Adrenomedullin modulates hemodynamic and cardiac effects of angiotensin II in conscious rats

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Luodonpää, Marja, Hanna Leskinen, Mika Ilves, Olli Vuolteenaho, and Heikki Ruskoaho. Adrenomedullin modulates hemodynamic and cardiac effects of angiotensin II in conscious rats. Am J Physiol Regul Integr Comp Physiol 286: R1085–R1092, 2004. First published January 29, 2004; 10.1152/ajpregu.00726.2003.—We examined whether adrenomedullin, a vasoactive peptide expressed in the heart, modulates the increase in blood pressure, changes in systolic and diastolic function, and left ventricular hypertrophy produced by long-term administration of ANG II or norepinephrine in rats. Subcutaneous administration of adrenomedullin (1.5 μg·kg⁻¹·h⁻¹) for 1 wk inhibited the ANG II-induced (33.3 μg·kg⁻¹·h⁻¹ sc) increase in mean arterial pressure by 67% (P < 0.001) but had no effect of norepinephrine-induced (300 μg·kg⁻¹·h⁻¹ sc) hypertension. Adrenomedullin enhanced the ANG II-induced improvement in systolic function, resulting in a further 9% increase (P < 0.01) in the left ventricular ejection fraction and 19% increase (P < 0.05) in the left ventricular fractional shortening measured by echocardiography, meanwhile norepinephrine-induced changes in systolic function were remained unaffected. Adrenomedullin had no effect on ANG II- or norepinephrine-induced left ventricular hypertrophy or expression of hypertrophy-associated genes, including contractile protein and natriuretic peptide genes. The present study shows that adrenomedullin selectively suppressed the increase in blood pressure and augmented the improvement of systolic function induced by ANG II. Because adrenomedullin had no effects on ANG II- and norepinephrine-induced left ventricular hypertrophy, circulating adrenomedullin appears to act mainly as a regulator of vascular tone and cardiac function.

catecholamines; hypertension; ventricular function

CARDIAC HYPERTROPHY is a physiological process adapting the heart to an increased hemodynamic workload. Initially, hypertrophy is a compensatory mechanism but eventually leads to pathologic myocyte hypertrophy characterized by increase in cell size, enhanced sarcomeric organization, and induction of the fetal gene program (14). Experimental and clinical evidence suggests that ANG II has an important role in cardiac and vascular pathology and that under some circumstances the renin-angiotensin system (RAS) is a predominant factor in development of left ventricular hypertrophy (LVH) (7). Also adrenergic signaling via sympathetic nervous system may play an important role in the adaptation of the heart to pressure and volume overload (14) and LVH is often associated with an increase in intracardiac sympathetic nerve activity and elevated plasma catecholamines (1).

Recently, several reports have suggested a role for adrenomedullin (AM) in paracrine and/or autocrine regulation of cardiac function and myocyte growth. High levels of AM mRNA (28) and 125I-labeled AM binding sites (23) have been identified in the heart. Both circulating immunoreactive (ir)-AM concentrations and cardiac AM mRNA levels increase in heart failure (11), suggesting a role for AM in regulating the function and structure of the failing myocardium. Consistent with this hypothesis, AM has been reported to exert a direct positive inotropic effect in isolated perfused rat heart in vitro (30) and to attenuate ANG II- and serum-stimulated protein synthesis in cardiac myocytes (33). Furthermore, we reported a selective inhibition of ANG II-induced hypertrophic changes in cultured cardiac myocytes by AM (15). However, the role of AM in development of LVH and regulation of cardiac gene expression in vivo is not known. The aim of the present study was to examine whether long-term administration of AM modulates the hypertension, cardiac function, and LVH induced by ANG II or α-adrenergic agonist norepinephrine (NE) in vivo in conscious rats.

METHODS

Animals and drugs. Male, 10- to 12 wk-old Sprague-Dawley (SD) rats from the colony of the Center of Experimental Animals at the University of Oulu, Finland, were used. The experimental design was approved by the Animal Experimentation Committee of the University of Oulu, Finland. The appearance and well-being of the animals were checked at least once a day. Despite the weight loss during ANG II administration, no signs of discomfort were observed. ANG II and NE were purchased from Sigma (St. Louis, MO) and AM (rat 1–50) from Phoenix Pharmaceuticals (Belmont, CA).

Telemetric monitoring and osmotic minipump implantation. Rats were anesthetized with 0.26 mg/kg fentanyl citrate, 8.25 mg/kg midazolam intraperitoneally. Aorta was cannulated cranial to the bifurcation through a midline abdominal incision and telemetry device (TA11PAC04, Data Sciences International, St. Paul, MN) was implanted. Buprenorphine (0.3 mg/kg sc) was used during the first day after operation for postsurgical analgesia. Mean arterial pressure (MAP) and heart rate (HR) were continuously measured through the recovery period. One week after implant operation, administration of vehicle (0.9% saline), AM (1.5 μg·kg⁻¹·h⁻¹), ANG II (33.3 μg·kg⁻¹·h⁻¹), or NE (300 μg·kg⁻¹·h⁻¹) or a combination of AM plus ANG II or AM plus NE was started with osmotic minipumps (Alzet model 2001, Alza Pharmaceuticals, Palo Alto, CA) placed subcutaneously during inhalation anesthesia with isoflurane. Hemodynamics were recorded every 10 min and averaged for every hour throughout the experiment. Results shown in this study represent 1-h average at 0600 every day.

Echocardiography. Transthoracic echocardiograms were performed before the implantation of the minipumps and at the end of the experiment (day 7) using Acuson Ultrasound System (Sequoia 512) and a 15-MHz linear transducer (15L8) (Acuson, Mountain View, CA). Rats were sedated with ketamine (50 mg/kg ip) and xylazine (10 mg/kg ip). A two-dimensional short axis view of the left ventricle was

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obtained at the level of the papillary muscle. Left ventricle (LV) end-systolic (LVESD) and end-diastolic (LVEDD) dimensions as well as interventricular septum (IVS) and posterior wall (PW) thickness were measured from the M-mode tracings. LV fractional shortening (FS) and ejection fraction (EF) were calculated from the following equations:

$$\text{FS} (\%) = \left( \frac{\text{LVEDD} - \text{LVESD}}{\text{LVEDD}} \right) \times 100$$

$$\text{EF} (\%) = \left( \frac{\text{LVEDD}^3 - \text{LVESD}^3}{\text{LVEDD}^3} \right) \times 100.$$

Mitral flow was recorded from an apical four-chamber view. Measurements of peak flow velocity of the early rapid diastolic filling wave (E) and the late diastolic filling wave (A) were made. All calculations were made from at least three consecutive cardiac cycles.

Tissue preparation. After 7-day administration of drugs, the rats were decapitated, the thoracic cavity was opened, and the heart was removed. Ventricles were divided into a right ventricular free wall portion and a left ventricular septal portion containing both right and left septa. All the cardiac tissue samples were blotted dry, weighed, immersed in liquid nitrogen, and stored at −70°C until extraction.

RNA analysis. RNA was isolated from tissue samples by ultracentrifugation through a cesium chloride cushion (6). For Northern blot analyses, 20- to 30-μg samples of total RNA were transferred to nylon membranes. The cDNA probes for rat skeletal muscle β-actin (Sk-β-A, 234 bp), rat cardiac β-actin (Ca-β-A, 158 bp), rat α-myosin heavy chain (α-MHC, 149 bp), rat α-myosin heavy chain (α-MHC, 91 bp), rat B-type natriuretic peptide (BNP, 433 bp), rat ribosomal 18S RNA (482 bp), and a full-length rat ANP cDNA probe Car-55 (9) were labeled with [32P]dCTP by random prime labeling. The membranes were hybridized and washed as previously described (16) and exposed to PhosphorImager screens. The screens were scanned with Molecular Imager FX (Bio-Rad Laboratories, Hercules, CA). The hybridization signals were normalized to signals of 18S RNA in each sample.

Table 1. Forward and reverse primers for quantitative PCR

<table>
<thead>
<tr>
<th>Probe</th>
<th>Sense Primer and Antisense Primer</th>
<th>Fluorogenic Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S</td>
<td>TGGTTGCAAAGCTGAAACTTAAAG</td>
<td>CGGCGGGTTTATCCTCA</td>
</tr>
<tr>
<td>AM-R</td>
<td>AGTCAATTAAAGCGGCGACCCG</td>
<td>CAACCTGGGCGGCTGACCC</td>
</tr>
<tr>
<td>RAMP-2</td>
<td>GAGGAGGCTCTGGGGATAGGG</td>
<td>AGCGGCGAGCAAAGTTGCTGG</td>
</tr>
<tr>
<td>ACE</td>
<td>AFTTCAGATTCACCAACCC</td>
<td>AAGGTGACTTTGAAACCAAGGTCAGAT</td>
</tr>
<tr>
<td>AT1</td>
<td>GGCGCCAAATCCGACTTCA</td>
<td>CATTTGACTGATGGCTGCTG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GTTGGATAGCAAGCTGGCAAAC</td>
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</tbody>
</table>

AM-R, adrenomedullin receptor; RAMP-2, receptor activity modifying protein; ACE, angiotensin converting enzyme; AT1, ANG II type 1 receptor.

R1086 INTERACTIONS OF ADRENOMEDULLIN WITH ANG II

Fig. 1. Mean arterial pressure (MAP) and heart rate (HR) in rats treated with adrenomedullin (AM; 1.5 μg·kg⁻¹·h⁻¹), ANG II (33 μg·kg⁻¹·h⁻¹, A and B), or NE (300 μg·kg⁻¹·h⁻¹, C and D) alone or in combination of AM. Vehicle, ■, AM; □, ANG II; ●, ANG II + AM; ★, NE; ●, NE + AM. Results are expressed as the means ± SE, n = 6 or 7 in each group. *P < 0.05; **P < 0.01; ***P < 0.001 vs. vehicle; #P < 0.01; ##P < 0.001 vs. ANG II [1-way ANOVA followed by least-significant difference (LSD)].
For quantitative PCR the probes for 18S, angiotensin converting enzyme (ACE), angiotensin II type 1 receptor (AT₁), adrenomedullin receptor (AM-R), and receptor activity modifying protein 2 (RAMP-2) were made. The forward and reverse primers for probes are shown in Table 1. The cDNA first strand was synthesized from 0.5 μg of RNA, and the reactions were performed with an ABI 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) using TaqMan chemistry. The results were normalized to 18S RNA quantified from the same samples.

Radioimmunoassays. The AM, N-terminal-proANP, and BNP radioimmunoassays were performed as previously described (16, 26, 35).

Statistics. Results are expressed as the means ± SE. Hemodynamic variables were analyzed with repeated-measures ANOVA. One-way ANOVA followed by least-significant difference post hoc test was used to determine the significance of differences in multiple comparisons. P < 0.05 was considered statistically significant.

RESULTS

MAP and HR. Initially, we identified a dose of AM that would have no effect on baseline MAP to minimize involvement of any compensatory hemodynamic and neurohumoral mechanisms activated by AM-induced hypotension. Basal values of MAP and HR for each group are shown in Fig. 1. No significant changes in blood pressure or HR were observed during the administration of vehicle or AM at a dose of 1.5 μg·kg⁻¹·h⁻¹ sc (repeated-measures ANOVA, P = 0.174) and these groups did not differ from each other (P = 0.811) (Fig. 1, A and B). Administration of ANG II (33.3 μg·kg⁻¹·h⁻¹) induced a marked hypertension. MAP increased within the first day and remained elevated throughout the 7-day period, reaching the maximal increase of 64 ± 7 mmHg (P < 0.001) at the end of administration (Fig. 1A). This increase in MAP was associated with a significant decrease in HR during first 2 days. Thereafter the HR returned to the basal level and was increased from the 6th day of the administration onward (Fig. 1B). Simultaneous treatment with AM had an inhibitory effect on ANG II-induced hypertension. The inhibition was seen on the 3rd day of administration, the decrease being 67% (P < 0.001) at 7 days (Fig. 1A). Initially, HR decreased similarly in AM- and ANG II-treated animals compared with ANG II-treated animals, but the increase in HR observed at the end of administration of ANG II was not seen in animals treated with combination of ANG II and AM (Fig. 1B). At a dose of 300 μg·kg⁻¹·h⁻¹, NE induced a 24 ± 5 mmHg (P < 0.01) increase in MAP. The elevation reached maximum at 24 h, and thereafter MAP declined slightly, but remained significantly elevated up to the end the study. AM did not have any significant effect on NE-induced increase in blood pressure (Fig. 1C). A compensatory decrease in HR was seen at 24 h in both NE- and NE plus AM-treated rats; thereafter HR did not significantly differ from vehicle-treated animals (Fig. 1D). Because administration of NE (300 μg·kg⁻¹·h⁻¹) raised blood pressure less than administration of ANG II, we used a higher dose of NE (500 μg·kg⁻¹·h⁻¹) to induce greater increase in MAP. With this dose, a maximal increase of 34 ± 4 mmHg MAP (P < 0.001) was seen at 24 h. Yet AM had no effect on NE-induced hypertension (NE: 148 ± 3 vs. 114 ± 2 mmHg, P < 0.001; NE+AM: 148 ± 8 vs. 113 ± 4 mmHg, P < 0.001, n = 6 for each group).

Systolic and diastolic function. Echocardiography was used to characterize the effect of AM on the ANG II- and NE-induced changes in systolic and diastolic function. ANG II significantly improved systolic function by increasing LVEF 8% (P < 0.05) (Fig. 2). AM enhanced the effect of ANG II, resulting in a further 9% increase in LVEF (P < 0.01) and 19% increase in LVFS (P < 0.05). Compared with ANG II, NE alone induced even greater increases both in LVEF (+23%, P < 0.001) and LVFS (+57%, P < 0.001) (Fig. 2). Interestingly, AM had no significant effect on NE-induced changes in systolic function. To examine further the interaction of NE and
AM on systolic function, we administered NE at a lower concentration (50 μg·kg⁻¹·h⁻¹) to study whether smaller increases in LVEF and LVFS may be enhanced by AM. NE at the concentration of 50 μg·kg⁻¹·h⁻¹ induced a 12% (P < 0.05) and 29% (P < 0.05) increase in LVEF and LVFS, respectively (Fig. 2). Administration of AM with the lower dose of NE did not lead to augmentation of the effects of NE. The E/A ratio was significantly lower in ANG II- and NE (300 μg·kg⁻¹·h⁻¹)-treated rats compared with control rats (−29%, P < 0.05 and −46%, P < 0.01, respectively), suggesting changes in diastolic function. AM did not modulate the effect of ANG II or NE on E/A ratio, and AM alone had no effect on systolic or diastolic function (Fig. 2).

**Left ventricular hypertrophy and expression of hypertrophy-associated genes.** The left ventricular weight-to-body weight ratio, used as an index of LVH, increased 36% (P < 0.001) and 14% (P < 0.05) by ANG II or NE, respectively (Table 2). Accordingly, echocardiographic measurements of the thickness of IVS and LVPW increased during ANG II infusion by 33% (P < 0.001) and 31% (P < 0.01), respectively. NE induced 36% (P < 0.001) increase in IVS and 38% (P < 0.01) increase in LVPW. AM did not modulate significantly the ANG II- or NE-induced changes in cardiac mass (Table 2). ANG II also induced hypertrophic changes in expression of cardiac contractile protein genes: β-MHC and Skα₁-A mRNA levels were upregulated, whereas α-MHC mRNA and Cα₁-A mRNA levels remained unchanged, resulting in 2.6-fold (P < 0.001) increase in β-MHC-to-α-MHC ratio and 2.8-fold (P < 0.001) increase in Skα₁-A-to-Cα₁-A ratio (Fig. 3, A and B). NE decreased α-MHC expression, thus causing a significant increase in β-MHC-to-α-MHC ratio (1.5 ± 0.14 vs. 1.0 ± 0.04, P < 0.05), whereas NE did not cause any significant change in the Skα₁-A-to-Cα₁-A ratio. AM did not alter the changes in contractile protein gene expression when administered either alone or in combination with ANG II or NE (Fig. 3, A and B).

Both ANG II and NE treatment induced upregulation of the ANP gene expression (Fig. 3C), a sensitive marker of left ventricular hypertrophy (2, 27). ANG II also increased left ventricular BNP mRNA levels (P < 0.001), whereas NE infusion for 7 days had no effect on BNP gene expression (Fig. 3D). AM did not affect either stimulated or basal ANP or BNP gene expression in left ventricle (Fig. 3, C and D).

**Plasma and tissue concentrations of AM and natriuretic peptides.** Plasma and left ventricular concentrations of AM were similar in vehicle and AM groups and administration of ANG II or NE did not significantly alter the concentration of ir-AM (Table 3). Plasma ir-BNP was increased 2.1-fold in response to ANG II treatment (P < 0.001), and the induction was abolished in rats treated with the combination of ANG II and AM. Administration of AM alone, NE, or NE plus AM had no effect on plasma ir-BNP. Concentration of ir-NT-proANP in plasma tended to increase in response to ANG II and NE treatments, but these changes were statistically not significant. The ir-BNP and ir-ANP concentrations in the left ventricle increased in response to ANG II and NE treatments and remained unchanged by AM (Table 3).

**Left ventricular expression of RAS, AM, and AM receptors.** To examine the possible mechanisms underlying the observed interaction between ANG II and AM, we measured mRNA levels of RAS components in left ventricular myocardium in response to administration of ANG II and AM. ACE gene expression was similarly upregulated in ANG II- and AM plus ANG II-treated rats compared with vehicle-treated animals, whereas mRNA levels of AT₁ receptors remained unchanged in response to administration of AM or ANG II (Table 4).

Neither AM, AM-R, nor RAMP-2 mRNA levels changed in response to administration of ANG II, AM, or their combination (Table 4).

**DISCUSSION**

The aim of the present study was to examine the role of AM in modulating the effects of two hypertrophic substances, ANG II and NE, on hemodynamic parameters, cardiac function, and development of LVH in conscious rats. We observed that long-term administration of AM suppressed the increase in blood pressure and enhanced the improvement of systolic function induced by ANG II but did not affect the development of LVH and cardiac gene expression. Moreover, the effects were selective for ANG II, because AM had no effects on NE-induced hypertension and changes in systolic function.

Intravenous infusion of AM results in potent and long-lasting hypotension, mainly via cAMP-mediated nitric oxide (NO) generation in the vasculature (29). Two-week intravenous administration of AM lowered blood pressure, plasma renin activity, and aldosterone concentrations significantly in two-kidney, one-clip hypertensive rats as well as in their normotensive sham-operated controls (12). In experimental model of heart failure in sheep, infusion of AM for several days resulted in reduction of peripheral resistance and MAP

**Table 2. Body weight and LVWs and echocardiographic measurements of the left ventricle**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vehicle</th>
<th>AM</th>
<th>ANG II</th>
<th>ANG II + AM</th>
<th>NE</th>
<th>NE + AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>369±6</td>
<td>354±10</td>
<td>366±14</td>
<td>353±8</td>
<td>369±8</td>
<td>370±9</td>
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<tr>
<td>1 wk</td>
<td>358±9</td>
<td>350±7</td>
<td>299±13*</td>
<td>292±10*</td>
<td>358±13</td>
<td>348±7</td>
</tr>
<tr>
<td>LVW, mg</td>
<td>736±17</td>
<td>727±15</td>
<td>828±20†</td>
<td>806±28†</td>
<td>846±25†</td>
<td>834±40†</td>
</tr>
<tr>
<td>LVW/body wt, mg/g</td>
<td>2.08±0.06</td>
<td>2.08±0.04</td>
<td>2.82±0.07*</td>
<td>2.81±0.16*</td>
<td>2.37±0.04‡</td>
<td>2.39±0.11‡</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>1.75±0.06</td>
<td>1.60±0.07</td>
<td>1.77±0.04</td>
<td>1.70±0.10</td>
<td>1.74±0.04</td>
<td>1.70±0.04</td>
</tr>
<tr>
<td>1 wk</td>
<td>1.73±0.04</td>
<td>1.75±0.06</td>
<td>2.30±0.10*</td>
<td>2.34±0.14*</td>
<td>2.36±0.04*</td>
<td>2.22±0.05*</td>
</tr>
<tr>
<td>LVPW, mm</td>
<td>1.70±0.04</td>
<td>1.67±0.03</td>
<td>1.72±0.08*</td>
<td>1.76±0.05</td>
<td>1.72±0.05</td>
<td>1.68±0.04</td>
</tr>
<tr>
<td>1 wk</td>
<td>1.80±0.09</td>
<td>1.75±0.09</td>
<td>2.35±0.15*</td>
<td>2.58±0.20*</td>
<td>2.48±0.16†</td>
<td>2.40±0.08†</td>
</tr>
</tbody>
</table>

Data expressed as means ± SE, n = 6 or 7 in each group. LVW, left ventricular weight; IVS, interventricular septum (diastole); LVPW, left ventricular posterior wall (diastole). *P < 0.001; †P < 0.01; ‡P < 0.05 vs. vehicle-treated [1-way ANOVA followed by least significant difference (LSD)].
Previous studies have also evaluated the acute interaction of intravenously administered AM with ANG II and NE. In anesthetized rats, a bolus injection of AM has been shown to inhibit the acute pressor effects induced by ANG II and NE (18). In addition, Charles et al. (3) recently reported that intravenously administered AM up to 2 h attenuated the vaso-pressor actions of ANG II, but not NE, in conscious sheep and that AM induces a different spectrum of hemodynamic, renal, and endocrine actions compared with another vasodilator, nitroprusside (3, 5). However, the effects of long-term administration of AM with ANG II or other pressor substances have not been studied previously. This is important because in pathophysiological situations such as pressure-overload hypertrophy, the RAS and sympathetic nervous system are chronically elevated (1, 7, 14). In the present study, long-term subcutaneous administration of AM up to 7 days attenuated the pressor responsiveness to ANG II, whereas no interaction was seen with NE. The effect of AM was seen after 2 days of administration of ANG II, likely due to the low, nonhypotensive dose of AM used in this study. We also observed that plasma concentration of ir-BNP increased in response to hypertension induced by ANG II and that administration of AM with ANG II resulted in normalization of BNP plasma levels, although the blood pressure remained above control levels.

Earlier studies have established that AM induces an increase in cardiac output in both rat and sheep (4, 20). Increased
cardiac output may be due to AM-induced vasodilatation and thereby decreased afterload but may also arise from direct cardiac effects of AM. Indeed, in isolated perfused rat heart, AM has been shown to enhance cardiac contractility via cAMP-independent mechanisms, which include mobilization of Ca\(^{2+}\) from intracellular Ca\(^{2+}\) stores, activation of protein kinase C (PKC), and activation of L-type Ca\(^{2+}\) channels (31). In the present study, we showed that AM augmented the ANG II-induced improvement in systolic function, measured by fractional shortening and ejection fraction, whereas no significant potentiation of NE-induced changes in cardiac systolic function was seen. Because diastolic function remained unaffected, AM appears to act on the mechanisms regulating the contraction rather than relaxation of the cardiac muscle cell.

AM elicits physiological actions through many signaling cascades including cAMP, PKC, and Ca\(^{2+}\)(29). The differential role of AM in ANG II-induced changes in hypertension and cardiac function may be explained via its action on distinct signaling cascades in vascular and cardiac tissues. In vascular smooth muscle cells, AM increases the cAMP, thus attenuating contraction rather than relaxation of the cardiac muscle cell. AM has been shown to enhance cardiac contractility via many signaling cascades as ANG II (13), and, therefore, the precise mechanisms for the selective interaction AM with ANG II remain to be studied.

AM has been shown to have a remarkable range of actions in the regulation of cellular growth and differentiation as well as in the modulation of hormone secretion in a number of tissues (for review, see Ref. 10). Long-term (4 wk) infusion of AM after myocardial infarction was shown to reduce the heart weight, the myocyte size, and collagen volume fraction in left ventricle without affecting the blood pressure or infarct size (21). In the present study, despite the attenuation of the pressor response, AM showed no inhibitory effect on ANG II- or NE-induced cardiac hypertrophy measured by LVW/body weight index, echocardiography, or expression of hypertrophy-associated genes. Previously, several in vitro studies have shown that AM inhibits the growth promoting effects of ANG II (17). Tsuruda et al. (33) showed that AM attenuates ANG II- and serum-stimulated protein synthesis in cardiac myocytes. Our earlier studies also demonstrated that AM selectively inhibits ANG II-induced myocyte hypertrophy confirmed by natriuretic peptide secretion and gene expression as well as protein synthesis and morphological changes in cultured cardiac myocytes (15). The lack of inhibition of LVH in this study may emphasize the divergent role of circulating vs. local cardiac AM in modulation of myocyte hypertrophy.

It has been reported earlier that ACE mRNA levels are high in hypertrophied left ventricle (24, 34) and that mechanical stretch as well as exogenously applied ANG II can upregulate ACE and AT\(_1\) gene expression in cultured cardiac myocytes (17). Recently, Mori et al. (19) showed that chronic infusion of subpressor dose of AM in DOCA-salt spontaneously hypertensive rats (SHR) decreased levels of renal cortical tissue ANG II and ACE mRNA in the renal cortex with a concomitant improvement of renal functional and morphological changes. Additionally, chronic infusion of AM has been shown to elicit similar renoprotective effects, including downregulation of renal tissue expression of ACE, renin, and angiotensinogen in Dahl salt-sensitive rats (22). In this study, an equivalent subpressor dose of AM had no cardioprotective effects against ANG II- or NE-induced LVH. Moreover, AM did not inhibit the upregulation of ACE mRNA in response to ANG II nor had any effects on AT\(_1\) receptor gene expression, suggesting that AM does not modulate ANG II responses via altered expression of at least these components of the RAS. The reason for these discrepant findings is unclear but may be due to different sensitivity of cardiac and renal tissues in responding to circulating AM or to differences between the experimental models, because DOCA-salt SHR develop a volume overload instead of a pressure overload seen in ANG II-treated rats. Nevertheless, the increased ACE gene expression in AM- and ANG II-treated rats may maintain the activation of intracardiac RAS and explain why AM-induced attenuation of pressor response to ANG II did not lead to inhibition of hypertrophic changes.

It has been shown earlier that chronic infusion of human recombinant AM decreases the elevated plasma levels of endogenous rat total AM and mature AM (21, 22), but it is not clear whether this downregulation is due to indirect mecha-

### Table 3. Plasma and tissue concentrations of AM and natriuretic peptides

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vehicle</th>
<th>AM</th>
<th>ANG II</th>
<th>ANG II + AM</th>
<th>NE</th>
<th>NE + AM</th>
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<tbody>
<tr>
<td>ir-AM</td>
<td>38 ± 1</td>
<td>41 ± 12</td>
<td>77 ± 20</td>
<td>62 ± 20</td>
<td>51 ± 20</td>
<td>47 ± 24</td>
</tr>
<tr>
<td>ir-BNP</td>
<td>19 ± 1</td>
<td>23 ± 3</td>
<td>40 ± 7†</td>
<td>22 ± 6€</td>
<td>20 ± 3</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>ir-nt-proANP</td>
<td>126 ± 9</td>
<td>121 ± 12</td>
<td>167 ± 16</td>
<td>144 ± 13</td>
<td>159 ± 13</td>
<td>147 ± 11</td>
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<td>Left ventricular, pmol/g</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ir-AM</td>
<td>0.19 ± 0.02</td>
<td>0.23 ± 0.03</td>
<td>0.24 ± 0.03</td>
<td>0.23 ± 0.4</td>
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<tr>
<td>ir-BNP</td>
<td>2.37 ± 0.14</td>
<td>2.87 ± 0.29</td>
<td>6.40 ± 0.90*</td>
<td>5.79 ± 1.00*</td>
<td>4.85 ± 0.70†</td>
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<tr>
<td>ir-ANP</td>
<td>11.4 ± 2.5</td>
<td>11.0 ± 1.2</td>
<td>88.0 ± 17.0*</td>
<td>113.0 ± 35.3*</td>
<td>65.2 ± 12.0†</td>
<td>58.5 ± 13.7‡</td>
</tr>
</tbody>
</table>

Data expressed as means ± SE, \(n = 6\) or 7 in each group. *\(P < 0.001\); †\(P < 0.01\); ‡\(P < 0.05\) vs. vehicle-treated; §\(P < 0.01\) vs. ANG II-treated (1-way ANOVA followed by LSD).

### Table 4. Gene expression of components of RAS and AM signaling system

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vehicle</th>
<th>AM</th>
<th>ANG II</th>
<th>ANG II + AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>1.00 ± 0.18</td>
<td>0.80 ± 0.18</td>
<td>2.03 ± 0.53*</td>
<td>2.01 ± 0.47*</td>
</tr>
<tr>
<td>AT(_1)</td>
<td>1.00 ± 0.12</td>
<td>1.03 ± 0.21</td>
<td>0.92 ± 0.27</td>
<td>1.05 ± 0.27</td>
</tr>
<tr>
<td>AM</td>
<td>1.00 ± 0.05</td>
<td>0.93 ± 0.09</td>
<td>0.94 ± 0.08</td>
<td>0.82 ± 0.05</td>
</tr>
<tr>
<td>AM-R</td>
<td>1.00 ± 0.07</td>
<td>0.92 ± 0.12</td>
<td>0.93 ± 0.08</td>
<td>0.91 ± 0.18</td>
</tr>
<tr>
<td>RAMP-2</td>
<td>1.00 ± 0.16</td>
<td>1.03 ± 0.15</td>
<td>0.76 ± 0.14</td>
<td>0.86 ± 0.14</td>
</tr>
</tbody>
</table>

Data expressed as means ± SE, \(n = 6\) or 7 in each group. *\(P < 0.05\) vs. vehicle-treated (1-way ANOVA followed by LSD).
nisms or a direct negative feedback of AM on its own production. In the study, we infused rat AM and thus could not distinguish between exogenous and endogenous AM, but in agreement with previous studies, there was no statistically significant increase in plasma levels of total AM. As suggested by Nakamura et al. (21), a slight increase of plasma mature AM, an active form of AM, may be attributable to biological effects seen during chronic administration of AM.

In conclusion, this study shows for the first time that long-term infusion of AM selectively interacts with ANG II by attenuating the hypertensive effects of ANG II and by augmenting the ANG II-induced improvement in cardiac systolic function. However, AM has no direct modulatory effects on ANG II- or NE-induced LVH or gene expression, suggesting that in pressure overload, circulating AM acts mainly as a regulator of vascular tone and cardiac function. Thus an orally active AM agonist or specific inhibitor of AM degradation might act as an antihypertensive drug and would be beneficial in the treatment of human hypertension and renal disease (19, 22), whereas a more targeted stimulation of myocardial AM activity may be needed to inhibit the development of LVH and cardiac failure.

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