Heart failure switches the RV $\alpha_1$-adrenergic inotropic response from negative to positive

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Wang GY, Yeh C, Jensen BC, Mann MJ, Simpson PC, Baker AJ. Heart failure switches the RV $\alpha_1$-adrenergic inotropic response from negative to positive. Am J Physiol Heart Circ Physiol 298: H913–H920, 2010. First published December 24, 2009; doi:10.1152/ajpheart.00259.2009.—Right ventricular (RV) failure is a serious common clinical problem that is poorly understood. Therefore, for failing and nonfailing hearts, we examined the distinctive inotropic responses induced in the RV myocardium after the stimulation of $\alpha_1$-adrenergic receptors (ARs). In RV trabeculae from nonfailing mouse hearts, $\alpha_1$-ARs induced a negative inotropic response, consistent with our previous study. In marked contrast, in RV trabeculae from failing hearts, 12 wk after coronary artery ligation, $\alpha_1$-ARs induced a positive inotropic response. Mechanistically, experiments with skinned trabeculae showed that $\alpha_1$-ARs decreased myofilament Ca$^{2+}$ sensitivity in the nonfailing RV myocardium, whereas $\alpha_1$-ARs increased Ca$^{2+}$ sensitivity in heart failure. This suggests that a switch in the Ca$^{2+}$ sensitivity response to $\alpha_1$-AR stimulation explained the switch in the RV $\alpha_1$-AR inotropic response in heart failure. Moreover, the MLCK inhibitor ML-9 prevented the switch of the RV inotropic response to stimulation of $\alpha_1$-ARs.

METHODS

RIGHT VENTRICULAR (RV) failure is a prevalent cause of cardiovascular collapse and a major public health problem (14, 20). RV failure frequently arises in patients with left ventricular (LV) failure and causes markedly worse symptoms and prognosis compared with patients with LV failure without RV dysfunction (9, 11, 13). Moreover, the prognosis of heart failure patients with pulmonary hypertension is strongly related to RV dysfunction (13).

Despite its clinical significance, RV failure is relatively understudied and poorly understood (31). The RV has been viewed merely as a weak LV (14). Conversely, we (33) reported that the RV and LV have fundamentally different inotropic responses to stimulation of $\alpha_1$-adrenergic receptors (ARs), which caused negative inotropic responses in the RV myocardium but positive inotropic responses in the LV myocardium. This and another study (31) have indicated that the RV and LV are categorically different and that RV failure cannot be understood by extrapolating data from LV failure. Thus, there is a need for greater understanding of the pathophysiology of RV failure (31). Toward this end, the goal of this study was to determine the impact of heart failure on the inotropic responses of the RV myocardium.

The most common cause of RV dysfunction is chronic left-sided heart failure (16). Multiple mechanisms may be involved, including 1) RV dysfunction secondary to pulmonary venous hypertension caused by LV failure, 2) a cardiomyopathic process affecting both ventricles, 3) myocardial ischemia affecting both ventricles, and 4) ventricular interdependence due to septal dysfunction (16, 31). For patients with moderate or advanced heart failure, RV dysfunction is a predictor of survival (9, 11).

We studied mouse hearts after LV failure and secondary RV failure caused by ligation of the left anterior descending (LAD) coronary artery. We found that, in heart failure, there was a dramatic switch in the RV inotropic response to stimulation of $\alpha_1$-ARs from a negative inotropic response (nonfailing) to a positive inotropic response (failing). In contrast, in the LV myocardium, the $\alpha_1$-AR inotropic response was not different for failing versus nonfailing hearts. Therefore, this study elucidates new features of the failing RV that cannot be extrapolated from studies of the failing LV and that may have implications for understanding and treating human disease.

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separated it from the adjacent noninfarcted myocardium, which remained pigmented. A free-running RV or LV trabecula was dissected (1 trabecula/heart). The width and thickness of trabecula from the RV (136 ± 10 and 96 ± 2 μm, respectively, n = 30) and LV (132 ± 11 and 107 ± 10 μm, respectively, n = 12) were not different (P > 0.05), and there were no differences between failing versus nonfailing groups. Trabeculae were mounted in a muscle chamber between a micromanipulator and force transducer, and sarcomere length was set to 2.1 μm (32). Trabeculae were superfused with Krebs-Henseleit solution (2 mM bath [Ca\(^{2+}\)] at 22°C) and field stimulated (pacing frequency: 0.5 Hz and voltage: 1.5 × threshold) (32).

**Inotropic responses.** After 30 min of equilibration, the α₁-AR agonist phenylephrine (PE; 10 μM) was added to the superfusate. For all experiments, 10–15 min after the addition of the agonist, contraction force stabilized at a new level, and the developed force (systolic minus diastolic) in this plateau region was measured over approximately six consecutive contractions and was expressed relative to the initial developed force. The β-AR antagonist timolol (10 μM) and, for some experiments, myosin light chain (MLC) kinase (MLCK) inhibitor ML-9 (1–(5-chloronaphthalene-1-sulfonyl)-1H-hexahydro-1,4-di-azepine; Sigma, 40 μM) (1) were present 30 min before and throughout agonist stimulation. Previously, ML-9 (10–50 μM) substantially eliminated α₁-AR-induced positive inotropic responses and eliminated α₁-AR-induced MLC2 phosphorylation (1).

**Myofilament function.** After PE treatment, after contraction force had reached a new steady level (~15 min), some trabeculae were then demembranated using 1% Triton X-100, and in vitro myofilament function was assessed 30 min later using steady-state contractions at 37°C. Myofilaments were homogenized in RIPA buffer containing protease inhibitors (no. 100024, Sigma). After centrifugation, samples of the supernatant and myofila-

<table>
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<tr>
<th>Body Weight, g</th>
<th>LV Weight, mg</th>
<th>LV Scar, %LV weight</th>
<th>Lung Weight, mg</th>
<th>Right Ventricular Weight, mg</th>
<th>Liver Weight, mg</th>
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<tbody>
<tr>
<td>Control mice</td>
<td>27.6 ± 0.5</td>
<td>91 ± 1</td>
<td>149 ± 4</td>
<td>23.5 ± 1</td>
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<td>n</td>
<td>22</td>
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<td>12</td>
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<td>Mice with MI</td>
<td>27.7 ± 0.4</td>
<td>110 ± 3*</td>
<td>34 ± 1</td>
<td>115 ± 2*</td>
<td>30 ± 1*</td>
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<td>n</td>
<td>39</td>
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Values are means ± SE; n, number of mice/group. Mice were euthanized 12 wk after myocardial infarction (MI) due to left anterior descending coronary artery ligation. LV, left ventricular. *P < 0.001 by t-test.

Cold cardioplegia solution was perfused antegrade before cardiac, and the explanted heart was placed immediately in ice-cold lactated Ringer solution. Samples from the LV and RV were cleaned rapidly of all epicardial fat, flash frozen in liquid nitrogen, and stored at −80°C.

**Quantitative real-time RT-PCR.** To assess the abundance of human MLCK mRNA, RNA was extracted from myocardial tissue in TRIzol reagent (Invitrogen, GibCO-BRL) and chloroform-isopropyl alcohol, purified on Qiagen Mini-Prep columns, treated with DNase (Turbo DNAfree, Ambion), and quantified using spectrophotometry (SmartSpec 3000, Bio-Rad). One microgram of RNA was reverse transcribed using SuperScript III Reverse Transcriptase (Invitrogen) in the presence of both random hexamers (Invitrogen) and oligo-dT (Roche). Oligonucleotide primers (left: 5’-CGGTGAGACGTGAAGCAGT-3’ and right: 5’-TACCCTGGTGTCAAAAGGAG-3’) were designed for the specific amplification of human smMLCK.

Quantitative real-time RT-PCRs contained 5% of the cDNA product, primers at 125 mM/μL reaction, and SYBR green master (Roche) with ROX reference dye. All reactions were performed in triplicate in an ABI PRISM 7900HT Sequence Detection System. Data were analyzed with SDS software version 2.3 (Applied Biosystems). The relative quantitation of PCR products used the ΔΔCt method, where Ct is the threshold cycle (22). Values for each mRNA are presented as arbitrary units (AU) relative to two reference genes: β-actin and TATA-binding protein (TBP).

**Statistical analysis.** Data are presented as means ± SE. Groups were compared using Students t-test or ANOVA with a Tukey post hoc test.

**RESULTS**

**Heart failure after LAD ligation.** Twelve weeks after MI, there was LV hypertrophy and increased lung weight, consistent with fluid in the lungs from LV failure (Table 1). Moreover, there was hypertrophy of the RV and increased liver weight, consistent with secondary RV failure (Table 1).

For nonfailing and failing hearts, we measured the force development of cardiac trabeculae isolated from the LV or RV. For LV and RV trabeculae, the induction of heart failure did not reduce electrically stimulated force development (5–7 mN/mm², P > 0.05).

The level of F\(_\text{max}\) has been reported to be reduced in heart failure (7). Consistent with this, we found that heart failure (12 wk post-MI) substantially reduced the F\(_\text{max}\) for skinned LV trabeculae (22 ± 1 mN/mm², n = 12) compared with controls (37 ± 1 mN/mm², n = 11, P < 0.001). However, heart failure (12 wk post-MI) did not reduce the F\(_\text{max}\) of RV trabeculae (36 ± 1 mN/mm², n = 12) compared with controls (36 ± 1 mN/mm², n = 12, P > 0.05). Conversely, for a subgroup of hearts studied at 20 wk post-MI, the F\(_\text{max}\) of RV trabeculae was reduced (22 ± 1 mN/mm², n = 3, P < 0.001). Thus, during the
development of heart failure, the reduction in $F_{\text{max}}$ is manifest later in the RV compared with the LV.

**$\alpha_1$-AR inotropic in RV trabeculae.** For the nonfailing RV myocardium, stimulation of $\alpha_1$-ARs with PE in the presence of a $\beta$-AR antagonist caused an overall negative inotropic response (Fig. 1A), consistent with our previous report (33). In marked contrast, in heart failure, the response to $\alpha_1$-ARs in the RV myocardium was switched to a net positive inotropic response (Fig. 1B).

Inotropic responses were quantitated by comparing the developed force (systolic minus diastolic force) before PE with that after PE when force had stabilized to a new level. The change in developed force induced by PE was expressed as a percentage of the developed force before PE.

The data from all experiments (Fig. 2A) showed that in the RV myocardium from failing hearts, the switch to a positive inotropic response induced by $\alpha_1$-ARs was highly statistically significant. However, for hearts subjected to small infarcts (scar weight $<$20% LV weight), the response of the RV myocardium to $\alpha_1$-ARs was not switched to a positive inotropic response (Fig. 2B), suggesting the existence of an injury threshold for switching of the RV inotropic response in heart failure.

Hearts with longer-term MI (20 wk) also had a switch to a positive $\alpha_1$-AR inotropic response (force increased ~77%), which was similar to that observed earlier at 12 wk post-MI (Fig. 2). Thus, in the RV myocardium in failing hearts, the switch to a positive $\alpha_1$-AR inotropic response occurred both before and after myofilament force development ($F_{\text{max}}$) was reduced.

$\alpha_1$-AR inotropic in LV trabeculae. In nonfailing hearts, the LV myocardium had a fundamentally different $\alpha_1$-AR inotropic response compared with the nonfailing RV myocardium. In the nonfailing LV myocardium, $\alpha_1$-ARs mediated a positive inotropic response (Fig. 2C), consistent with our previous report (33). Moreover, in the LV myocardium, the inotropic response to $\alpha_1$-ARs was similar in nonfailing versus failing hearts (Fig. 2C). Thus, in heart failure, the $\alpha_1$-AR inotropic response was switched in the RV myocardium but not in the LV.

**$\alpha_1$-AR effects on RV and LV myofilament properties.** Mechanistically, we investigated the role of myofilament properties in the switch to a positive $\alpha_1$-AR inotropic response in the RV myocardium from failing hearts. First, $\alpha_1$-AR inotropic responses to PE were monitored using intact cardiac trabeculae. After force had stabilized at a new level, trabeculae were demembranated (skinned) to assess myofilament function. A comparison was made with experiments of trabeculae without PE pretreatment before being skinned.

In both LV and RV trabeculae from nonfailing or failing hearts, PE pretreatment before skinning had no effect on $F_{\text{max}}$ compared with untreated trabeculae ($P < 0.6$ by ANOVA). In contrast, as detailed below, PE appreciably affected myofilament $Ca^{2+}$ sensitivity.

Figure 3A shows the relationship between myofilament force and bath $[Ca^{2+}]$ for skinned RV trabeculae from nonfailing and failing hearts both with and without PE pretreatment before being skinned. In nonfailing RV trabeculae, PE pretreatment before skinning resulted in a rightward shift of the relationship between myofilament force and bath $[Ca^{2+}]$ compared with no PE pretreatment (Fig. 3A), consistent with our previous report (33). Moreover, fitting the force-Ca$^{2+}$ relationship to the Hill equation revealed that PE caused a significant increase in EC$50$ (Fig. 3B). These results indicate that PE pretreatment before skinning caused decreased myofilament Ca$^{2+}$ sensitivity.

In marked contrast, in RV trabeculae from failing hearts, PE pretreatment before skinning resulted in a leftward shift of the
sensitivity induced by $\alpha_1$-ARs could contribute to the switch to a positive $\alpha_1$-AR inotropic response.

However, in the LV myocardium, heart failure did not result in a switch in the effect of $\alpha_1$-AR stimulation on myofilament properties. After $\alpha_1$-AR stimulation, myofilament Ca$^{2+}$ sensitivity assessed by $EC_{50}$ was not different for trabeculae from nonfailing versus failing hearts (Fig. 3C). Consistent with these findings, the inotropic response to $\alpha_1$-ARs in the LV was not appreciably affected in heart failure (Fig. 2C).

In the absence of $\alpha_1$-AR stimulation, $EC_{50}$ was lower in the LV myocardium from failing hearts versus nonfailing hearts (1.09 ± 0.08 vs. 1.37 ± 0.03 µM, $n = 6$ hearts/group, $P < 0.01$). This finding is consistent with some studies (3, 21, 30) reporting increased myofilament Ca$^{2+}$ sensitivity with heart failure. However, $EC_{50}$ values were not lower in the RV myocardium from failing hearts versus nonfailing hearts.

MLCK in the switch to positive $\alpha_1$-AR inotropy in the RV. Stimulation of $\alpha_1$-ARs results in MLCK-dependent phosphorylation of cardiac MLCK2, which sensitizes the myofilaments to Ca$^{2+}$ (1, 5, 33). Therefore, to investigate the role of MLCK in the switch to a positive $\alpha_1$-AR inotropic response in the RV myocardium in heart failure, we acutely treated trabeculae with the MLCK inhibitor ML-9 (1).

Before ML-9 treatment, RV trabeculae from failing hearts had a positive $\alpha_1$-AR inotropic response (Fig. 4A). However, with ML-9 treatment, the positive $\alpha_1$-AR inotropic response was abolished and replaced by a negative $\alpha_1$-AR inotropic response (Fig. 4, B and D). After washout of ML-9, the positive $\alpha_1$-AR inotropic response was restored (Fig. 4C).

These findings suggest that the presence of a positive $\alpha_1$-AR-induced inotropic response in the RV myocardium from failing hearts depends on MLCK function. We measured smMLCK, which is also present in cardiomyocytes. In nonfailing mouse hearts, the RV myocardium had a lower level of smMLCK than the LV myocardium (Fig. 5A). In contrast, in failing hearts, the level of smMLCK was markedly increased in the RV myocardium (Fig. 5A) and was higher than in the LV myocardium. This finding is consistent with the idea that in heart failure, increased smMLCK function in the RV myocardium contributed to the switch to a positive $\alpha_1$-AR inotropic response.

To investigate the relevance to human disease, we studied surgical samples from failing human hearts (ejection fraction: 25 ± 2%, $n = 11$) and nonfailing human hearts (ejection fraction: 59 ± 3%, $n = 9$, $P < 0.0001$). Interestingly, human hearts (Fig. 5B) had a strikingly similar pattern of smMLCK abundance compared with mouse hearts. Specifically, in nonfailing human hearts, smMLCK was lower in the RV than in the LV. However, in the RV myocardium from failing human hearts, smMLCK was markedly upregulated (Fig. 5B).

Using quantitative RT-PCR, we also measured smMLCK mRNA levels in the human myocardium. Compared with nonfailing hearts, the RV myocardium from failing hearts had significantly elevated smMLCK mRNA, which may have contributed to the increased smMLCK abundance (Fig. 5C).

DISCUSSION

The major finding of this study was that for the mouse RV myocardium, the negative inotropic response to $\alpha_1$-AR stimulation was fundamentally switched in heart failure to a positive
inotropic response. This switch occurred even before heart failure caused significant impairment of myofilament force development in the RV myocardium. Moreover, this switch was linked to increased myofilament Ca\(^{2+}\) sensitivity induced by α1-ARs. These findings illustrate that the failing RV manifests unique properties that cannot be extrapolated from studies of LV failure.

Heart failure fundamentally changed myofilament properties in the RV myocardium. The function of the myofilaments is a critical determinant of myocardial contractility. Therefore, we studied the role of myofilament properties in the switch to a positive inotropic response to α1-AR stimulation in the RV myocardium from failing hearts.

In the RV myocardium from nonfailing hearts, α1-AR stimulation led to both decreased myofilament Ca\(^{2+}\) sensitivity and a negative inotropic response, consistent with our previous report (33). In marked contrast, in the RV myocardium from failing hearts, α1-AR stimulation caused increased myofilament Ca\(^{2+}\) sensitivity and a positive inotropic response. These findings suggest that in the RV myocardium from failing hearts, the switch to increased myofilament Ca\(^{2+}\) sensitivity mediated by α1-ARs contributes to the switch to a positive inotropic response mediated by α1-ARs.

There is controversy regarding the effects on EC\(_{50}\) in heart failure, with reports of decreased EC\(_{50}\) (increased myofilament Ca\(^{2+}\) sensitivity) in heart failure in some, but not all, studies (3, 7, 12, 17, 21, 30). We found that the effects of heart failure on EC\(_{50}\) depended on the experimental context. Specifically, in the absence (but not in the presence) of α1-AR stimulation, EC\(_{50}\) values were lower in the LV myocardium from failing versus nonfailing hearts. Moreover, in the presence (but not in the absence) of α1-AR stimulation, EC\(_{50}\) values were lower in the RV myocardium from failing versus nonfailing hearts. Thus, heart failure can increase myofilament Ca\(^{2+}\) sensitivity; indeed, in LV trabeculae from failing hearts, increased Ca\(^{2+}\) sensitivity may have contributed to our finding that electrically evoked force development was not impaired despite a marked reduction in F\(_{\text{max}}\). Nevertheless, our findings also suggest that there are appreciable ventricle-specific effects and agonist effects that influence the impact of heart failure on EC\(_{50}\).

Increased MLCK in the RV myocardium in heart failure. Contractile force is generated by the muscle protein myosin. Myosin molecules have two globular motor domains (heads) connected by tapering neck regions to a rod region (6). Each myosin head has two associated polypeptides: MLCs, which wrap around the neck region (23). These MLCs are designated the essential light chain (MLC-1) and the regulatory light chain (MLC2). In vertebrate striated muscles, MLC2 phosphorylation increases the sensitivity of the myofilaments to Ca\(^{2+}\), thereby potentiating contraction (28).

Fig. 4. Myosin light chain kinase (MLCK) inhibitor ML-9 blocks the switch to a positive inotropic response to α1-AR stimulation in the RV myocardium from failing hearts. A: slow time-based recording of the α1-AR inotropic response in the failing RV. B: same trabecula after treatment with ML-9. C: same trabecula after washout of ML-9. D: summary of α1-AR inotropic responses in the failing RV before and after MLCK inhibitor ML-9 treatment. n = 5 hearts/group. ***P < 0.001.
Cardiac MLC2 is phosphorylated by MLCK. Moreover, MLCK is activated by $\alpha_1$-AR stimulation (1). Therefore, we studied the role of MLCK in the emergence of a positive inotropic response to $\alpha_1$-AR stimulation in the RV myocardium in heart failure.

MLCK exists in multiple isoforms. The skeletal muscle MLCK isoform is expressed in skeletal muscle but not in the heart (18). smMLCK is expressed ubiquitously, including in cardiomyocytes (18). A recently discovered cardiac MLCK isoform has cardiac-restricted expression (cardiac MLCK) (4, 26). The kinase that phosphorylates cardiac MLC2 physiologically has been difficult to identify (19) and was not resolved in the present study. For example, in cardiomyocytes, loss of cardiac MLCK expression by RNA inhibition only reduced cardiac MLC2 phosphorylation by 50%, suggesting that other kinases, such as smMLCK, also play a role (4). We monitored smMLCK; thus, our findings are limited by a need for information on both the cardiac MLCK isoform and the counterbalancing effects of MLC2 phosphatase activity.

Within the LV, regional differences in MLCK abundance and MLC2 phosphorylation have been previously reported, with high levels of MLCK and MLC2 phosphorylation in the apex and epicardium versus lower levels toward the base and endocardium (8). These spatial gradients were suggested to underlie regional differences in LV contractility, with greater regional contractility associated with higher levels of MLC2 phosphorylation (8).

In this study, we found a spatial gradient of smMLCK in nonfailing hearts with a higher level of smMLCK in the LV versus the RV. This LV/RV gradient of MLCK may contribute to the fundamentally different $\alpha_1$-AR inotropic responses we observed in the nonfailing LV versus RV myocardium, with the robust positive $\alpha_1$-AR inotropic response in the LV myocardium linked to high levels of smMLCK. Consistent with this, we (33) previously found that pharmacological inhibition of MLCK abolished the positive $\alpha_1$-AR inotropic response in the LV myocardium. The resulting MLCK-independent negative $\alpha_1$-AR inotropic response of the LV myocardium resembled that in the nonfailing RV myocardium (33).

The present study identified a switch in the spatial gradient of smMLCK in heart failure, with a higher level of smMLCK found in the RV compared with the LV. As heart failure involves increased vascularization and interstitial fibrosis, potentially, smMLCK expressed in smooth muscle cells and nonmyocytes could also contribute to our measurements of MLCK. However, these sources are unlikely to account for our finding a higher level of smMLCK in the RV versus LV in failing hearts, because we studied a heart failure model involving greater injury to the LV than in the RV. Our findings suggest that in heart failure, smMLCK is increased in RV cardiomyocytes, and this contributes to the switch to a positive $\alpha_1$-AR inotropic response. Consistent with this, in heart failure, the switch of the RV myocardium to a positive $\alpha_1$-AR inotropic response was abolished by the MLCK inhibitor ML-9.

In addition to augmentation of contractility, increased MLCK function may also stabilize myofilament structure (2, 4, 26). Interestingly, in failing mouse hearts, the RV myocardium had increased smMLCK, and $F_{\text{max}}$ was better preserved compared with the LV myocardium.

Consistent with the findings in failing mouse hearts, in human hearts we also found high levels of smMLCK in RV samples from failing hearts versus lower levels of smMLCK in RV samples from nonfailing hearts. Moreover, we found that smMLCK mRNA levels were relatively increased in the RV.
from failing human hearts. Increased mRNA levels could contribute to increased smMLCK in the RV in heart failure.

Reemergence of a developmental phenotype in the RV in heart failure. α1-ARs mediate a positive inotropic response in the mouse neonatal RV myocardium, in contrast to a negative inotropic response in the nonfailing adult RV myocardium (29). We found that in heart failure, the RV myocardium manifests a return to the early developmental phenotype with a reemergence of the positive inotropic response to α1-AR stimulation.

In utero, the RV is the main pumping chamber (24). Thus, the presence of a positive α1-AR inotropic response during development may reflect an adaptation of the RV to a high hemodynamic load during development. However, in the adult, the hemodynamic load on the RV is much reduced, and maturation is accompanied by a transition of the RV myocardium to a negative α1-AR inotropic response. In heart failure, the RV becomes again exposed to a high hemodynamic load. In the RV myocardium from failing hearts, the return of the early developmental phenotype, consisting of a positive α1-AR inotropic response, may represent a beneficial adaptation of the RV to heart failure. As catecholamines are elevated in heart failure and β-AR responses are downregulated, the switch to a positive α1-AR inotropic response may help the failing RV adapt to increased pulmonary pressures.

Limitations. This study found that in the RV myocardium, the negative inotropic response to α1-ARs in nonfailing hearts becomes switched to a positive inotropic response in failing hearts. Moreover, the mechanism involves α1-AR-induced increases of myofilament Ca$^{2+}$ sensitivity, possibly mediated by MLCK. However, there are several study limitations. Multiple MLCK isoforms, as well as MLCK2 phosophatase activity, likely contribute to the level of cardiac MLCK2 phosphorylation, and their roles in the failing RV need to be established. The MLCK inhibitor used in this study (ML-9) can also inhibit a variety of exchangers, transporters, and channels; therefore, findings with ML-9 have to be interpreted with caution (10, 27). Nevertheless, we and others (1, 33) have previously reported that ML-9 did not affect β-AR-induced positive inotropic responses. Thus, the elimination of α1-AR-induced positive inotropic responses in the presence of ML-9 was not due to a nonspecific effect of the inhibitor on contraction (1). Future studies could take a more specific molecular approach to inhibit MLCK function by studying transgenic mice in which the phosphorylation sites on cardiac MLCK2 are eliminated (25). Regulation of multiple myofilament proteins (e.g., troponin, cardiac MLCK2, and myosin binding protein C) may contribute to the distinctive RV inotropic responses that we have observed. Moreover, other mechanisms, such as changes in Ca$^{2+}$ handling in the failing RV, may also play a role. Finally, it will be important to validate our findings in intact animals.

Conclusions. In heart failure, the RV inotropic response to α1-AR stimulation switches from negative to positive. The mechanism involves α1-AR-induced decreases of myofilament Ca$^{2+}$ sensitivity in the nonfailing RV becoming switched to α1-AR-induced increases of myofilament Ca$^{2+}$ sensitivity in the failing RV. The switch to a positive α1-AR inotropic response may help the failing RV adapt to pulmonary hypertension.


