β₁-Adrenoceptor blockade mitigates excessive norepinephrine release into cardiac interstitium in mitral regurgitation in dog

Gerald H. Hankes, Jeffrey L. Ardell, José Tallaj, Chih-Chang Wei, Inmaculada Aban, Merrilee Holland, Patricia Rynders, Ray Dillon, Rene Cardinal, Donald B. Hoover, J. Andrew Armour, Ahsan Husain, and Louis J. Dell’Italia. β₁-Adrenoceptor blockade mitigates excessive norepinephrine release into cardiac interstitium in mitral regurgitation in dog. Am J Physiol Heart Circ Physiol 291: H147–H151, 2006; doi:10.1152/ajpheart.00951.2005.—Mitral regurgitation (MR) is associated with increased neuronal release of norepinephrine (NE) and epinephrine (EP) into myocardial interstitial fluid (ISF) that may be necessary in sustaining left ventricular (LV) function via activation of cardiomyocyte β-adrenergic receptors (ARs). However, activation of neuronal β-ARs on cardiac neurons may lead to further catecholamine release, with an attendant risk of functional deterioration. We hypothesize that a beneficial effect of β-AR blockade may therefore mitigate excessive catecholamine release from cardiac adrenergic neurons in dogs with MR. We measured the effects of chronic β-receptor blockade (β-RB) on ISF NE and EP release using in vivo microdialysis in open-chest anesthetized dogs after 4 wk of MR with or without extended release of metoprolol succinate (100 mg/day) as well as in control dogs. Fractional shortening increased by 30% in both MR and MR + β-RB dogs after 4 wk of MR. In MR + β-RB dogs, stellate-stimulated heart rate change was attenuated compared with control and MR dogs, whereas peak change of LV pressure over time (+dP/dt) increased equally in all groups. Stellate-stimulated ISF NE increased fivefold over baseline in MR versus twofold in control dogs (<0.05), but the NE release was significantly attenuated in MR + β-RB dogs. In contrast, stellate-stimulated increases in ISF EP did not differ in control, MR, and MR + β-RB dogs. This study demonstrates that β-RB attenuates ISF NE release from cardiac neurons and that the LV functional response to MR is not dependent on an excess increase in ISF NE. Thus β₁-RB may exert a beneficial effect by attenuating untoward effects of excessive sympathetic effector neural NE release while sustaining early LV functional adaptation to MR.

interstitial fluid; epinephrine

MITRAL REGURGITATION (MR) is characterized by initial left ventricular (LV) dilation and augmented stroke volume that is mediated by the Starling mechanism and facilitated by regurgitation into the left atrium (1). Studies in animals and humans have suggested that this initial recruitment of preload reserve is quickly followed by increased sympathetic drive in early phases of MR (12, 13, 15). Thus it is hypothesized that the MR heart is subjected to early increased release of norepinephrine (NE) and epinephrine (EP) into the interstitial fluid (ISF) space, thereby contributing to sustaining LV function. We have reported that catecholamine release into cardiac ISF in response to ANG II is enhanced with 4-wk MR compared with ANG II effects in normal canines (22) and that, moreover, the catecholamine release was attenuated (to lower than normal values) after chronic treatment with extended release of metoprolol succinate (22), a relatively selective β₁-adrenergic receptor (AR) blocker (β₁-RB) (19). Canine intrathoracic neurons contain both β₁- and β₂-ARs (25) as well as ANG II receptors that are all involved in eliciting effenter sympathetic responses and catecholamine release in the heart (2, 6).

Using the microdialysis technique in healthy open-chest canines, we have shown that there is compartmentalized NE and EP release into the LV ISF space during electrical stimulation of the stellate ganglion (6, 22). In the present study, we report that excess NE is released into the cardiac interstitium in the early compensated stage of MR (4 wk) and, moreover, that chronic treatment with a β₁-AR blocker decreases such excess neurally mediated release of NE but without diminishing the functional LV responses to stellate ganglion stimulation.

METHODS

Experimental Preparation

Mitral valve regurgitation was induced at Auburn University College of Veterinary Medicine in conditioned mongrel dogs of either sex (19–26 kg) by chordal rupture using a fluoroscopic-guided catheterization method previously described in by Dell’Italia and colleagues (4, 17, 21, 22). Dogs were randomly assigned to one of three groups: I) unoperated controls (n = 6), 2) 4 wk of MR (n = 6), and 3) 4 wk of MR treated with β₁-AR blocker (extended release metoprolol succinate, 100 mg orally, once daily; n = 8), starting 24 h after MR induction. In these same animals, our group has previously reported the results of M-mode echocardiography that was performed in the conscious state at baseline and at the time of euthanasia as well as the effects of ANG II infusion in the anesthetized state (22). Dogs were transferred to the University of Alabama at Birmingham for euthanasia. Metoprolol succinate was withheld on the day of the terminal experiments. This study was approved by the Animal Services Committees at the University of Alabama at Birmingham and Auburn University.

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Terminal Study: Instrumentation

Animals were maintained at a deep plane of general anesthesia using isoflurane and were mechanically ventilated (Harvard Apparatus, South Natick, MA) as described previously (22). All catheters for LV pressure and coronary sinus and aortic blood samples were inserted as described previously (6, 22). After a median sternotomy, all preganglionic inputs to stellate ganglia were severed and bipolar stimulating electrodes were inserted into both ganglia as described previously (6). Both cervical left and right vagi were sectioned. The heart was suspended in a pericardial cradle. Microdialysis probes (Chirans, Terumo, Tokyo, Japan) were inserted into the LV anterior wall and perfused at 2.5 μl/min as described previously (6, 22). Based on in vitro experiments, 18.8% recovery was used in the calculation of ISF NE and EP values (6, 22).

Experimental Protocol

Dialysate was collected for 10 min before bilateral stellate ganglia stimulation (5 ms, 4 Hz, 8–14 V for 10 min). For each animal, stimulation voltage was set at times two threshold as described previously (6, 22). During stimulation, dialysate was collected separately for the first 3 min, the second 3 min, and the last 4 min. After stimulation, dialysate was collected sequentially for the first and second 5 min. Aortic and coronary sinus blood samples were taken at 8 min of baseline and at 8 min of stellate ganglion stimulation. At the end of the physiological experiments, the heart was arrested with intracardiac KCl and quickly extirpated, placed in phosphate-buffered ice slush, and the coronaries were flushed with oxygenated Krebs solution. The LV was cut into 1-cm cubes and snap frozen in liquid nitrogen for subsequent analysis of catecholamine content and β-AR autoradiography.

Biochemical Analyses

ISF and tissue catecholamine levels. ISF NE and EP values at baseline (2 collections) and during stimulation (3 collections) were averaged for one baseline value and one stimulation value. ISF and plasma NE and EP concentrations were determined with the Biotrak catecholamines radioenzymatic assay (Amersham Pharmacia Biotech) as previously described (6, 22).

Tissue catecholamine content. Ventricular tissue NE and EP contents were measured by high-performance liquid chromatography using a Waters 460 electrochemical detector (Milford, MA) as described previously (3).

Receptor Autoradiography

Labeling of β1- and β2-ARs in 20-μm thick sections of unfixed LV myocardium was done by a modification of published protocols (14, 20) using I-[125I]iodocyanopindolol (PerkinElmer Life Sciences; Boston, MA), which is a membrane permeant ligand and measures both extracellular and intracellular receptors. Total binding was determined by incubation of sections for 90 min at 37°C in a Tris-salt buffer containing 0.1 nM [125I]cyanopindolol, 10 μM pargyline, and 10 μM serotonin. Incubation buffers for other sections also contained 3 μM timolol (nonspecific binding) or 0.5 μM CGP-20712A, a β1-AR antagonist. Radioligand binding sites were identified by film autoradiography and quantified by using a microcomputer-assisted imaging device (Imaging Research) as described previously (20). Total β-AR levels were calculated by subtracting nonspecific binding from total binding. Nonspecific binding was subtracted from [125I]cyanopindolol binding in the presence of CGP-20712A to determine the abundance of β2-ARs. β1-AR density was calculated by subtraction of β2-AR density from that for total β-ARs. Data are presented as femtomoles [125I]cyanopindolol bound per milligram of tissue.

Statistical Analysis

Analysis of NE and EP was compared in control, MR, and MR + β1-RB groups during basal and stellate stimulation separately, using the Kruskal-Wallis test. This statistical test is an analog of ANOVA but does not require normal distribution and constant variance of data. This analysis was required because the data violated assumptions of ANOVA, namely normality and constant variance, which is not unexpected for these physiological variables. If the Kruskal-Wallis test was significant at the 5% level, pairwise comparisons were performed by using Wilcoxon test, which is an analog of the t-test but does not require normal data distribution. Type 1 error was set at 5%. Bonferroni adjustment was utilized to address the issue of inflating type 1 error due to multiple testing in the pairwise comparisons stage. Note that in the comparison of three groups, there are a total of three pairs. Hence, in the pairwise comparisons, only P values <0.0167 (≈0.05/3) were considered significant to achieve an overall 5% level of significance.

RESULTS

Hemodynamic Response to Stellate Ganglia Stimulation

In comparison with the control group, peak change of LV pressure over time (+dP/dt) was significantly lower at baseline...
in both the MR and MR + β₁-RB, which was in parallel with significantly lower baseline mean arterial pressure (Fig. 1). Stellate-mediated increases in dP/dt did not differ from control in both the MR and MR + β₁-RB groups. The chronotropic responses were similar in the control and MR group, whereas the increase in heart rate was significantly blunted in the MR + β₁-RB dogs (last dose, 36 h before experiment).

**NE Responses to Stellate Stimulation**

Baseline transcardiac plasma NE levels measured in MR dogs with and without β₁-RB were not statistically different from controls (Table 1). During stellate ganglion stimulation, significant transcardiac NE differences of a similar magnitude (aortic-coronary sinus levels ≥ 1,000 pg/ml) were detected in all three groups (data not shown). ISF NE levels increased over baseline in all groups. However, the peak ISF NE level achieved during stellate ganglion stimulation in the 4-wk MR group was significantly greater than in the control group, whereas peak ISF NE levels in the MR + β₁-RB group were not different from controls (Fig. 2). It is noteworthy that the stimulated ISF NE increments were preserved or increased (in comparison to the control group) despite a significant 45% decrease in the tissue NE stores in MR dogs with and without β₁-RB (Table 1).

**EP Responses to Stellate Stimulation**

Similar baseline transcardiac plasma EP levels and similar tissue EP levels were measured in all three groups (Table 1). In response to stellate ganglion stimulation, EP ISF levels increased significantly in all three groups without differences among them (Fig. 3).

**β-Adrenoreceptor Density**

When compared with the density of the control group, LV β₁-AR density was reduced by 50% at 4-wk MR in the two groups with or without +β₁-RB, whereas β₂-AR values were not significantly different among all three groups (Fig. 4).

**DISCUSSION**

Our study reports that NE release by stellate ganglion stimulation into the cardiac interstitum of MR dogs is twofold higher than in control dogs. This finding is consistent with the notion of exaggerated sympathetic efferent responses sustaining cardiac function in the early phases of MR. The higher stimulated NE interstitial levels attained in the untreated MR group are associated with relatively greater than control ISF EP levels (although not statistically significant). The enhancement of NE release occurred in the face of a 45% decrease in LV tissue NE content. Neuronal NE stores form the major part of tissue NE content, which is therefore subject to pathological modifications of the rates of release, turnover, and synthesis. Previous studies in other forms of heart failure have demon-

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**Table 1. Baseline Ao and CS and LV NE and EP levels in MR dogs**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MR</th>
<th>MR + β₁-RB</th>
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<tbody>
<tr>
<td>Ao NE</td>
<td>300±70</td>
<td>335±77</td>
<td>271±53</td>
</tr>
<tr>
<td>CS NE</td>
<td>232±33</td>
<td>389±54</td>
<td>337±61</td>
</tr>
<tr>
<td>Ao EP</td>
<td>175±38</td>
<td>339±72</td>
<td>175±49</td>
</tr>
<tr>
<td>CS EP</td>
<td>119±19</td>
<td>246±104</td>
<td>186±65</td>
</tr>
<tr>
<td>LV NE</td>
<td>927±84</td>
<td>519±53*</td>
<td>530±58*</td>
</tr>
<tr>
<td>LV EP</td>
<td>63±25</td>
<td>29±5</td>
<td>87±35</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, sample size. Ao, aorta; CS, coronary sinus; LV, left ventricular; NE and EP, norepinephrine and epinephrine, respectively (in pg/ml); MR, mitral regurgitation; β₁-RB, β₁-receptor blockade. *P < 0.05 vs. control.
noted that at this early stage of MR, stimulated heart rate compared with control dogs. It should be noted that diastolic dimension in the early stage of MR could also be an important variable to consider in the pathophysiology of adverse LV remodeling in the dog with experimentally induced MR. Taken together with the previous report, this study suggests that the β1-RB attenuates the risk of potentially untoward effects from excessive sympathetic efferent neural release of NE into the ISF as well as from ANG II-stimulated NE release in the volume overload of MR.

The current investigation also demonstrates that β1-RB mitigates excessive sympathetic neuronal NE release in response to electrical stimulation of the stellate ganglia in the early phase of the volume overload of MR. These results suggest that, in addition to the direct effects mediated on cardiomyocyte β-ARs, a major target for β1-RB in MR may be the β-ARs on cardiac neurons (25). This mechanism of action thus provides an added benefit of decreasing the potentially long-term toxic effects of elevated NE in the interstitium of the heart. Work performed in the tachycardia-induced heart failure model indicates that chronic sympathetic efferent neural activation induces a state of oxidative stress in the cardiac ISF (10), which can directly activate matrix metalloproteinases (18, 24). The canine MR model is marked by activation of matrix metalloproteinases and a 50% decrease in extracellular matrix as early as 2 wk that persists for 6 mo (21). We have shown that β1-AR blockade significantly attenuates extracellular matrix dissolution in the MR model at 4 wk (22).

We postulate that increased ISF NE levels in the conscious dog are reflected by our neural-stimulated levels in the anesthetized state, thereby accounting for the decrease in β1-AR density. Although chronic β1-RB in MR dogs attenuated the rise in neuronal NE release in response to stellate stimulation, downregulation of the β1-AR was not reversed by β1-RB. This suggests that suppression of sympathetic drive was not complete, as evidenced by our persistent decrease in tissue NE levels. Nevertheless, β1-RB did result in a decrease in stellate-stimulated heart rate compared with control dogs. It should be noted that at this early stage of MR, β1-RB failed to attenuate the small (15%) increase in LV end-diastolic dimension in our MR dogs at 4 wk (22). This adaptive increase in LV end-diastolic dimension in the early stage of MR could also be a stimulus for β1-AR downregulation, despite the attenuation of excessive ISF NE release after β1-AR blockade.

In dogs with MR and MR + β1-RB treatment, the increase in stellate-evoked +dP/dt_{max} was similar to control at this early compensated stage of MR. However, the positive chronotropic response was slightly decreased in MR + β1-RB compared with controls but without differing significantly from untreated MR animals. This could be due to a greater sensitivity of pacemaker mechanisms to the reduced NE release into cardiac ISF in the MR + β1-RB group, whereas the preservation of the ISF NE response could be a contributing factor to the increase in stellate evoked increase in dP/dt_{max}. In the present study, ISF NE release was equivalent in MR and MR + β1-RB dogs, despite a tendency for lower LV EP tissue levels in the untreated MR group. This could be an indication that most of the EP present in adrenergic nerve terminals is readily available for release even when the stores are reduced in this model. It is noteworthy that there was also a tendency for higher circulating levels of EP and lower LV EP levels among the untreated MR canines. This may be related to the previously reported downregulation of the cardiac renin-angiotensin system and reduction of ANG II-mediated plasma and ISF EP release after chronic β1-RB in this model (22).

In summary, there are attendant risks to excessive sympathetic efferent neural activation, including receptor desensitization, eventually leading to diminished functional responses to the sympathetics (9). In addition to its effect on β1-AR signaling, NE itself generates toxic oxidative metabolites in the cardiac ISF space that can reduce cardiac nerve and myocyte viability (11) and promote extracellular matrix degradation (18, 24). Dell’Italia and colleagues (21) have previously reported that extracellular matrix degradation appears to be important in the pathophysiology of adverse LV remodeling in the dog with experimentally induced MR. Taken together with the previous report, this study suggests that the β1-RB attenuates the risk of potentially untoward effects from excessive sympathetic efferent neural release of NE into the ISF as well as from ANG II-stimulated NE release in the volume overload of MR.

**REFERENCES**


