β₁-Adrenergic Receptor Blockade Attenuates Angiotensin II–Mediated Catecholamine Release Into the Cardiac Interstitium in Mitral Regurgitation

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Background—This study tested the hypothesis that β₁-adrenergoreceptor blockade modulates the angiotensin II (Ang II)–evoked neural release of norepinephrine (NE) and epinephrine (Epi) into the cardiac interstitial fluid (ISF) space in experimentally induced mitral regurgitation (MR) in the dog.

Methods and Results—Normal dogs (n=8) were compared with dogs with MR of 2 (n=8) and 4 (n=6) weeks’ duration and with dogs with MR treated with β₁-receptor blockade (RB; extended-release metoprolol succinate, 100 mg QD; MR + β₁-RB) that was started 24 hours after MR induction for 2 (n=6) and 4 weeks (n=8). Left ventricular end-diastolic dimension increased 20% as plasma Ang II levels increased >5-fold in both MR and MR + β₁-RB dogs at 2 and 4 weeks. Ang II infusion into the left atrium produced increases in ISF NE and Epi in normal dogs, which were further increased in 2- and 4-week MR dogs but were restored to normal in 4-week MR + β₁-RB dogs. Ang II infusion produced 4-fold increases in circulating NE and Epi in 2- and 4-week MR dogs that returned to normal in 4-week MR + β₁-RB dogs. Left ventricular angiotensin-converting enzyme activity and ISF Ang II were increased in 4-week MR dogs but were decreased in 4-week MR + β₁-RB dogs.

Conclusions—β₁-RB decreases renin-angiotensin system sympathostimulation and activation by attenuating the Ang II–mediated NE and Epi release into the cardiac ISF and circulation and by decreasing left ventricular angiotensin-converting enzyme expression in the early phases of volume overload. (Circulation. 2003;108:225-230.)

Key Words: angiotensin ■ nervous system, autonomic ■ receptors, adrenergic, beta ■ heart failure ■ renin

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ympathetic neuronal activation on initiation of cardiac stress increases ventricular inotropy and systemic vascular resistance, mediated by way of the release of catecholamines, thereby maintaining cardiac output and blood pressure. However, sustained sympathetic effenter neural activation has several adverse consequences, including direct myocyte toxicity, apoptosis, and extracellular matrix remodeling.1 In addition to sympathetic activation mediated by cardiac reflexes, there is also evidence that angiotensin II (Ang II) activates intrathoracic cardiac adrenergic effenter postganglionic neurons, thereby resulting in the liberation of catecholamines from their postganglionic nerve terminals.2,3 Indeed, Ang II type-1 (AT₁) receptor density in intrinsic cardiac ganglia is 9-fold higher than that found in ventricular myocardial tissue.4

This anatomic relationship of AT₁ receptors with cardiac neurons might account, in part, for the fact that Ang II–induced myocyte necrosis and fibrosis5,6 are prevented by β-adrenergoreceptor blockade. Furthermore, the myocyte necrosis and coronary vascular damage that occur within the first 3 days after elevation of circulating Ang II are prevented by AT₁ receptor blockade and significantly attenuated by β₁-receptor blockade (β₁-RB).6 Yet, in this animal model, the Ang II–induced increase in plasma norepinephrine (NE) does not occur until day 4, and it is prevented by AT₁ receptor blockade.6 These studies suggest that the deleterious effects of exogenously infused Ang II are mediated by enhanced local catecholamine release through activation of AT₁ receptors on cardiac adrenergic neurons.

Using the cardiac microdialysis technique in open-chest dogs, we have shown that Ang II activates intrathoracic adrenergic neurons to release NE and epinephrine (Epi) into the cardiac interstitial fluid (ISF) space and that transcathartic plasma NE and Epi release substantially underestimates...
sympathetic efferent neuronal NE and Epi release into the cardiac ISF space, especially during Ang II stimulation. Therefore, to effectively evaluate the dynamic interaction between the renin-angiotensin system (RAS) and the adrenergic system in the evolution of cardiac pathology, it is essential to assess ISF NE and Epi in conjunction with induced changes in left ventricular (LV) function and remodeling. We have previously shown that volume overload induced by mitral regurgitation (MR) in the dog results in upregulation of the cardiac RAS, remodeling of the extracellular matrix, and adverse cardiac remodeling and function. However, angiotensin-converting enzyme (ACE) inhibition or AT1 receptor blockade does not improve adverse cardiac remodeling and function, whereas β1-RB results in significant improvement in LV function in chronic MR. These studies suggest that although the cardiac RAS is upregulated, the adrenergic system is more central to the pathophysiology of volume overload. In the current study, we hypothesize that β1-RB modulates the Ang II–mediated release of catecholamines into the ISF which, in turn, is associated with a beneficial effect on LV function and remodeling in LV volume overload of MR in the dog.

**Methods**

**Experimental Preparation**

MR was induced at the College of Veterinary Medicine, Auburn University, Auburn, Ala, in conditioned mongrel dogs of either sex (19 to 26 kg) by chordal rupture with the use of a fluoroscopically guided catheterization method previously described from our laboratory. Dogs were randomly assigned to 1 of 5 groups: (1) 2 weeks of MR (n=8); (2) 2 weeks of MR and treatment with a β1-adrenergic receptor blocker (extended-release metoprolol succinate, 100 mg PO, once daily; n=6) starting 24 hours after MR induction; (3) 4 weeks of MR (n=6); (4) 4 weeks of MR plus the β1-adrenergic receptor blocker toprol (100 mg PO, once daily; n=8); and (5) unoperated controls (n=8). Two-dimensional and M-mode echocardiography (2.25-MHz transducer, ATL Ultramark VI) was performed at baseline and 2 weeks (groups 1 and 2) and 4 weeks (groups 3 and 4) after induction of MR. The dogs were transferred to the University of Alabama at Birmingham for sacrifice. This study was approved by the Animal Services Committees at the University of Alabama at Birmingham and College of Veterinary Medicine, Auburn University.

**Terminal Catheterization Study**

Animals were maintained on a deep plane of general anesthesia with isoflurane and were mechanically ventilated (Harvard Apparatus, Inc) with continuous ECG monitoring as previously described. In brief, both cervical vagi were sectioned, and the heart was suspended in a pericardial cradle through a median sternotomy. PE50 tubing was inserted into the left atrium and secured with a purse string suture. Microdialysis probes (Clirans, Terumo Corp) were inserted into the LV anterior wall as described previously. On the basis of previous in vitro experiments, the infusion rate was 2.5 μL/min, and 18.8% recovery was used in the calculation of ISF NE and Epi.

**Experimental Protocol**

After a 10-minute baseline dialysate collection, Ang II was infused into the left atrium for 10 minutes (100 μmol/L, Ang II at 1 mL/min). Dialysate was collected separately for the first 3 minutes, the second 3 minutes, and the last 4 minutes of the Ang II infusion. Subsequently, dialysate was collected for the first 5 minutes and the second 5 minutes. Aorta and coronary sinus blood samples were taken at 8 minutes of baseline and 2 minutes before the end of Ang II infusion. Mean arterial pressure, high-fidelity LV pressure, LV dP/dt, and heart rate were monitored continuously. At the end of the in vivo experiments, the heart was arrested with intracardiac KCl and quickly extirpated and placed in phosphate-buffered ice slush, and the coronary arteries were flushed with oxygenated Krebs’ solution. The LV was cut into 1-cm cubes and snap-frozen in LN2 for subsequent biochemical studies. The LV free wall incorporating the circumflex artery (5×5 cm) was excised and prepared for formalin fixation.

**Biochemical Analyses**

**LV ACE and Chymase Activities**

LV ACE and chymase activities were measured by assays developed in our laboratory.

**Ang Peptide Levels**

Plasma Ang I and Ang II concentrations were determined by a method described from our laboratory that combines solid-phase extraction, high-performance liquid chromatography, and radioimmunoassay.

**ISF and Tissue Catecholamine Levels**

ISF and plasma NE and Epi concentrations were determined with the Biotrak catecholamine radioenzymatic assay (Amersham Pharmacia Biotech) as previously described from our laboratory.

**Collagen Analysis**

Interstitial collagen volume percent was quantitatively evaluated by using a 20× objective (600× on the video screen) of an Olympus AH3 research microscope with a monochrome video CCD72 camera interfaced to a computer equipped with an Image One (Universal Imaging) morphometry system. Endocardial and epicardial halves of the LV myocardium were examined by means of picrosirius red-stained sections on a minimum of 20 randomly selected, digitized images from each animal.

**Statistical Analysis**

All data are presented as mean±SEM. One-way repeated-measures ANOVA with post hoc comparison was used to compare hemodynamics and Ang II, Epi, and NE concentrations throughout the stages of the protocol. A value of P<0.05 was considered significant.

**Results**

**Hemodynamics**

Mean arterial pressure was significantly decreased in all MR groups compared with controls, whereas mean pulmonary artery pressure and pulmonary capillary wedge pressure did not differ across MR groups and controls. Cardiac output and peak +LV dP/dt did not differ from controls in the untreated MR dogs at 4 weeks but was significantly depressed in all other MR groups. Mean systemic vascular resistance showed trends demonstrating an increase at 2 weeks in MR and MR+β1-RB dogs and at 4 weeks in MR+β1-RB dogs and a decrease in 4-week MR dogs. There was a progressive trend toward increases in LV mass in MR and MR+β1-RB dogs at 2 and 4 weeks (Table 1).

**Echo LV Function**

Percent increases in LV end-diastolic dimension and LV fractional shortening were equivalent in MR and MR+β1-RB dogs at both 2- and 4-week time points. LV end-systolic dimension did not change, and the ratio of LV wall thickness to end-diastolic dimension decreased to a similar extent across all groups of dogs (Table 1).
### TABLE 1. LV Function Hemodynamics and RAS Expression in MR Dogs

<table>
<thead>
<tr>
<th></th>
<th>Normal Dogs</th>
<th>2 Weeks</th>
<th>4 Weeks</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MR</td>
<td>MR + β₁-RB</td>
<td>MR</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>94 ± 6</td>
<td>76 ± 4</td>
<td>74 ± 2</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>84 ± 3</td>
<td>64 ± 7*</td>
<td>65 ± 4*</td>
</tr>
<tr>
<td>Mean pulmonary arterial pressure, mm Hg</td>
<td>13 ± 2</td>
<td>15 ± 2</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>Pulmonary capillary wedge pressure, mm Hg</td>
<td>8 ± 2</td>
<td>10 ± 1</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Systemic vascular resistance, dyne·s·cm⁻¹</td>
<td>2007 ± 219</td>
<td>2620 ± 235</td>
<td>3123 ± 517</td>
</tr>
<tr>
<td>Cardiac output, L/min</td>
<td>3.6 ± 0.4</td>
<td>2.1 ± 0.01*</td>
<td>1.9 ± 0.5*</td>
</tr>
<tr>
<td>LV mass, g/kg</td>
<td>4.4 ± 0.2</td>
<td>4.8 ± 0.3</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td>Pulmonary vascular resistance, dyne·s·cm⁻¹</td>
<td>117 ± 24</td>
<td>181 ± 34</td>
<td>336 ± 65*</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>4 ± 1</td>
<td>9 ± 2</td>
<td>15 ± 3*</td>
</tr>
<tr>
<td>LV +dP/dt, mm Hg/s</td>
<td>1863 ± 149</td>
<td>1157 ± 113*</td>
<td>993 ± 176*</td>
</tr>
<tr>
<td>LV end-diastolic dimension, % change</td>
<td>...</td>
<td>14.5 ± 4.7†</td>
<td>18.2 ± 2.4†</td>
</tr>
<tr>
<td>Fractional shortening, % change</td>
<td>...</td>
<td>22.7 ± 6.3†</td>
<td>43.6 ± 11.0†</td>
</tr>
<tr>
<td>Wall thickness/end-diastolic dimension, % change</td>
<td>...</td>
<td>−15.9 ± 5.7†</td>
<td>−25.0 ± 3.8†</td>
</tr>
<tr>
<td>Sample size, n</td>
<td>6</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

Values presented as mean ± SEM. 
*P<0.05 vs Normal Dogs. 
†P<0.05, % change vs baseline study.

### Renin-Angiotensin System

Aortic and coronary sinus plasma Ang II levels (Table 2) were elevated >3-fold in all MR groups. Ang I levels were increased 16-fold in MR dogs at 2 weeks compared with control animals, whereas in the 2- and 4-week MR + β₁-RB groups, plasma Ang I levels were similar to those in control animals. LV chymase activity was increased 2-fold in all MR groups, whereas LV ACE activity was increased in 4-week MR dogs but was reduced in 4-week MR + β₁-RB dogs. LV ISF Ang II levels were increased in all MR groups compared with controls; however, ISF Ang II levels in the 4-week MR + β₁-RB group were decreased compared with those in 4-week MR dogs.

### Cardiac Hemodynamic Response to Ang II

Infusion of Ang II induced increases in heart rate, mean arterial pressure, and peak +LV dP/dt in control animals, but these changes were significantly attenuated in MR and MR + β₁-RB dogs at 2 and 4 weeks (Figure 1).

### ISF and Plasma NE Responses to Ang II

In control animals, Ang II infusion increased ISF NE, which was similar in 2-week MR dogs, but which was significantly higher in 2-week MR + β₁-RB and 4-week MR dogs (Figure 2); these levels returned to normal in 4-week β₁-RB dogs. In control dogs, aortic and coronary sinus plasma (data not shown) NE levels did not change during Ang II infusion (Figure 4). However, Ang II induced a 3-fold increase in aortic and coronary sinus NE in 2-week MR and MR + β₁-RB dogs and in 4-week MR dogs; these levels were also restored to normal in 4-week MR + β₁-RB dogs (Figures 2 and 4).

### TABLE 2. Plasma Aorta and Coronary Sinus Ang I and II Levels and LV Chymase and ACE Activities in MR Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>2 Weeks</th>
<th>4 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MR</td>
<td>MR + β₁-RB</td>
<td>MR</td>
</tr>
<tr>
<td>Ao Ang I</td>
<td>270 ± 29</td>
<td>3478 ± 598*</td>
<td>805 ± 192</td>
</tr>
<tr>
<td>CS Ang I</td>
<td>256 ± 31</td>
<td>3558 ± 828*</td>
<td>856 ± 191</td>
</tr>
<tr>
<td>Ao Ang II</td>
<td>60 ± 3</td>
<td>330 ± 64*</td>
<td>235 ± 51*</td>
</tr>
<tr>
<td>CS Ang II</td>
<td>50 ± 8</td>
<td>204 ± 36*</td>
<td>164 ± 35*</td>
</tr>
<tr>
<td>ISF Ang II</td>
<td>846 ± 77</td>
<td>1691 ± 209*</td>
<td>1397 ± 227*</td>
</tr>
<tr>
<td>LV chymase activity</td>
<td>13.5 ± 1.5</td>
<td>29.3 ± 2.0*</td>
<td>32.9 ± 1.5*</td>
</tr>
<tr>
<td>LV ACE activity</td>
<td>1.23 ± 0.02</td>
<td>1.28 ± 0.05</td>
<td>1.24 ± 0.04</td>
</tr>
<tr>
<td>Sample size, n</td>
<td>6</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

Values presented as mean ± SEM. Ao indicates aorta; CS, coronary sinus. 
*P<0.05 vs Normal Dogs. 
†P<0.05 vs Control. 
‡P<0.05 vs 4-week MR.
ISF and Plasma Epi Responses to Ang II

In control animals, Ang II increased ISF Epi 10-fold as plasma aortic and coronary sinus Epi levels tended to increase. In untreated MR dogs at 2 and 4 weeks, ISF and aortic and coronary sinus Epi levels increased significantly but returned to normal in 4-week MR/H11001/H92521-RB dogs (Figures 3 and 4).

LV Collagen

Endocardial volume percent collagen decreased in both MR and MR+/β1-RB dogs at 2 weeks compared with control animals. At 4 weeks, there was a further significant reduction in LV epicardial collagen in untreated MR dogs, which was prevented in the dogs treated β1-RB (Figure 5).

Discussion

The current study demonstrates that in early stages of adaptation to the volume overload of MR, reflex-mediated sympathoexcitation is critical for maintenance of compromised cardiac function. β1-RB in the early, 2-week stage of MR blunts the cardiac-supportive effects of ISF catecholamines, as reflected in depressed LV inotropic function, an increase in end-diastolic pressure, and a reflex increase in baseline ISF NE levels. In the 4-week, untreated MR dogs, the adaptive processes responded further, such that cardiac output was transiently sustained at control levels through neurohumoral remodeling, but at a cost. There was upregulation of the cardiac RAS, coupled with the functional upregulation of the synergism between it and the sympathetic nervous system, which was abrogated by the 4 weeks of treatment with β1-RB while preserving LV function and attenuating extracellular matrix remodeling.

In control animals, Ang II increased heart rate, peak +dP/dt, and mean arterial pressure. This was associated with significant increases in ISF NE and Epi, as plasma NE was unchanged and plasma Epi increased to a small extent. However, in all of the MR animals, there were significant reductions in the chronotropic and inotropic effects of Ang II, with near obliteration of the vasoconstrictor response. Nevertheless, administration of Ang II resulted in greater NE and Epi release into the ISF and circulation in untreated MR dogs at 2 and 4 weeks and in MR+/β1-RB dogs at 2 weeks. Conversely, 4 weeks of treatment with β1-RB normalized the Ang II–mediated release of NE and Epi into the cardiac ISF and the circulation. Thus, more prolonged treatment with β1-RB resulted in functional downregulation of the Ang II–sensitive neuronal release of catecholamines that could have a long-term myocardial-protective effect.

Levels of interstitial catecholamines reflect the dynamic interplay between neural release mechanisms, reuptake pro-
cesses, and diffusion between the ISF and vascular compartments. In no instance did we find evidence of NE or Epi spillover into the coronary sinus, suggesting that the catecholamine reuptake mechanism was intact. Moreover, because Epi content is a small fraction of the NE content in intracardiac nerves,12 its recycling is critical for continued release. The augmented catecholamine release in the progression of MR is more likely reflective of the synergism between the RAS and sympathetic nervous system. Ang II acts by way of AT1 receptors on the sympathetic nerve soma and at their end terminals to facilitate catecholamine release.2 Our data suggest that intracardiac Ang II production is facilitated initially by increases in LV chymase activity and subsequently by LV ACE activity. It is also possible that adaptations of AT1 receptors on neural ganglia cells in the heart occur during the progression of MR, which could account in part for the increase in NE release.

Despite the increase in ISF NE and Epi, +LV dP/dt did not increase with Ang II administration in animals with MR. This could be a manifestation of β-adrenergic desensitization and/or lack of an afterload-stimulating effect secondary to a decreased vasoconstrictor effect of Ang II. In a similar study, there was a decreased vasoconstrictor and hypertrophic response to Ang II infusion in the first 2 weeks after myocardial infarction in the rat.13 However, there was also increased plasma atrial natriuretic peptide and nitric oxide synthase activity that would oppose the vasoconstrictive and hypertrophic effects of Ang II. Alternatively, a change in expression and activation of the AT1 receptor in the vasculature and heart could explain the suppression of the vasoconstrictor and inotropic effects of Ang II.14 Taken together, the induction of hormone systems that are counterregulatory to the effects of increased, circulatory Ang II might, in the early phase of volume overload, prevent excessive vasoconstriction in response to the decrease in cardiac output.

β1-RB did not prevent the significant increase in plasma Ang II in all MR groups. However, β1-RB did decrease the marked increase in plasma Ang I levels in the 2- and 4-week untreated MR dogs. β1-RB also attenuated the increase in ISF Ang II in MR dogs, most likely due in part to the suppression of renin production and release from the kidney. It is now well appreciated that renin receptors exist in the heart and that uptake of renin is an important regulatory mechanism in the formation of Ang II in the heart.15 β1-RB was also associated with a decrease in LV ACE activity compared with the 4-week untreated MR dogs. We and others have shown that LV ACE expression is related to elevated diastolic wall stress in volume overload in the dog7,8 and to the severity of heart failure in the human heart.16 Thus, lesser activation of LV

Figure 4. Aortic plasma NE and Epi levels at baseline and Ang II infusion in control, 2- and 4-week MR, and 2- and 4-week MR + β1-RB dogs. +P<0.05 vs baseline.

Figure 5. Volume percent collagen of LV endocardium and epicardium in control, 2- and 4-week MR, and 2- and 4-week MR + β1-RB dogs. #P<0.05 vs control; *P<0.05, epicardium in 4-week MR vs 4-week MR + β1-RB dogs.
ACE activity at 4 weeks of MR by β1-RB might be reflective of the maintenance of the epicardial collagen matrix, thereby reducing the potential for myocyte slippage and its resultant stretch activation of LV ACE activity.

At 2 and 4 weeks after induction of MR, LV diameter increased and the ratio of wall thickness to LV end-diastolic dimension decreased 20% as endocardial collagen content decreased 50% in both MR and MR + β1-AR dogs at 2 weeks. By 4 weeks of MR, the epicardial collagen content had also decreased by 50% in untreated MR dogs, an effect that was prevented by β1-RB. It is this progressive degradation of extracellular matrix, reflective of matrix metalloproteinase (MMP) activation, that results in adverse LV chamber remodeling and a disproportionate increase in LV diameter relative to wall thickness that contributes to the eventual development of congestive heart failure. Indeed, we have demonstrated that MMP-2 activity is increased at 2 and 4 weeks after induction of MR in the dog. This occurs at a time when LV fractional shortening increases because LV end-diastolic dimension increases as LV end-systolic dimension remains unchanged, suggesting an adaptive use of preload reserve. However, during the ensuing 3 months, we found a further decrease in the ratio of LV wall thickness to end-diastolic dimension and a decrease in fractional shortening that were not attenuated by RAS blockade. It is of interest that 3 months of β1-RB, which was started after 3 months of MR, not only improved cardiomyocyte function but also attenuated the LV chamber remodeling in the MR dog model.

The results of the current investigation demonstrate that Ang II–mediated NE release is increased in MR and restored to normal by concurrent β1-RB therapy. In addition, the suggestion that β1-RB influences the rate of transcardiac collagen degradation is particularly intriguing. NE and Ang II might act directly or indirectly to activate MMPs through interactions with inflammatory cytokines or reactive oxygen species. Szenzaki and coworkers demonstrated that high-dose β1-RB during Ang II infusions prevented MMP activation as well as tissue damage and inflammation in the pacing canine model. Future studies will evaluate the effects of β1-RB on MMP and RAS activation and the progression of LV remodeling in MR volume overload in the dog.

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References
