table 1.
Values are mean±SD.
*P<0.05 vs sham, †P<0.05 vs non-treated AB, ‡P<0.05 vs AB treated with AM group.

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**Figure 5.** AM immunoreactivity using a monoclonal antibody that recognizes the C-terminal structure of AM in the ventricle. AM immunoreactivity is more intense in hypertrophied myocytes, and the hypertrophied ventricles showed perivascular fibrosis. A, Aortic banding ventricle; B, sham-operated ventricle; and C, control ventricle (nonimmune mouse IgG was used).
Adrenomedullin in Left Ventricular Hypertrophy

Adrenomedullin (AM) is a hypotensive peptide that is produced and secreted from vascular smooth muscle cells: augmented production by tumor necrosis factor-alpha. These results suggest that increased plasma AM may lead to the regression of LVH.

In conclusion, we demonstrated that LV AM levels gradually increased in a time-dependent manner similar to the LV/BW ratio in the development of hypertrophy. The immunohistochemistry revealed that hypertrophied myocytes may be a major source of increased ventricular AM levels during the progression of LVH.

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References


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