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Human Myocardium Has a Robust $\alpha 1A$ -Subtype Adrenergic Receptor Inotropic Response

Paul M.L. Janssen^{1,*}, Benjamin D. Canan¹, Ahmet Kilic², Bryan A Whitson², and Anthony J. Baker^{3,*}

¹Department of Physiology and Cell Biology, The Ohio State University, Columbus, Ohio

²Department of Surgery, The Ohio State University, Columbus, Ohio

³Veterans Affairs Medical Center, San Francisco, and Department of Medicine, Univ. Calif. San Francisco, San Francisco

Abstract

Recent studies report that a single subtype of $\alpha 1$ -adrenergic receptor ($\alpha 1$ -AR), the $\alpha 1A$ -subtype, mediates robust cardioprotective effects in multiple experimental models of heart failure, suggesting that the $\alpha 1A$ -subtype is a potential therapeutic target for an agonist to treat heart failure. Moreover, we recently found that the $\alpha 1A$ -subtype is present in human heart. The goal of this study was to assess the inotropic response mediated by the $\alpha 1A$ -subtype in human myocardium, and to determine if the response is down-regulated in myocardium from failing human heart. We measured in-vitro contractile responses of cardiac muscle preparations (trabeculae) isolated from the right ventricle from non-failing and failing human hearts. Addition of the $\alpha 1A$ -subtype agonist A61603 (100 nM) resulted in a large positive inotropic response (force increased ≈ 2 -fold). This response represented $\approx 70\%$ of the response mediated by the β -adrenergic receptor agonist isoproterenol (1 μ M). Moreover, in myocardium from failing hearts, $\alpha 1A$ -subtype responses remained robust, and only slightly reduced relative to non-failing hearts. We conclude that $\alpha 1A$ -subtype-mediated inotropy could represent a significant source of inotropic support in the human heart. Furthermore, the $\alpha 1A$ -subtype remains functional in myocardium from failing human hearts and thus, might be a therapeutic target to support cardioprotective effects in heart failure patients.

Keywords

alpha-1-adrenergic; contraction; inotropic; right ventricle; human

INTRODUCTION

The myocardial contractile response to endogenous catecholamines is driven by cardiomyocyte beta-1 adrenergic receptors ($\beta 1$ -ARs) and alpha-1-ARs ($\alpha 1$ -ARs). $\beta 1$ -

Correspondence to Anthony J. Baker, Ph.D., University of California, San Francisco, VA Medical Center, Cardiology Division (111C), 4150 Clement St, San Francisco, CA 94121. Phone: (415) 221-4810 x24790, Anthony.Baker@ucsf.edu.

*PMLJ and AJB contributed equally to this work

Disclosures

None

adrenergic responses have been extensively studied and found to have consistent effects among studies. These effects include a robust positive inotropic effect (PIE).

In contrast, $\alpha 1$ -AR responses have been less studied and show considerable variation among studies (11). For example, there is marked species-dependent variation in the magnitude and direction of $\alpha 1$ -AR inotropic responses, ranging from a robust positive inotropic effect in rabbit and rat, to no response in dog (12). Moreover, in studies of mice, a marked regional variation was found, with a positive inotropic effect (PIE) in left ventricular (LV) myocardium, but a negative inotropic effect (NIE) in right ventricular (RV) myocardium (33). The presence of disease altered the $\alpha 1$ -AR response; the NIE of non-failing RV mouse myocardium was switched to a PIE in mouse models of RV failure induced by pulmonary fibrosis (6), or secondary to LV failure post-infarction (32).

Cardiac myocytes have two predominant $\alpha 1$ -AR subtypes ($\alpha 1A$ and $\alpha 1B$) and each subtype can mediate markedly different inotropic effects. For example, in rabbit the $\alpha 1A$ -subtype mediates a NIE, but the $\alpha 1B$ - subtype mediates a PIE (29), whereas in human the $\alpha 1A$ -subtype mediates a PIE, but the $\alpha 1B$ - subtype mediates a NIE (30).

Recently, $\alpha 1$ -ARs have gained new significance by the suggestion that $\alpha 1$ -ARs can mediate powerful cardioprotective effects in heart failure (4, 16, 23). Specifically, chronic stimulation of the $\alpha 1A$ -subtype protects against cell death and improves function in mouse heart failure models based on doxorubicin toxicity (5, 22), trans-aortic constriction (21), or RV failure due to pulmonary fibrosis (7). These findings raise the possibility that $\alpha 1A$ -subtype signaling in humans might be a novel therapeutic target to treat heart failure.

The goal of this study was to assess the inotropic effect of $\alpha 1A$ -subtype stimulation in human myocardium and determine how the inotropic effect is changed in human myocardium from failing hearts. We used the $\alpha 1A$ -subtype-selective agonist A61603 that is highly potent and selective for the $\alpha 1A$ -subtype (22). We found a robust positive inotropic response to A61603 in myocardium from both non-failing and failing human hearts. The $\alpha 1A$ -subtype inotropic response represented $\approx 70\%$ of the response elicited by stimulation of the $\beta 1$ -AR using isoproterenol.

We conclude that $\alpha 1A$ -subtype-mediated inotropy could represent a significant source of inotropic support in the human heart. Furthermore, the $\alpha 1A$ -subtype remains functional in myocardium from failing human hearts. As the $\alpha 1A$ -subtype can mediate cardioprotective effects in animal heart failure models, the presence of a functional $\alpha 1A$ -subtype in myocardium from failing human hearts suggests that the $\alpha 1A$ -subtype, might be a therapeutic target to support cardioprotective effects in heart failure patients.

METHODS

Human Hearts

All human tissues were experimented on with approval from the Institutional Review Board (IRB) of The Ohio State University and conform to the declaration of Helsinki. Informed consents were acquired from cardiac transplant patients. Explanted hearts from patients

undergoing cardiac transplantation at The Ohio State University Wexner Medical Center were obtained directly in the operating room and immediately (within seconds) flushed with cardioplegic solution after removal from donors/patients as described previously (10, 19). The hearts were transferred to the laboratory (within 10–30 minutes) in cold cardioplegic solution containing (in mM): 110 NaCl, 16 KCL, 16 MgCl₂, 10 NaHCO₃, and 0.5 CaCl₂. All hearts were procured and treated with identical protocols, personnel, solutions and timing regardless of the source. All end-stage failing hearts (n = 6) were acquired from patients in the operating room. Non-transplantable donor hearts (n = 6) were acquired in the operating room in collaboration with Lifeline of Ohio Organ Procurement. The biometric characteristics of these hearts are provided in Table 1.

Trabecula preparation

The right ventricle of the heart was transferred from the cardioplegic solution to a cold modified Krebs-Henseleit solution (K-H) bubbled with 95% O₂-5% CO₂ containing (in mM): 137 NaCl, 5 KCl, 0.25 CaCl₂, 20 NaHCO₃, 1.2 NaH₂PO₄, 1.2 MgSO₄, 10 dextrose, and 20 BDM (2,3-butanedione monoxime) and pH of 7.4. A linear, small, and free-running trabecula was isolated under a stereo dissection microscope, and kept in this solution at 0–4 °C until the time of the experiment, which was typically 2 hours or less. Approximately 10 trabeculae from each heart were harvested for use in separate parallel studies. One muscle per heart was used for this study. For both non-failing and failing groups, data from 6 hearts were averaged per group. Muscles were transferred into custom-made setups as previously described for animal models (15) and the perfusion solution was changed to a modified K-H but without BDM. This solution was maintained at 37 °C and continuously bubbled with 95% O₂-5% CO₂ resulting in pH of 7.4. Continuous electrical stimulation was initiated (frequency of 0.5 Hz, and stimulation voltage ~20% above threshold) and CaCl₂ concentration of the solution was slowly raised to 2 mM over ~15 minutes. Muscles were gradually stretched (over a few minutes) until an increase in developed force was exceeded by an increase in resting tension. This length (optimal length) roughly corresponds to sarcomere length of ~2.2 μm, which is near or at the *in vivo* sarcomere length at end-diastole (24). Dimensions of the muscles from the non-failing hearts used were 347 ± 81 μm wide, 232 ± 55 μm thick, and 2.7 ± 0.7 mm long. Dimensions of the muscles from the failing hearts were not significantly different; 329 ± 35 μm wide, 247 ± 56 μm thick, and 2.4 ± 0.5 mm long.

Inotropic stimulation

After assessment of baseline contractility, we used supramaximal doses of both agonists. First, 100 nM of A61603 (Sigma) was added, and data was recorded when contractions had stabilized. Thereafter, the drug was washed out, and muscle contractile parameters returned back to baseline. Next, 1 μM isoproterenol was added, and data were again collected.

Statistical analysis

Data are presented as mean ± SE. Statistical tests (ANOVA with post hoc analysis using Tukey correction for multiple comparisons, and paired and unpaired t-tests) were performed using Prism 7 software (GraphPad Software, Inc., La Jolla, CA) with a significance level set at $P < 0.05$.

RESULTS

Figure 1 shows typical example records of the contractile responses to A61603 for a single muscle from a non-failing heart (A), and for a single muscle from a failing heart (B). After washout of A61603, Figure 1 also shows the time course of the contractile response to isoproterenol (ISO) for these same muscles (in panels C and D respectively).

For all muscles studied, the effects of A61603 and ISO on force development are summarized in Figure 2. Shown are the individual experimental values (one muscle per heart) and the grouped data (mean $\pm$ SE, $n = 6$ hearts per group). Baseline absolute contraction force (mN/mm^2) was similar in myocardium from non-failing ($16 \pm 3 \text{ mN/mm}^2$) vs. failing hearts ($15 \pm 3 \text{ mN/mm}^2$) (Figure 2A). Both A61603 and ISO elicited a robust positive inotropic effect (PIE) in non-failing and failing myocardium. However, the inotropic responses showed considerable variability between subjects. After A61603 addition, there was a trend for greater absolute force level in non-failing myocardium ($39 \pm 9 \text{ mN/mm}^2$) versus failing myocardium ($26 \pm 3 \text{ mN/mm}^2$), but this trend did not reach statistical significance ($P = 0.21$). Likewise, there was a trend for greater force level after ISO in non-failing ($51 \pm 7 \text{ mN/mm}^2$) vs failing ($42 \pm 3 \text{ mN/mm}^2$) myocardium which did not reach statistical significance ($P = 0.27$). Basal contractions and responses to ISO were similar to those observed in our recent studies (10, 18–20).

To minimize inter-subject variability, the contractile responses to A61603 and ISO were expressed relative to the baseline force before agonist (Figure 2B). After addition of A61603, force was increased ≈ 2 -fold relative to the baseline force (Figure 2B) (2.4-fold for non-failing vs. 1.9-fold for failing). Following washout of A61603, after addition of isoproterenol, force was increased to a higher level (≈ 3 -fold relative to the baseline) (3.6-fold for non-failing vs. 3.2-fold for failing). However, the higher level of force after ISO compared to after A61603 only reached statistical significance for the failing group, but not for the non-failing group.

Isoproterenol is known to induce a high level of activation of contraction. Here, we compared the activation of contraction due to A61603 with that due to ISO (Figure 2C). The increase of force elicited by A61603 was $\approx 70\%$ relative to the increase of force elicited by isoproterenol (74% for non-failing vs. 63% for failing). There was no statistical difference between the non-failing and failing group. These data suggest that for both non-failing and failing groups the $\alpha 1A$ -subtype is potentially a significant source of inotropic support.

Figure 3 shows representative records of single electrically-evoked contractions both before and after addition of agonists for two trabeculae, one from a non-failing heart and one from a failing heart. For both non-failing and failing heart, A61603 increased the contraction amplitude without affecting the time course of contraction or relaxation. In contrast, as expected, ISO increased the contraction amplitude and appreciably accelerated the timing of the phases of contraction and relaxation. Both agonists caused small reductions in diastolic force level. For non-failing muscles, the drop of diastolic force after addition of A6 or ISO (1.4 ± 0.4 and $0.9 \pm 0.3 \text{ mN/mm}^2$ respectively) did not differ versus failing muscles (2.3 ± 0.6 and $1.6 \pm 0.6 \text{ mN/mm}^2$ respectively).

The contraction and relaxation phases were quantitated from the time between the start and peak of the contraction (Figure 4 A), and from the time from the peak of contraction to 50% relaxation (Figure 4B). Figure 4 shows that the pooled data for the contraction/relaxation timing parameters had less inter-subject variability compared to the inotropic responses (Figure 2). The time to peak contraction (Fig. 4A) was not different between myocardium from non-failing vs. failing hearts. For each muscle, a change in timing parameters from before versus after agonist were evaluated using a paired t-test. For both non-failing and failing myocardium, the time to peak was not changed by addition of A61603 ($P > 0.7$, paired t-test). However, the time to peak was significantly decreased after addition of isoproterenol ($P < 0.05$, paired t-test). The time to 50% relaxation (Fig. 4B) was higher in myocardium from failing vs. non-failing hearts indicating impaired relaxation. Relaxation time was not changed by addition of A61603 ($P > 0.4$, paired t-test). After addition of isoproterenol, relaxation time was appreciably reduced ($P < 0.05$, paired t-test), and there was no difference in relaxation time between non-failing and failing groups.

DISCUSSION

We found that the $\alpha 1A$ -subtype was functional in human myocardium from both non-failing and failing hearts. The $\alpha 1A$ -subtype produced a robust positive inotropic response representing about $\approx 70\%$ of the response produced by the $\beta 1$ -AR. The $\alpha 1A$ -subtype response was not appreciably downregulated in myocardium from failing hearts. These findings suggest that in the human heart the $\alpha 1A$ -subtype might represent a source of significant inotropic support.

Previous studies in experimental animals suggest that the $\alpha 1A$ -subtype might be a novel therapeutic target to treat heart failure. In this context, we found that for human heart the $\alpha 1A$ -subtype remains functional in myocardium from failing hearts and thus might be a therapeutic target to treat patients with heart failure.

$\alpha 1A$ -subtype inotropy in human myocardium

Previously, $\alpha 1$ -AR mediated inotropic responses involving increases of contraction in the range of 30–60% were reported for rat and rabbit myocardium (2, 3, 26). Similar $\alpha 1$ -AR mediated inotropic responses were reported for human myocardium (3). For myocardium from failing rat hearts and from failing human hearts, the inotropic response to $\alpha 1$ -AR stimulation was reported to be relatively increased compared to that evoked by β -AR stimulation (3, 28). Moreover, human allografts explanted 10 years after transplantation showed $\alpha 1$ -AR mediated positive inotropic responses of similar size as native explanted hearts (≈ 50 –60%) demonstrating that $\alpha 1$ -AR inotropic responses survived long-term immune-suppressant therapy (27).

Previously, we found that for myocardium dissected from cultured human cardiac tissue slices, the $\alpha 1A$ -subtype elicited a positive inotropic effect (PIE) whereas the $\alpha 1B$ subtype elicited a negative inotropic effect (NIE). In the current study using trabeculae from explanted hearts we confirmed the presence of a PIE elicited by stimulation of the $\alpha 1A$ -subtype. Moreover, we found that the inotropic response mediated by the $\alpha 1A$ -subtype was appreciable, representing about $\approx 70\%$ of the response produced by the $\beta 1$ -AR. We also

found that a robust $\alpha 1A$ -subtype inotropic response was present in myocardium from failing hearts.

The response to $\alpha 1A$ -subtype stimulation did not involve a change in the timing of contraction or relaxation. This is consistent with the known lack of an effect of $\alpha 1$ -AR stimulation on heart rate in-vivo. In contrast, stimulation of $\beta 1$ -ARs markedly accelerated the timing of contraction and relaxation. Faster twitch kinetics are compatible with the known increase in heart rate that occurs during $\beta 1$ -AR stimulation in-vivo.

Relation to previous studies

In studies of mouse myocardium, we previously reported that $\alpha 1$ -AR stimulation elicits contrasting inotropic responses in LV versus RV myocardium (33). Specifically, $\alpha 1$ -AR agonist elicited a PIE in LV myocardium but a NIE in RV myocardium. Moreover, stimulation of the single $\alpha 1A$ -subtype also resulted in contrasting responses, again with a PIE in LV, but a NIE in RV (33). These responses in mouse differ from those observed in human. We recently reported that for human LV myocardium $\alpha 1A$ -subtype stimulation elicited a PIE (30). In the current study, we found that for human RV myocardium, $\alpha 1A$ -subtype stimulation also elicited a robust PIE. Thus, contrasting inotropic responses of LV versus RV myocardium following $\alpha 1A$ -subtype stimulation were not observed in human.

For mouse RV myocardium, we found that the NIE of $\alpha 1$ -AR stimulation was switched to a PIE in myocardium from failing hearts (32). Moreover, we found that that this switch was due to the $\alpha 1A$ -subtype which elicited a NIE in non-failing RV myocardium but a PIE in RV myocardium from failing hearts (6). For human myocardium, in this study we did not observe appreciable difference in the $\alpha 1A$ -subtype inotropic response in RV myocardium from non-failing versus failing human hearts.

Collectively, these findings of different effects of $\alpha 1A$ -subtype stimulation in mouse versus human suggests that caution is needed when extrapolating to human from studies using mice.

Previous studies suggest that the 3 cardiac $\alpha 1$ -AR subtypes have different roles in cardiac inotropy. The $\alpha 1A$ -AR subtype mediates a PIE in this study, consistent with previous studies (6, 17). In contrast, the $\alpha 1B$ -AR subtype generally mediates a NIE (6, 13, 25) including in human myocardium (30). The $\alpha 1D$ -AR subtype mediates coronary vasoconstriction (31). Thus, the inotropic response of human myocardium to non-subtype-selective $\alpha 1$ -AR agonist stimulation is likely complicated by both a PIE mediated by the $\alpha 1A$ -AR subtype and a NIE mediated by the $\alpha 1B$ -AR subtype.

$\alpha 1A$ -subtype mediates cardioprotective effects

In studies of mouse models of heart failure, several lines of evidence suggest that the $\alpha 1A$ -subtype can mediate powerful cardioprotective effects. Overexpression of the $\alpha 1A$ -subtype increases contractility in-vivo (17), protects against pressure-overload-induced dysfunction (8) and limits post-infarct cardiomyopathy (9). Moreover, $\alpha 1A$ -subtype stimulation protects cardiac myocytes against necrosis induced by oxidative-stress, or toxicity from norepinephrine or doxorubicin (14). Chronic administration of an $\alpha 1A$ -subtype agonist

protects against cardiomyopathy due to doxorubicin toxicity (5, 22), trans-aortic constriction (21), or RV failure due to pulmonary fibrosis (7). Notably, these chronic administration studies used a low dose of agonist that did not raise blood pressure through vascular $\alpha 1$ -AR activation.

In human, a large clinical trial (ALLHAT) of an $\alpha 1$ -AR antagonist was stopped prematurely because of an increase in heart failure in the $\alpha 1$ -AR-antagonist-treated group (1). This result suggests that a reduced level of $\alpha 1$ -AR activation is injurious, and is consistent with a cardioprotective effect mediated by $\alpha 1$ -ARs.

Together, these findings suggest the possibility that cardioprotective $\alpha 1A$ -subtype signaling in humans might be a novel therapeutic target to treat heart failure. In view of this possibility, the current study suggests that the $\alpha 1A$ -subtype is active in failing human myocardium.

Limitations

We studied RV endocardial trabeculae which might have different inotropic properties compared to other RV regions and might also differ from LV myocardium. Moreover, for the heart failure patients in this study, the extent of pathological involvement of the RV is unclear.

Some heart failure patients were implanted with an LVAD which might lessen the extent of RV injury. However, there were no RV functional differences noted between heart failure patients with or without LVAD.

Downregulation of β -ARs occurs in heart failure. However, the β -AR inotropic response was not reduced in myocardium from heart failure patients. Nevertheless, the relaxation time was increased in the heart failure group, consistent with injury to RV myocardium.

The agonist ISO was always evaluated after A61603. In pilot studies, we found that the effects of ISO took a long time to wash out and were not completely reversed. A long wash out time would result in considerable time-dependent loss of function (run-down) that occurs due to progressive impairment of excitation-contraction coupling (18). Therefore, in this study, ISO was used after A61603. We used a single maximal dose of each agonist. Our recent study found that A61603 is highly potent in both non-failing and failing human myocardium with an EC₅₀ of 5 nM for activation of cell signaling that is well below the 100 nM dose used in the current study (30).

Conclusion

The $\alpha 1A$ -subtype mediates a robust positive inotropic effect in human myocardium. Thus, $\alpha 1A$ -subtype-mediated inotropy could represent a significant source of inotropic support in the human heart. Furthermore, the $\alpha 1A$ -subtype remains functional in myocardium from failing human hearts. As the $\alpha 1A$ -subtype mediates cardioprotective effects in animal heart failure models, the presence of a functional $\alpha 1A$ -subtype in myocardium from failing human hearts suggests that the $\alpha 1A$ -subtype, might be a therapeutic target to treat patients with heart failure.

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Figure 1. Representative slow time base recordings of contraction force

Force developed by electrically paced trabeculae showing the inotropic responses to addition of the agonists A61603 (100 nM) or Isoproterenol (ISO, 1 μ M). Individual contractions are not evident on this time scale.

Figure 2. Inotropic effects of A61603 and ISO on myocardium from non-failing (NF) and failing (F) hearts

Individual experimental data (one muscle per heart) and pooled data (mean $\pm$ SE, $n = 6$ hearts per group) for: **(A)** baseline forces and forces after addition of A61603 or ISO; **(B)** data for each muscle scaled relative to its pre-drug (baseline) contraction force; **(C)** responses to A61603 scaled relative to the response to ISO. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ for comparisons relative to baseline; † $P < 0.05$; †† $P < 0.01$ for comparisons of ISO relative to A61603.

Figure 3. Representative fast time base recordings of twitch contractions
(A) electrically-evoked twitch contraction records of trabecula from non-failing heart at baseline (no drug) and after addition of 100 nM A61603. Following washout of A61603, the effect after addition of 1 μ M ISO is shown; (B) contraction records of trabecula from failing heart.

Figure 4. Effect of A61603 and ISO on the timing of contraction and relaxation
 Individual experimental data and pooled (mean $\pm$ SE) data: (A) contraction time quantitated from the time interval from the start of contraction to peak of contraction; (B) relaxation time quantitated from the time interval from the peak of contraction to the time at 50% decrease of force. * $P < 0.05$; *** $P < 0.001$ for comparisons relative to baseline; †† $P < 0.01$; ††† $P < 0.001$ for comparisons of ISO relative to A61603; # for comparison between non-failing and failing.

Table 1

Patient Characteristics

Non-Failing	Race	Sex	Age	Heart Weight (g)	Cause of Death	LVEF (%)
380071	Caucasian	Female	43	603	Anoxia, respiratory arrest	55
712301	Caucasian	Male	67	527	Blunt injury, ICH	Not measured
632941	Caucasian	Female	68	402	Natural causes, CVA/Stroke	Not measured
313956	Caucasian	Female	38	406	Drug overdose	60
574165	Caucasian	Male	62	584	Blunt injury/MVA	Not measured
394176	Caucasian	Male	57	748	Blunt injury/Non-MVA	Not measured

Failing	Race	Sex	Age	Heart Weight (g)	Etiology	LVEF (%)
774694	Caucasian	Female	50	486	Ischemic	< 25
514955	Caucasian	Female	67	556	Ischemic	< 25
911614	Caucasian	Male	65	716	Non-ischemic	< 25
956256	Caucasian	Male	64	714	Non-ischemic	< 25
203056	African American	Male	51	704	Non-ischemic	< 25
724569	Caucasian	Male	64	636	Non-ischemic	< 25

LVEF = left ventricular ejection fraction. ICH = intracerebral hemorrhage

MVA = motor vehicle accident