Cardiac adrenomedullin gene expression and peptide accumulation after acute myocardial infarction in rats

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Adrenomedullin (AM) is a potent vasodilator peptide that was originally isolated from human pheochromocytoma (8). Subsequent studies have shown that AM peptide and mRNA are distributed in a variety of tissues, including the heart (6, 24). Binding studies have demonstrated the presence of specific receptors for AM in the heart (20). We have recently shown that cardiac myocytes produce and secrete AM (17), and the production of AM has been reported to be increased in the failing heart (16) and hypertrophied ventricles (12). AM increases left ventricular contractility in vivo (13, 21) and exerts a direct inotropic effect in vitro (27). On the other hand, AM has been shown to be a possible endogenous suppressor of myocyte hypertrophy (28) and fibroblast proliferation (29), which might affect the remodeling process of the heart (22). These findings suggest that AM synthesis is augmented in ventricular disorders and that AM may play a role in modulating cardiac function and the structure of the heart as a paracrine and/or autocrine factor.

Recently, we and others have demonstrated that plasma AM level increases in the early phase of acute myocardial infarction (MI; see Refs. 9 and 11) and that a high plasma level is strongly related to congestive heart failure, complicating MI (9) and leading to a poor prognosis (14). These findings raise the possibility that cardiac AM synthesis is rapidly induced in the infarcted and/or noninfarcted myocardium, particularly after a large MI, and that AM may play a role in the pathophysiology of MI. However, little information is available regarding cardiac AM synthesis and potential stimulation of AM after MI. Thus the purpose of this study was 1) to examine the time course of left ventricular AM production in infarcted and noninfarcted myocardium using an experimental rat model of MI and 2) to investigate the effects of an angiotensin-converting enzyme inhibitor (ACEI), which is known to cause a reduction of preload and afterload and inhibition of myocyte hypertrophy via blocking ANG II production (25), on AM levels in the infarcted and noninfarcted regions.

**METHODS**

Model of MI. Male Wistar rats weighing 180–230 g were used in this study. MI was produced by a previously described method (18). 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. 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 underwent sham operation consisting of thoracotomy and cardiac exposure but without coronary artery ligation. The surviving rats were maintained on standard rat chow.

Study protocol. First, to examine the time course of AM peptide levels in the infarcted and noninfarcted myocardium, rats were killed at the following six time points: 6 h (MI, n = 9; sham, n = 7), 24 h (MI, n = 9; sham, n = 7), 3 days (MI, n = 8; sham, n = 7), 1 wk (MI, n = 9; sham, n = 7), 2 wk (MI, n = 9; sham, n = 7), and 4 wk (MI, n = 9; sham, n = 7) after the operation. Second, AM mRNA levels in the infarcted and noninfarcted regions were also evaluated 6 h, 1 wk, and 4 wk after surgery (MI, n = 5; sham, n = 5). Third, AM immunoreactivity in the infarcted and noninfarcted regions was assessed 1 and 4 wk after operation. Finally, to assess the effect of ACEI on ventricular AM synthesis, delapril was administered to MI rats for 1 wk after surgery (n = 9). Delapril was dissolved in drinking water at 1.0 g/l.

Hemodynamic studies. Rats were anesthetized by intraperitoneal injection of pentobarbital sodium (30 mg/kg body wt) and were 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 (PE-50) was inserted in the right carotid artery for measurement of heart rate and mean arterial pressure. Next, the catheter was advanced in the left ventricle for measurement of left ventricular end-diastolic pressure. These hemodynamic variables were measured using a pressure transducer (model P 23 ID; Gould) connected to a polygraph and were recorded with a thermal recorder (7758 B System; Hewlett-Packard). After completion of hemodynamic measurements, 4 ml of blood were drawn from the carotid artery for measurements of plasma AM. The heart was arrested by the injection of 2 mmol KCl through the carotid artery, and the cardiac ventricles were excised. The size of MI was determined in all rats with MI, except for those 6 h postinfarction, as reported previously (3). In brief, incisions were made in the left ventricle so that the left ventricular tissue could be pressed flat. The circumference of the entire flat left ventricle and the visualized infarcted area, as judged from both epicardial and endocardial sides, was outlined on a clear plastic sheet. The difference in weight between the two marked areas on the sheet was used to determine infarction size and was expressed as a percentage of left ventricular surface area. These tissues were frozen in liquid nitrogen and stored at −80°C until RIA or RNA blot analysis.

RIA for AM. Tissues for RIA were weighed and boiled in 10 vol of 1 mol/l acetic acid for 10 min to inactivate intrinsinc proteases. The tissues were homogenized with a Polytron mixer. The homogenate was centrifuged at 3,000 g for 30 min, and the supernatant was centrifuged again at 15,000 g for 10 min. The supernatant was evaporated in a vacuum until dry. The RIA for rat AM in tissue was performed as reported previously (24). Plasma AM was determined by RIA after extraction with Sep-Pak C18 cartridges (Millipore; Waters, Milford, MA), as previously reported (13, 24).

Northern blot analysis for AM mRNA. Total RNA was extracted from the left ventricle by the acid guanidium thiocyanate-phenol-chloroform method (26). Total RNA (20 µg/lane) was electrophoresed on 1% agarose and then was transferred to a nylon membrane. Hybridization and washing of the membrane were performed using a [32P]cDNA probe for rat AM mRNA, as reported previously (16). The band intensity was estimated by a radioimage analyzer (BAS-5000; Fuji Film, Tokyo, Japan). To normalize the rat AM signal to the loaded amount and transfer efficiency, the same membrane was rehybridized with an oligonucleotide probe for 18S rRNA.

Immunohistochemistry. Immunohistochemical studies were performed to localize AM protein in left ventricular myocardium after MI. The left ventricle was immediately fixed with 10% Formalin. Ventricular tissues were embedded in paraffin, and 4-µm-thick sections were cut and mounted on glass slides treated with silica. The slides were incubated overnight at 60°C and were deparaffinized with graded concentrations of xylene and ethanol. Immunohistochemical analysis was performed using a monoclonal antibody recognizing AM-(46–52) (dilution of ascites, 1:200), as reported previously (10). Nonimmune mouse IgG was used as a control. The presence of immunoreactive AM was assessed by light microscopy by trained observers.

Statistical analysis. Numerical values are expressed as means ± SD unless otherwise indicated. Comparisons of means between two groups were made by unpaired Student’s t-test. Comparisons of variables among the six groups were made using the one-way ANOVA, followed by the Scheffe’s multiple comparison test. Correlation coefficients between ventricular AM level and infarct size or hemodynamic variables were calculated by linear regression analysis. A P value <0.05 was considered significant.

RESULTS

Infarct size, ventricular weight, and hemodynamic variables. The physiological profiles of the experimental groups are summarized in Table 1. There were no significant differences in infarct size within MI groups over time. Left ventricular weight tended to increase from 6 h to 2 wk postinfarction and was significantly increased at 4 wk compared with sham-operated rats. Left ventricular end-diastolic pressure was significantly higher in MI rats than in sham-operated rats throughout the 4-wk period, whereas mean arterial pressure was significantly lower in MI rats than in sham-operated rats.

Time course of ventricular AM levels in infarcted and noninfarcted myocardium. In sham-operated rats, ventricular AM level was slightly elevated on days 1 and 3 and returned to near baseline thereafter (Fig. 1). In MI rats, the ventricular AM level increased 1.5-fold in the infarcted region and 1.7-fold in the noninfarcted region as early as 6 h postinfarction compared with sham-operated rats. Subsequently, AM level in the infarcted region reached its peak (2.6-fold) 1 wk postinfarction and thereafter decreased to normal. In the noninfarcted region, however, AM level remained elevated for at least 4 wk compared with sham-operated rats. In MI rats, ventricular AM level at 3 and 7 days was significantly higher in the infarcted region than in the noninfarcted region, whereas the AM level at 2 and 4 wk was significantly higher in the noninfarcted region than in the infarcted region.

Ventricular AM mRNA levels in infarcted and noninfarcted myocardium. Ventricular AM mRNA expression was increased 11-fold in the infarcted region and 6-fold in the noninfarcted region as early as 6 h postinfarction compared with sham-operated rats (Fig. 2). AM mRNA level remained elevated 2.3-fold in the infarcted region and 1.9-fold in the noninfarcted region 1 wk after MI compared with sham-operated rats. Ventricular AM
mRNA levels 4 wk after MI tended to be increased compared with those of sham-operated rats, although these changes did not reach statistical significance.

Immunohistochemical analysis. AM immunoreactivity was intense in myocytes surrounding the infarct and in surviving myocytes in the infarcted region 1 wk after MI compared with sham-operated rats (Fig. 3). Intense immunostaining for AM was also observed in myocytes in the noninfarcted region 1 and 4 wk after MI. No immunostaining was observed in necrotic myocytes and fibrous tissue.

Relations between ventricular AM level and infarct size and hemodynamic variables. When all data on MI rats were included, ventricular AM level in the noninfarcted region was also positively correlated with infarct size and hemodynamic variables. When all data on MI rats were included, ventricular AM level at 1 wk postinfarction (Fig. 5). In MI rats treated with ACEI, ventricular AM level did not significantly differ between the infarcted and noninfarcted regions.

Plasma AM level after MI. Plasma AM levels from 24 h to 4 wk were significantly higher in MI rats than in sham-operated rats (Fig. 6). Unlike ventricular AM levels, plasma AM levels were maximal 4 wk after MI. When all data on MI rats were included, plasma AM levels were not significantly correlated with ventricular AM levels in the infarcted region (r = −0.25, P = not significant, n = 50) or those in the noninfarcted region (r = −0.04, P = not significant, n = 50).

**DISCUSSION**

In this study, we demonstrated that 1) ventricular AM peptide and mRNA levels were significantly increased as early as 6 h postinfarction, 2) AM level in the infarcted region reached its peak at 1 wk and decreased thereafter, whereas AM level in the noninfarcted region remained elevated throughout 4 wk after MI, and 3) intense immunostaining for AM was limited to myocytes in both the infarcted and noninfarcted regions. We also demonstrated that 4) AM level in the noninfarcted region correlated positively with infarct size and left ventricular end-diastolic pressure and that 5) an oral ACEI suppressed the overproduction of AM 1

### Table 1. Baseline characteristics of MI and sham-operated rats

<table>
<thead>
<tr>
<th>Time</th>
<th>Number of Rats</th>
<th>Body Wt, g</th>
<th>Infarct Size, %</th>
<th>LV Wt/Body Wt, g/kg</th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>LVEDP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Hours</td>
<td>MI</td>
<td>9</td>
<td>192 ± 8</td>
<td>2.2 ± 0.1</td>
<td>427 ± 36</td>
<td>98 ± 16*</td>
<td>18 ± 5*</td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>7</td>
<td>189 ± 10</td>
<td>2.0 ± 0.2</td>
<td>446 ± 32</td>
<td>121 ± 12</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>24 Hours</td>
<td>MI</td>
<td>9</td>
<td>186 ± 15</td>
<td>36 ± 12</td>
<td>2.5 ± 0.2</td>
<td>456 ± 30</td>
<td>94 ± 15*</td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>7</td>
<td>177 ± 14</td>
<td>21.0 ± 0.2</td>
<td>460 ± 32</td>
<td>132 ± 22</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>3 Days</td>
<td>MI</td>
<td>8</td>
<td>183 ± 16</td>
<td>30 ± 12</td>
<td>2.3 ± 0.2</td>
<td>449 ± 30</td>
<td>96 ± 16*</td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>7</td>
<td>182 ± 16</td>
<td>21.0 ± 0.2</td>
<td>424 ± 44</td>
<td>121 ± 8</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>1 Week</td>
<td>MI</td>
<td>9</td>
<td>202 ± 16</td>
<td>31 ± 13</td>
<td>2.1 ± 0.3</td>
<td>420 ± 30</td>
<td>106 ± 18*</td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>7</td>
<td>200 ± 14</td>
<td>2.0 ± 0.2</td>
<td>443 ± 31</td>
<td>89 ± 8*</td>
<td>11 ± 3*</td>
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<tr>
<td>ACEI</td>
<td></td>
<td>9</td>
<td>33 ± 12</td>
<td>2.0 ± 0.2</td>
<td>401 ± 27</td>
<td>106 ± 22</td>
<td>16 ± 8*</td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>7</td>
<td>32 ± 14</td>
<td>1.8 ± 0.1</td>
<td>403 ± 29</td>
<td>124 ± 9</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>MI</td>
<td>9</td>
<td>212 ± 26*</td>
<td>33 ± 12</td>
<td>2.0 ± 0.2</td>
<td>401 ± 27</td>
<td>106 ± 22</td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>7</td>
<td>253 ± 7</td>
<td>1.8 ± 0.1</td>
<td>403 ± 29</td>
<td>124 ± 9</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>4 Weeks</td>
<td>MI</td>
<td>9</td>
<td>249 ± 35*</td>
<td>38 ± 6</td>
<td>2.2 ± 0.2</td>
<td>401 ± 31</td>
<td>106 ± 15*</td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>7</td>
<td>286 ± 11</td>
<td>1.8 ± 0.1</td>
<td>411 ± 41</td>
<td>123 ± 7</td>
<td>4 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SD. MI, acute myocardial infarction; ACEI, angiotensin-converting enzyme inhibitor; LV, left ventricular; HR, heart rate; MAP, mean arterial pressure; LVEDP, left ventricular end-diastolic pressure. *P < 0.05 vs. sham-operation group; †P < 0.05 vs. ACEI group.

Fig. 1. Time course of left ventricular adrenomedullin (AM) levels in infarcted and noninfarcted myocardium after acute myocardial infarction (MI). Values are means ± SE. *P < 0.05 vs. sham-operated rats; †P < 0.05 vs. noninfarcted region.
wk postinfarction in association with decreases in left ventricular end-diastolic pressure and mean arterial pressure.

AM is produced by the normal heart (6, 24), and its production is augmented in the failing heart (16) and hypertrophied ventricle (12). Specific receptors for AM are present in the heart (20). In an in vitro study, AM has been shown to inhibit myocyte hypertrophy (28) and fibroblast proliferation (29). These findings suggest the possible involvement of cardiac AM in the pathophysiology of ventricular disorders after MI. Indeed, we have recently shown that plasma AM level increases in the early phase of MI (9, 11). However, little information is available regarding cardiac AM synthesis and its potential stimuli after MI.

The present study is the first demonstration of the rapid induction of cardiac AM gene expression and protein synthesis after MI. MI rats 6 h after surgery showed marked elevation of left ventricular end-diastolic pressure, indicating the presence of acute ventricular overload in this model. A recent experimental study has shown that ventricular AM peptide and mRNA levels increase in response to acute cardiac overload produced by infusion of arginine vasopression for 2 h (23). Taken together, these results suggest that cardiac AM synthesis is rapidly induced, at least in part, by acute mechanical load after MI. Despite the marked increase in AM mRNA levels in the infarcted region 6 h postinfarction, its protein levels were demonstrated to be modestly increased in the present study. Although the mechanisms responsible for this discrepancy remain undetermined, it is interesting to speculate that the marked increase in AM mRNA might reflect compensatory responses to the rapid degradation of cardiac AM protein in an acute phase of MI.

MI is known to cause dilatation and thinning of the infarcted region in the early phase, i.e., infarct expansion (22), leading to increased wall stress in the infarcted region and the border zone between the infarcted and noninfarcted regions (1). In the present study, AM peptide level in the infarcted region reached its peak 1 wk after MI. Intense immunostaining for AM was observed predominantly in surviving myocytes in the infarcted region and in myocytes surrounding the infarct 1 wk after MI. Similarly, earlier studies have shown that AM immunoreactivity was intense in myocytes, but not nonmyocytes, in the failing heart (7) and the hypertrophied heart (12). These findings suggest that the ventricular myocyte, not the nonmyocyte, may be a major source of increased ventricular AM after MI. An experimental study has shown that both ventricular brain natriuretic peptide (BNP) and BNP mRNA expression are increased in the surviving myocytes in the infarcted region and the border zone (4). Thus the localization of AM synthesis after MI mimics that of BNP. These results suggest that, like BNP, AM synthesis in myocytes may be related to increased mechanical force in the process of infarct expansion after MI.

On the other hand, MI is also known to produce eccentric hypertrophy of the noninfarcted region over 1 mo after MI (22). In the present study, MI rats 4 wk postinfarction showed significant elevation of left ventricular weight compared with sham-operated rats, indicating the presence of progressive ventricular remodeling in this model. Surprisingly, AM level in the noninfarcted region remained elevated for at least 4 wk after MI, although AM level in the infarcted region decreased to normal at 2 and 4 wk after MI. Four weeks postinfarction, intense immunostaining for AM was observed mainly in myocytes in the noninfarcted region. In addition, AM level in the noninfarcted region correlated positively with infarct size, which is related to ventricular remodeling after MI (2). The AM level also correlated positively with left ventricular end-

![Fig. 2. A: representative images of left ventricular AM gene expression in infarcted and noninfarcted myocardium 6 h, 1 wk, and 4 wk after MI. B: quantitative analysis of ventricular AM gene expression in infarcted and noninfarcted myocardium after MI. *P < 0.05 vs. sham-operated rats; †P < 0.05 vs. noninfarcted region.](http://ajpregu.physiology.org/
diastolic pressure. These results suggest that cardiac AM synthesis in the late phase of MI may be related to reactive myocyte hypertrophy in the noninfarcted region in the process of left ventricular remodeling.

We have recently shown that AM synthesis in cardiac myocytes is enhanced by various cytokines such as interleukin (IL)-1β and tumor necrosis factor-α (TNF-α; see Ref. 5). In addition, Ono et al. (19) have shown that mRNA levels of IL-1 and TNF-α in the infarcted region rise steeply 1 wk postinfarction and thereafter fall to near baseline. In contrast, cytokine gene expression remained elevated in the noninfarcted region. Thus the time course of IL-1 and TNF-α synthesis in the infarcted and noninfarcted regions appears to mimic that of AM synthesis observed in the present study. These results suggest that the high cardiac levels of IL-1 and

Fig. 3. Color photomicrographs showing immunohistochemical staining of left ventricular AM after MI. Intense immunostaining for AM was observed in myocytes surrounding the infarct (A, arrows), in surviving myocytes in the infarcted region (B, arrows), and in myocytes in the noninfarcted region (C and D). A: border zone of infarcted and noninfarcted myocardium 1 wk after MI; B: infarcted myocardium 1 wk after MI; C: noninfarcted myocardium 1 wk after MI; D: noninfarcted myocardium 4 wk after MI; E: normal myocardium 1 wk after sham operation; F: section stained with mouse IgG as negative control. Magnification ×200 (A, C, D, E, and F) and ×400 (B).
TNF-α may partly contribute to the augmented production of AM after MI.

Interestingly, the overproduction of AM 1 wk postinfarction in both the infarcted and noninfarcted regions was significantly suppressed by an ACEI, which is known to cause a reduction of cardiac preload and afterload and inhibition of myocyte hypertrophy via blocking ANG II production (25). ACEI also decreased mean arterial pressure and left ventricular end-diastolic pressure. Because cardiac mechanical stress is an important stimulus for cardiac AM synthesis (23) and because hypertrophied myocytes are a major source of AM production (12), the inhibitory effect of ACEI on cardiac AM synthesis may be attributable, at least in part, to a decrease in ventricular load and inhibition of myocyte hypertrophy.

In the present study, plasma AM levels did not change in parallel with ventricular AM levels after MI. Immunoreactive AM has been detected in a variety of tissues, including vascular smooth muscle cells, endothelial cells, heart, lungs, and kidneys. These findings indicate that circulating AM levels do not necessarily depend on cardiac AM synthesis in rats with MI.

It remains unclear how endogenous AM functions in the failing ventricle after MI. AM has recently been reported to increase myocardial contractility in vivo (13, 21) and to exert a direct inotropic effect in vitro (27). Considering that ventricular AM peptide and mRNA increase in response to acute cardiac overload after MI, AM in cardiac myocytes may function as an endogenous positive inotropic substance to oppose deterioration of cardiac performance. On the other hand, in vivo, we have shown that AM significantly reduces proliferation and collagen synthesis of cultured cardiac fibroblasts, possibly via a cAMP-dependent pathway (29). Ventricular AM level in the infarcted region 1 wk postinfarction was ~10⁻⁹ M, which significantly increases cAMP level in fibroblasts (17). Taken together with the potent inhibitory effect of AM on myocyte hypertrophy (28), increased AM in myocytes may play a role in a paracrine or autocrine fashion in inhibiting fibroblast proliferation and myocyte hypertrophy in the process of left ventricular remodeling after MI. Further studies are necessary to elucidate the pathophysiological role of cardiac AM after MI.

Limitations. In the present study, we did not examine the change in left ventricular volume, which is an indicator of progressive ventricular remodeling. In addition, AM synthesis was examined for a relatively short period (4 wk). Nevertheless, MI rats 4 wk postinfarction had increased left ventricular weight compared with sham-operated rats, indicating the presence of progressive ventricular remodeling in this model.

In summary, cardiac AM synthesis was rapidly induced in both the infarcted and noninfarcted regions...
after MI. The subsequent ventricular AM in the two regions demonstrated different time-concentration curves in 4 wk after MI. The AM may be synthesized predominantly by cardiac myocytes, but not by fibroblasts, at least in part, in association with increased ventricular load in the process of left ventricular remodeling after MI.

Perspectives

We have recently shown that short-term infusion of AM has beneficial hemodynamic and renal effects in patients with congestive heart failure (15), suggesting the potential therapeutic benefit of AM in acute congestive heart failure. However, long-term cardiovascular effects of AM still remain unknown. In the present study, sustained elevation of ventricular AM levels was noted in myocytes surrounding the infarct, in surviving myocytes in the infarcted region, and in myocytes in the noninfarcted region after MI. In vitro, AM has been shown to inhibit fibroblast proliferation and myocyte hypertrophy. Thus it is interesting to speculate that the sustained elevation of ventricular AM has cardioprotective effects on progressive left ventricular remodeling in a paracrine and/or autocrine fashion. Long-term effects of AM on the failing ventricle should be confirmed by another study.

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