The α1A-adrenergic receptor subtype mediates increased contraction of failing right ventricular myocardium

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Dysfunction of the right ventricle (RV) is closely related to prognosis for patients with RV failure. Therefore, strategies to improve failing RV function are significant. In a mouse RV failure model, we previously reported that α1A-adrenergic receptor (α1-AR) inotropic responses are increased. The present study determined the roles of both predominant cardiac α1-AR subtypes (α1A and α1B) in upregulated inotropy in failing RV. We used the mouse model of bleomycin-induced pulmonary fibrosis, pulmonary hypertension, and RV failure. We assessed the myocardial contractile response in vitro to stimulation of the α1A-subtype (using α1A-subtype-selective agonist A61603) and α1B-subtype [using α1A-subtype selective α1A-AR agonist phenylephrine (PE)]. In wild-type nonfailing RV, a negative inotropic effect of α1-AR stimulation with PE (force decreased ~50%) was switched to a positive inotropic effect (PIE) with bleomycin-induced RV injury. Upregulated inotropy in failing RV occurred with α1A-subtype stimulation (force increased ~200%), but not with α1B-subtype stimulation (force decreased ~50%). Upregulated inotropy mediated by the α1A-subtype involved increased activator Ca2+ transients and increased phosphorylation of myosin regulatory light chain (a mediator of increased myofilament Ca2+ sensitivity). In failing RV, the PIE elicited by the α1A-subtype was appreciably less when the α1A-subtype was stimulated in combination with the α1B-subtype, suggesting functional antagonism between α1A- and α1B-subtypes. In conclusion, upregulation of α1-AR inotropy in failing RV myocardium requires the α1A-subtype and is opposed by the α1B-subtype. The α1A-subtype might be a therapeutic target to improve the function of the failing RV.

α1A-adrenergic; inotropic; right ventricle; myosin regulatory light chain

NEW & NOTEWORTHY

For failing right ventricle (RV) myocardium, α1A-adrenergic receptor (α1-AR) inotropic responses were increased. Of the two predominant cardiac α1-AR subtypes (α1A and α1B), increased α1-AR inotropy for failing RV myocardium required the α1A-subtype and was opposed by the α1B-subtype. The α1A-subtype might be a therapeutic target to improve the function of the failing RV.

FAILURE OF THE RIGHT VENTRICLE (RV) is a serious and common clinical problem (15, 24); nevertheless, RV failure remains relatively understudied and poorly understood (37). RV dysfunction is a predictor of survival for patients with moderate or advanced heart failure (7, 9) and is strongly related to the prognosis of heart failure patients with pulmonary hypertension (14). Therefore, strategies to improve RV function may be beneficial for improving the survival and functional state of patients with severe RV failure.

Recent studies suggest that in heart failure, a higher level of α1A-adrenergic receptor (AR) activation is beneficial (3, 20, 29). In heart failure β-ARs are markedly downregulated; in contrast, α1-AR levels are not decreased (4). Consequently, α1-ARs, which represent ≈10% of all ARs in nonfailing hearts, are increased to ≈25% of ARs in heart failure (29). Thus α1-ARs may play a relatively greater role in heart failure. Consistent with this, in myocardium isolated from failing human hearts, α1A-AR mediated inotropy was upregulated and equalled β-AR mediated inotropy (34). Moreover, we reported that in a mouse model of RV failure induced secondary to LV failure, the RV myocardial inotropic response to α1-ARs was upregulated (38).

There are two predominant α1-AR subtypes on cardiac myocytes (α1A and α1B). The goal of this study was to determine the roles of both α1-AR subtypes in upregulation of α1-AR inotropic responses in the failing RV. We used the RV-specific mouse heart failure model of bleomycin-induced pulmonary fibrosis, pulmonary hypertension, and RV failure (17). We found that upregulated α1-AR inotropy in the failing RV is associated exclusively with the α1A-subtype and involves increased Ca2+ transients and increased phosphorylation of cardiac myosin regulatory light chain. In contrast, inotropic responses mediated by the α1B-subtype were not changed compared with nonfailing RV. Therefore, this study demonstrates that the α1A-subtype, but not the α1B-subtype, plays a role in augmenting contraction in RV failure and suggests that the α1A-subtype might be a therapeutic target for improving the function of the failing RV.

METHODS

This institution is accredited by the American Association for the Accreditation of Laboratory Animal Care (Institutional Public Health Service Assurance Number is A3476-01). The study was approved by the Animal Care and Use Subcommittee of the San Francisco Veterans Affairs Medical Center (Protocol 13-013) and conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Revised 2011).

Animals and RV injury model. Adult male wild-type (C57BL/6J; Jackson Laboratory) and α1A-subtype knock-out mice (AKO) (31) were used, age 10–14 wk, body weight ~28 g, at the beginning of the experiment. We used a right ventricle (RV)-specific heart failure model. The fibrogenic antibiotic bleomycin was introduced into the lungs by endotracheal instillation to cause pulmonary fibrosis, pulmo-
In the present study, similar results were obtained using either protocol.

In vivo hemodynamics. Echocardiography was performed on conscious, gently restrained mice using an Acuson S2000 (Siemens) with a 5- to 14-MHz multi-dimension matrix transducer. RV and LV dimensions were measured using two-dimensional-guided M-mode, acquired from five consecutive cardiac cycles in the long axis view. Fractional shortening for each chamber was calculated from the diastolic minus systolic chamber dimension expressed as a percentage of the diastolic dimension. The echocardiographer was blinded to the treatments of all mice.

RV trabeculation preparation and measurement of calcium transients. Mice were anesthetized with 100 mg/kg ip pentobarbital sodium and heparinized (100 U). Hearts were removed and immediately immersed in ice-cold arrest solution containing (in mM) 120 NaCl, 30 KCl, and 0.1 CaCl₂ and then perfused through the aorta with a Krebs-Henseleit solution containing (in mM) 137 NaCl, 10 KCl, 1.2 MgSO₄, 1.2 NaH₂PO₄, 10 glucose, 20 NaHCO₃, 0.2 CaCl₂, and 30 2,3-butanedione monoxime. The perfusate was oxygenated with 95% O₂-5% CO₂ to give a pH of 7.4 at 22°C. The RV free-wall was removed, and a trabecula that was free-running between the RV and tricuspid valve was dissected.

Bleomycin (Sigma-Aldrich, St. Louis, MO) was solubilized in sterile normal saline. Most animals were given a single instillation of 0.075 U of bleomycin in 100 μl saline or saline alone, and animals were harvested after 14–30 days. A subset of animals (reported in Fig. 3A) were studied <2 wk after bleomycin instillation, but the data from this subset were not pooled with all other data obtained >2 wk after bleomycin instillation. Where indicated in the results, a few animals received a lower dose of bleomycin (0.04 U) repeated every 2 wk (up to 8 instillations) (8). The repetitive instillation protocol was suggested to be a better model for idiopathic pulmonary fibrosis. In the present study, similar results were obtained using either protocol.

2 wk after bleomycin instillation. Where indicated in Fig. 3A, data obtained

In vivo was slightly reduced to ~80% of initial (P < 0.002).

Fura-2 fluorescence was measured at an emission wavelength of 510 nm, with the excitation wavelength alternated between 340 nm and 380 nm (Photon Technology International, Edison, NJ). Fluorescence signals at each wavelength were integrated at 100 points/s. Muscle autofluorescence was subtracted before the fura-2 fluorescence ratio was computed. Fura-2 ratio was measured during contractions before and intermittently during the inotropic responses. Fura-2 AM can result in nonspecific loading into multiple cell compartments (which was not determined in this study). The rapid fura-2 fluorescence transient during contraction was used as a measure of the cytosolic Ca²⁺ transient.

Inotropic responses. Cardiac α₁-ARs were stimulated by addition of a maximal dose (10 μM) of phenylephrine (PE). In wild-type mice, PE stimulates both predominant α₁-AR subtypes on cardiac myocytes (α₁A and α₁B). A maximal dose of the subtype-selective agonist A61603 (100 nM) was used to selectively stimulate the α₁A-subtype. In knockout mice lacking the α₁A-subtype (AKO), PE was used to selectively stimulate the remaining α₁B-subtype. The β-AR antagonist timolol (10 μM) was present in all experiments. Acute inotropic responses were assessed when contraction force stabilized, typically ~20 min after agonist stimulation. For some experiments, inotropic responses were expressed using the absolute force level per unit area of myocardium (in mN/mm²). For other experiments, the cross-sectional area of the preparation could not be determined (e.g., due to a complex/branched trabecula structure); therefore, inotropic responses were also assessed from the developed force (systolic minus diastolic force) after addition of agonist, expressed relative to the developed force before agonist. Both measures gave similar results.

Phosphorylation status of myosin regulatory light chain. Measurements of regulatory light chain (RLC) phosphorylation in each trabecula were performed by urea/glycerol-PAGE and immunoblotting, as previously described (22). This method separates phosphorylated RLC from nonphosphorylated RLC, allowing a direct quantitative measure of phosphorylated RLC as a fraction of total RLC. As the separation results from a single phosphate, data may also be calculated as mole of phosphate per mole RLC. After contractions were recorded, a trabecula was immediately placed in 10% ice-cold trichloroacetic acid and 10 mM dithiothreitol. The precipitated trabecula was washed free of acid with three 5-min washes in ethyl ether and resuspended by vigorous agitation in 15 μl of urea sample buffer of 8 M Urea, 20 mM Tris base, 23 mM glycine, 0.2 mM EDTA, and 10 mM dithiothreitol using an orbital shaker (IKA Vibrax VXR) set at 1,400 rpm for 30 min at room temperature. Complete denaturation and solubilization was achieved by addition of urea crystals and prolonged agitation. Protein samples were centrifuged at 10,000 g for 2 min, and 5 μl of supernantant fraction containing denatured proteins was used for Western blotting to detect RLC phosphorylation. RLC phosphorylation was quantified using chemiluminescence and image analysis.

Fig. 1. Bleomycin (Bleo) model of right ventricular (RV) failure. Echocardiographic assessment of RV and left ventricular (LV) fractional shortening before and 2 wk after bleomycin instillation was completed. A: 2 wk after bleomycin instillation, RV fractional shortening in vivo was reduced to 45% of the value before bleomycin (P = 0.002). After saline instillation, RV fractional shortening in vivo was slightly reduced to ~80% of initial (P = 0.04). B: LV in vivo fractional shortening was not affected by bleomycin or saline instillation, demonstrating the RV-specific nature of the bleomycin model. *P < 0.05; **P < 0.01; ns, not significant.
directly loaded onto the glycerol gel system for separation of phosphorylated from nonphosphorylated RLC proteins. Briefly, polyacrylamide gels containing 40% glycerol were pre-electrophoresed for 1 h at 400 V at room temperature. After transfer, proteins were fixed onto the polyvinylidene difluoride membrane for 1 h at 0.3 A 4°C. After transfer, proteins were fixed onto the polyvinylidene difluoride membrane with 0.4% glutaraldehyde/PBS for 15 min at room temperature. The membrane was then rinsed 3× in PBS and immunoblotted with antibody to cardiac RLC (Enzo Life, F109 3E1). RLC was detected using ECL Plus (Pierce), and fluorescent signal acquired by Storm (GE Healthcare). RLC and p-RLC bands were auto-detected and quantified by ImageQuant TL (GE Healthcare).

Statistical analysis. Data are presented as means ± SE. Statistical tests (paired and unpaired t-tests, linear regression, and χ² test) were performed using Prism 6 software (GraphPad Software, La Jolla, CA) with a significance level set at P < 0.05.

RESULTS

Model of bleomycin-induced RV failure. The RV-specific bleomycin model of RV failure was reported to cause pulmonary fibrosis, pulmonary hypertension, RV hypertrophy, decreased RV ejection fraction, and RV failure within ~2 wk (17). Consistent with this we found that 2 wk after bleomycin instillation, there was a greater than twofold increase in lung weight relative to body weight, a 55% reduction in RV fractional shortening (Fig. 1A), and high mortality (~50%). There was a 21% reduction in fractional shortening in the saline-treated group (Fig. 1A), suggesting that instillation of saline into the lung may result in mild injury. However, the reduction in fractional shortening due to bleomycin was considerably greater than the reduction due to saline (P = 0.018). With confirmation of the RV-specificity of this model, LV fractional shortening was not affected (Fig. 1B).

Upregulation of α1-AR inotropy in RV failure. We studied cardiac trabeculae from failing RV (bleomycin-treated) or nonfailing RV (saline-treated) and measured the acute α1-AR inotropic response to PE, a nonselective α1-AR agonist that stimulates both predominant α1-AR subtypes on cardiac myocytes (α1A and α1B). We also measured the inotropic response to the α1A-subtype-selective agonist A61603. All experiments were in the presence of the β-AR blocker timolol.

Figure 2 shows representative recordings of contractions of RV trabeculae with agonist stimulation. For nonfailing RV (saline treated), stimulation of the α1A-subtype by A61603 elicited a negative inotropic effect (NIE) that was identical to that elicited by combined stimulation of both the α1A- plus α1B-subtypes using PE. Thus, for nonfailing RV, stimulation of the α1A-subtype singly, or in combination with the α1B-subtype, elicited a NIE, consistent with our previous reports (25, 39).

In contrast, for failing RV there was a switch to a positive inotropic effect (PIE) (Fig. 2). This is consistent with our previous finding of increased α1-AR inotropy in a model of RV failure induced secondary to LV failure (38). Interestingly, the PIE elicited by A61603 (α1A agonist) was greater than the PIE elicited by PE (α1A plus α1B mixed agonist) (discussed below).

The timing of contraction and relaxation were not affected by α1-AR stimulation, as evidenced by the similar time course of contraction and relaxation both before and after agonist (Fig. 2B) and from the data in Table 1 summarizing the time to peak contraction and the relaxation time.

Upregulation of α1-AR inotropy develops 2 wk after bleomycin instillation. Figure 3A summarizes the inotropic response elicited by A61603 at various times after bleomycin instillation. For RV myocardium studied less than 2 wk after bleomycin, a PIE was observed. For RV myocardium studied more than 2 wk after bleomycin, a PIE was not observed. Accordingly,
UPREGULATION OF \(\alpha_1\)-AR INOTROPY IN FAILING RV

Table 1. No effect of \(\alpha_1\)-AR stimulation on the timing of contraction or relaxation of nonfailing or failing RV myocardium

<table>
<thead>
<tr>
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<th>Time To Peak, ms</th>
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<tr>
<td></td>
<td>Saline (4–5)</td>
<td>Basal</td>
<td>A61603</td>
<td>PE</td>
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<tr>
<td></td>
<td>137 ± 1</td>
<td>139 ± 1</td>
<td>136 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bleomycin (3–7)</td>
<td>137 ± 1</td>
<td>149 ± 2</td>
<td>138 ± 3</td>
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<tr>
<td></td>
<td></td>
<td>Basal</td>
<td>A61603</td>
<td>PE</td>
</tr>
<tr>
<td></td>
<td>103 ± 6</td>
<td>103 ± 6</td>
<td>101 ± 6</td>
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Values are means ± SE. Measurements of the time to peak force (interval from the start of a contraction to the peak contraction force) and 50% relaxation time (RT50, interval from the peak contraction force to the time when force had relaxed by 50%) are shown. Nonfailing or failing right ventricle (RV) myocardium was obtained from animals 2 wk after a single tracheal instillation of saline or bleomycin, respectively. For these experiments, \(\alpha_1\)-adrenergic receptor (\(\alpha_1\)-AR) stimulation elicited a negative inotropic response in nonfailing RV, but a positive inotropic response in failing RV. However, the timing of contraction and relaxation was not statistically different than before agonist (number/group indicated in parentheses). PE, phenylephrine.

all of the other data presented in this study involved mice more than 2 wk after bleomycin or saline instillation.

Figure 3A also shows that the switch to a PIE elicited by A61603 did not occur in a subset of hearts. Nevertheless, there was a markedly increased lung weight in animals whether \(\alpha_1\)-A-subtype inotropic response was increased (converter group) or not (nonconverter group) (see Table 2), suggesting that for some animals, the lack of upregulation of the \(\alpha_1\)-A-subtype inotropic response was not due to a lack of bleomycin-induced lung injury.

Figure 3B summarizes the inotropic response elicited by A61603 for hearts studied at least 2 wk after bleomycin instillation. As described above, bleomycin-treated hearts were divided into converter or nonconverter groups based on whether A61603 elicited a PIE or NIE, respectively. Moreover, inotropic responses assessed using absolute force units (Fig. 3B) were consistent with inotropic responses assessed using relative force units.

Upregulation of \(\alpha_1\)-AR inotropy in failing RV requires the \(\alpha_1\)-A-subtype. Figure 4 summarizes the contractile response to stimulation of the \(\alpha_1\)-A-subtype singly by A61603 or in combination with the \(\alpha_1\)-B-subtype using PE. For nonfailing RV, both A61603 and PE elicited a NIE (Fig. 4A) and there was no statistical difference between the responses to these agonists. After bleomycin treatment, \(\approx 60\% \) of animals had a markedly increased \(\alpha_1\)-A-subtype inotropic response than saline-treated controls (converter group, Fig. 4C). For the remainder, the \(\alpha_1\)-A-subtype inotropic response was similar to nonfailing RV myocardium (nonconverter group, Fig. 4B). For RV myocardium from bleomycin-treated animals, the nonconverter group was similar to the nonfailing RV group in having a NIE elicited both by A61603 or PE (Fig. 4B). In contrast, for the converter group, after 2 wk of bleomycin treatment, there was a switch in the \(\alpha_1\)-A-subtype inotropic response to a PIE (Fig. 4C). As noted in Fig. 2, the pooled data in Fig. 4C show that the PIE elicited by the \(\alpha_1\)-A-subtype agonist A61603 was appreciably greater than that induced by combined stimulation of both \(\alpha_1\)-A and \(\alpha_1\)-B-subtypes with PE. The difference between the inotropic response to A61603 (reflecting the \(\alpha_1\)-A-subtype) versus the inotropic response to PE (reflecting \(\alpha_1\)-A plus \(\alpha_1\)-B-subtypes) may reflect the inotropic effect mediated by the \(\alpha_1\)-B-subtype alone. The difference between the inotropic response to A61603 minus the response to PE is calculated to be a NIE attributed to the \(\alpha_1\)-B-subtype (Fig. 4C).

Taken together, these data suggest that the \(\alpha_1\)-A-subtype mediates a NIE in nonfailing RV, but in failing RV the \(\alpha_1\)-A-subtype inotropic response can be switched to a PIE. In contrast, the \(\alpha_1\)-B-subtype mediates an NIE in nonfailing RV, and the \(\alpha_1\)-B-subtype response is not switched in failing RV. Thus upregulation of \(\alpha_1\)-AR inotropy in the failing RV in-
NIE elicited by PE was observed for all AKO mice that had mice was unresponsive to the A61603 or PE. As expected, RV myocardium from AKO P/H11021§Significantly different that nonconverter (number/group indicated in parentheses). ***Significantly different than vehicle (P < 0.0001); §Significantly different that nonconverter (P < 0.05).

volves functional antagonism between the α1-A-AR subtypes, with a PIE mediated solely by the α1A-subtype and antagonized by a NIE mediated by the α1B-subtype.

To confirm the key role of the α1A-subtype in upregulation of α1-AR inotropy in the failing RV, we used knockout mice lacking the α1A-subtype (AKO). For AKO mice, the predominant remaining α1-AR subtype on cardiac myocytes is the α1B-subtype. Therefore, inotropic stimulation of myocardium from AKO mice using PE would stimulate the remaining α1B-subtype. Figure 5 shows original records of the contractile response of AKO myocardium following stimulation with A61603 or PE. As expected, RV myocardium from AKO mice was unresponsive to the α1A-subtype-selective agonist A61603. For mice 2 wk after tracheal instillation of saline or bleomycin, there was a NIE elicited by PE. The summary of all data (Fig. 6) shows that for AKO myocardium, stimulation of the remaining α1B-subtype using PE resulted in a NIE (force decreased ~50%) in both nonfailing RV myocardium (Fig. 6A) and failing RV myocardium (Fig. 6B). Figure 6B shows that a NIE elicited by PE was observed for all AKO mice that had been instilled with bleomycin. Thus, for RV myocardium from animals instilled with bleomycin, a PIE in response to PE was observed for most wild-type hearts but not observed for AKO hearts. This difference is statistically significant (P < 0.05, χ² test). We conclude that the α1B-subtype elicits a NIE, in both nonfailing and failing RV. This is consistent with the data in Fig. 4 suggesting the α1B-subtype has a NIE in both nonfailing and failing RV. Thus upregulation of α1-AR inotropy in the failing RV requires the α1A-subtype. Moreover, in failing RV, the α1B-subtype does not switch to a PIE but elicits a NIE. Together these data suggest that in the failing RV functional antagonism can emerge between the α1A-subtype (that mediates a PIE) and the α1B-subtype (that mediates an NIE). Specifically, when the α1A-subtype is stimulated in combination with the α1B-subtype the inotropic response is lower than when the α1A-subtype is stimulated in the absence of the α1B-subtype.

Table 2. Effect of tracheal instillation of bleomycin or saline on body and organ weights

<table>
<thead>
<tr>
<th></th>
<th>Percent Change in Body Weight</th>
<th>Lung Weight/Body Weight, mg/g</th>
<th>RV Weight/Body Weight, mg/g</th>
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<tbody>
<tr>
<td>Saline (23)</td>
<td>6.4 ± 0.9</td>
<td>5.7 ± 0.2</td>
<td>1.11 ± 0.03</td>
</tr>
<tr>
<td>Bleomycin</td>
<td></td>
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</tr>
<tr>
<td>nonconverter (8)</td>
<td>-9.4 ± 3.4**§</td>
<td>12.1 ± 1.1***</td>
<td>1.22 ± 0.04</td>
</tr>
<tr>
<td>Converter (12)</td>
<td>-15.0 ± 3.2***§</td>
<td>13.2 ± 1.4***</td>
<td>1.37 ± 0.04§§</td>
</tr>
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</table>

Values are means ± SE. A single instillation of bleomycin (0.075 U) or saline was given. After at least 2 wk, the effect of bleomycin was assessed on lung weight and on the RV free wall weight. Bleomycin resulted in a decrease in body weight, an increase in lung weight, and increased RV weight. The bleomycin group is divided into 2 subgroups, depending on whether the α1-AR subtype-mediated effects on Ca²⁺ involved either increased Ca²⁺-sensitivity (2). Therefore, for trabeculae that manifested a PIE elicited by A61603, we measured the extent of phosphorylation of cardiac RLC. Figure 8 shows that for nonfailing RV myocardium, the RLC phosphorylation level was ~50% as previously reported for contracting.
myocardium (10). Moreover, the RLC phosphorylation level was not changed by A61603 stimulation. This indicates that the NIE elicited by A61603 stimulation in nonfailing RV did not involve decreased RLC phosphorylation, but instead, other mechanisms such as decreased Ca\textsuperscript{2+} transients may be important. Interestingly, the RLC phosphorylation level for the nonfailing RV (≈15% of total RLC) was substantially lower than nonfailing RV (≈40% of total RLC, P = 0.03). Moreover, for hearts that manifested a PIE in response to α1A-subtype stimulation, there was a marked increase in the level of RLC phosphorylation (P = 0.036). Thus increased RLC phosphorylation may contribute to the PIE elicited by the α1A-subtype in the failing RV.

**DISCUSSION**

RV failure results in downregulation of β-ARs in humans and multiple experimental models in rodents (30). In contrast, α1-AR inotropic responses are upregulated in failing RV (38). Of the two predominant subtypes of α1-AR on cardiac myocytes (α1A and α1B), the current study indicates that upregulation of α1-AR inotropy in failing RV myocardium is mediated solely by the α1A-subtype. An increased level of contraction mediated by the α1-A-subtype might provide inotropic support for the failing RV. Moreover, the α1-A-subtype may represent a novel therapeutic target to augment the function of the failing RV. Because the prognosis of heart failure patients with pulmonary hypertension is strongly related to RV dysfunction (14), strategies to improve RV function may be beneficial for improving survival of patients with severe RV failure.

**α1-AR signaling in heart failure.** It has been generally believed that α1-ARs, along with all G protein-coupled receptors that signal through Goq, play a pathogenic role in heart failure. In contrast, this view has been challenged by the suggestion that α1-AR signaling is beneficial in heart failure (3, 20, 29). Consistent with α1-ARs mediating a cardioprotective effect, knockout of α1-AR prevents normal cardiac growth (28) and worsens cardiomyopathy after pressure overload (27). Furthermore, a large clinical trial (ALLHAT) of an α1-AR antagonist was stopped prematurely because of an increase in heart failure in the α1-AR antagonist-treated group (1). These findings suggest that a higher level of α1-AR activation is cardioprotective in heart failure. Consistent with this, in human heart failure, where β-ARs are downregulated, α1-AR mediated inotropy can equal β-AR mediated inotropy, raising the possibility that a relative increase in α1-AR inotropy has a beneficial effect (34). In the context of the RV, we find that α1-AR inotropic responses are upregulated in the failing RV (38), which might help the failing RV adapt to increased pulmonary pressures.

**Cardioprotective role of α1A-subtype signaling.** Previous studies suggest that α1A-subtype signaling has cardioprotective effects. α1A-subtype overexpression enhances contractility without causing hypertrophy (23), protects against pressure-overload-induced dysfunction (11), limits postinfarct cardiomyopathy (12), and protects against ischemic injury (32). Recent studies found that the α1A-subtype mediates a prosurvival effect in cardiac myocytes subjected to multiple pathological conditions (18). Furthermore, α1A-subtype agonist treatment prevents apoptosis in a heart failure model in vivo (6) and prevents cell death and fibrosis and improves in vivo function and survival in multiple heart failure models (26). Consistent with beneficial effects mediated by the α1A-subtype, in the present study we found that the α1A-subtype, but not the α1B-subtype, mediated increased myocardial contraction in the failing RV.

**Functional antagonism between α1A-AR subtypes in failing RV.** We found that for nonfailing RV, the α1A- and α1B-subtypes both elicited a NIE of similar magnitude when stimulated singly or in combination, consistent with our previous study (25). Nevertheless, in failing RV we found upregulation of α1-AR inotropy and the emergence of functional antagonism.
between $\alpha_1A$- and $\alpha_1B$-subtypes. In the failing RV, the $\alpha_1A$-subtype inotropic response was switched from a NIE to a PIE in $\approx 60\%$ of hearts. However, the inotropic response mediated by the $\alpha_1B$-subtype was not observed to be switched and remained as a NIE in both nonfailing and failing RV. Therefore, for the failing RV, the inotropic response to nonsubtype selective $\alpha_1$-AR stimulation might be complex and consist of stimulatory effects mediated by the $\alpha_1A$-subtype that are antagonized by inhibitory effects mediated by the $\alpha_1B$-subtype.

Our findings are consistent with previous studies that suggested that the two predominant cardiac $\alpha_1$-AR subtypes can mediate different inotropic responses in LV myocardium. Previously, the $\alpha_1A$-subtype was linked to a PIE (23), and the $\alpha_1B$-subtype linked to a NIE (16, 33). Furthermore, in rat myocardium, stimulation of the $\alpha_1A$-subtype causes increases in the amplitude of the $Ca^{2+}$ transient and changes in the amplitude of contraction ($R^2 = 0.787, P < 0.0001$). For all experiments involving myocardium from hearts at least 2 wk after bleomycin or saline instillation into the trachea, following $\alpha_1A$-subtype stimulation, there was a significant positive linear relationship between changes in the amplitude of the $Ca^{2+}$ transient and changes in the amplitude of contraction ($R^2 = 0.787, P < 0.0001$) (the relationship remained significant after excluding the most positive value). Some animals received biweekly bleomycin instillation (triangles).

Our findings are consistent with previous studies that suggested that the two predominant cardiac $\alpha_1$-AR subtypes can mediate different inotropic responses in LV myocardium. Previously, the $\alpha_1A$-subtype was linked to a PIE (23), and the $\alpha_1B$-subtype linked to a NIE (16, 33). Furthermore, in rat myocardium, stimulation of the $\alpha_1A$-subtype causes increases in the amplitude of the $Ca^{2+}$ transient and changes in the amplitude of contraction ($R^2 = 0.787, P < 0.0001$). For all experiments involving myocardium from hearts at least 2 wk after bleomycin or saline instillation into the trachea, following $\alpha_1A$-subtype stimulation, there was a significant positive linear relationship between changes in the amplitude of the $Ca^{2+}$ transient and changes in the amplitude of contraction ($R^2 = 0.787, P < 0.0001$) (the relationship remained significant after excluding the most positive value). Some animals received biweekly bleomycin instillation (triangles).

Previous studies suggest that phosphorylation of myosin RLC mediates increased myofilament $Ca^{2+}$ sensitivity and contributes to a PIE mediated by $\alpha_1$-ARs (5, 25). In contrast, in the failing RV, $\alpha_1A$-subtype stimulation resulted in an increase in the $Ca^{2+}$ transient and a PIE. This suggests that a switch in the effect of $\alpha_1A$-subtype stimulation on $Ca^{2+}$ handling, from inhibitory in nonfailing RV to stimulatory in failing RV, contributed to the switch in the inotropic response from a NIE in nonfailing RV to a PIE in failing RV.

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of upregulation of α₁-AR inotropy as evidenced by the finding that a PIE elicited by α₁-ARs was not observed before 2 wk after bleomycin instillation. Interestingly, the RV injury model of bleomycin-induced pulmonary fibrosis is associated with a high level of mortality within the first 2 wk after bleomycin instillation (17), and before upregulation of α₁-AR inotropy develops. Potentially, upregulation of α₁-AR inotropy may be protective and contribute to a lower mortality in the period beyond 2 wk after bleomycin instillation.

There was greater RV hypertrophy in hearts that did manifest a switch to a PIE elicited by α₁-ARs (Table 2). Potentially, both the increased inotropic response and the greater RV mass might contribute to a beneficial effect on RV function.

Limitations. The current study found upregulation of myocardial α₁A-subtype inotropic responses using cardiac trabeculae studied in vitro. Further study is warranted to search for a functional in vivo correlate. The current study used cardiac trabeculae under in vitro conditions of low temperature and low pacing rate. The level of absolute force development observed was considerably lower than that of previous studies using more physiological conditions (35, 36).

The current study was performed using mouse myocardium. Although, α₁-AR levels and regulation in mouse myocardium are similar to those in human (19, 21), future studies should determine whether α₁A-subtype signaling is upregulated in RV myocardium from human hearts with RV failure. Further study is needed to determine the significance of our finding upregulation of α₁A-subtype inotropy in the failing RV. In this regard, chronic agonist stimulation of the α₁A-subtype may be beneficial in the failing heart (6, 26), and upregulation of α₁A-subtype function in the failing RV may contribute to a beneficial effect. Finally, the mechanisms mediating upregulation of α₁A-subtype inotropy remain unclear. For example, the roles of changes in RLC phosphorylation and Ca²⁺ transients were not resolved in this study. Moreover, the underlying mechanisms by which the α₁A-subtype mediates a decreased Ca²⁺ transient in nonfailing RV but an increased Ca²⁺ transient in failing RV need to be defined.

Conclusion

In conclusion, the upregulated α₁-AR inotropic response of failing RV is mediated by the α₁A-subtype and not the α₁B-subtype. The α₁A-subtype may represent a novel therapeutic target to augment the function of the failing RV.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

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