Intraventricular and interventricular cellular heterogeneity of inotropic responses to α₁-adrenergic stimulation

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Previoussly, we found interventricular differences in inotropic responses to stimulation of α₁-adrenergic receptors (α₁-ARs) with an overall positive inotropic effect (PIE) in left ventricular (LV) myocardium in contrast to an overall negative inotropic effect (NIE) in right ventricular (RV) myocardium (27, 28). Alterations in myofilament Ca²⁺ sensitivity played a role in the contrasting α₁-AR inotropic responses of the LV versus RV myocardium (27, 28). However, the role of Ca²⁺ handling in α₁-AR inotropic responses remains unclear. Previously, an interventricular difference in Ca²⁺ handling was reported, with smaller Ca²⁺ transients noted in RV myocytes versus LV myocytes (15). Here, we investigated the role of Ca²⁺ handling in the differing α₁-AR inotropic responses of the RV versus LV. We used adult mouse ventricular myocytes from the RV or LV free wall and monitored Ca²⁺ transients and contractile responses in response to stimulation of α₁-ARs.

Interestingly, we found that for both RV and LV myocytes, there was considerable cellular heterogeneity in the effects of α₁-AR stimulation on inotropic responses. Moreover, compared with the LV, the RV had a significantly greater proportion of cells in which α₁-AR stimulation elicited a NIE (68% vs. 36%). Differences in α₁-AR inotropic responses appeared to be driven by effects on Ca²⁺ transients; moreover, α₁-AR effects on Ca²⁺ loading of the sarcoplasmic reticulum (SR) played a role.

This study demonstrates cellular heterogeneity of α₁-AR inotropic responses, which is a new aspect of biological heterogeneity among myocytes that potentially might extend to other types of receptors.

METHODS

The Animal Studies Subcommittee of the San Francisco Veterans Affairs Medical Center approved all procedures. Previously, we measured α₁-AR inotropic responses using multicellular myocardial samples (trabeculae) dissected from the LV or RV (27, 28). The present study used single myocytes isolated from the LV or RV and measured α₁-AR inotropic responses and fura-2- assessed Ca²⁺ transients.

Myocyte isolation. Twelve-week-old male C57Bl/6 mice were deeply anesthetized with pentobarbital (100 mg/kg ip) mixed with heparin (100 units). A midline thoracotomy was performed, and the heart was rapidly removed, immersed in ice-cold arrest solution containing (in mM) 120 NaCl, 30 KCl, and 0.1 CaCl₂, and mounted to a cannula by the aorta. Myocytes were isolated by enzymatic digestion of the heart by retrograde perfusion of collagenase solution through the coronary vasculature (20). After collagenase treatment, the heart was placed in a “stop buffer” to halt enzymatic digestion (20). The free wall of the RV or LV was dissected, placed in 10 ml of stop buffer, and gently teased with forceps followed by repeated pipetting to release the cells. Cells from only one ventricle were studied per animal. The cell isolation buffers contained 10 mM 2,3-butanedione monoxime to prevent cell contraction. Cells were used within 4 h of isolation.

The RV yielded ~350,000 cells. The LV free wall (representing ~50% of the LV) yielded ~720,000 cells. These yields suggest a cell yield for the entire heart of ~1.8 million cells, which is similar to the cell yield obtained in our previous study (17) of mouse hearts. Moreover, the ratio between RV versus LV cell yield is consistent with the ratio of RV versus LV weight for the mouse heart in our previous study (27).

Overall, we studied 163 cells from 64 animals. Few cells (2–3 cells) were studied per heart due to the difficulty of maintaining stable cell contractions for the 20–30 min required for an equilibration period followed by recording the response to an agonist. Technical
problems, such as cell movement and arrhythmias, resulted in a success rate ∼50%.

**Fura-2 loading.** Myocytes were loaded with fura-2 by exposure to 1 μM fura-2 AM for 20 min. After being washed, myocytes were equilibrated for 20 min to allow for deesterification of the indicator and used within 1.5 h after completion of the loading protocol.

**Contractility and fluorescence measures.** Myocytes were superfused in a small glass-floored chamber with Krebs-Henseleit solution containing (in mM) 112 NaCl, 5 KCl, 1.2 MgCl₂, 10 glucose, 24 NaHCO₃, 1.2 Na₂SO₄, 2.0 NaH₂PO₄, and 1 CaCl₂. The perfusate was oxygenated with 95% O₂-5% CO₂ to give a pH of 7.4 at 22°C. The chamber was mounted on an inverted Nikon Diaphot microscope, and cells were visualized at ×40 magnification. Cells were electrically stimulated with 4-ms square-wave pulses at 25 V and at a frequency of 0.5 Hz. Cells were selected based on a rod-shaped appearance with clear striations and contractions (≈2% basal length) in response to electrical stimulation. Cell contraction and fura-2 fluorescence were monitored using an IonOptix Hyperswitch system (Milton, MA). Cell contraction was computed by monitoring changes in muscle sarcomere length measured from the myocyte striation spacing. Fura-2 fluorescence was converted to Ca²⁺ concentration using the formula of Grynkiewicz (12). The concentration of caffeine passed through a micropipette placed close to the cell (1).

**RESULTS**

**RV cells have smaller Ca²⁺ transients and slower relaxation than LV cells.** Figure 1 shows the contraction and Ca²⁺ handling baseline characteristics of LV versus RV cells. Con-
sistent with a previous study (15), RV cells had smaller Ca$^{2+}$ transients and slower relaxation than LV cells. New findings were that compared with LV cells, the decline phase of the Ca$^{2+}$ transient was slower in RV cells and the diastolic sarcomere length was longer in RV cells.

Consistent with a previous report (15), for electrically stimulated cells, RV cells had significantly lower systolic Ca$^{2+}$ levels compared with LV cells (1.11 ± 0.06 μM, n = 30, vs. 1.35 ± 0.1 μM, n = 26, P < 0.05; Fig. 1A).

Figure 1B shows that for contracting cells, the diastolic sarcomere length of RV cells was 24 nm longer than for LV cells (1.823 ± 0.005 μm, n = 31, vs. 1.799 ± 0.007 μm, n = 28, P < 0.01). This was not due to differences in diastolic Ca$^{2+}$ levels, which did not differ between LV and RV cells (Fig. 1A). Furthermore, this difference in sarcomere length was evident in quiescent cells maintained in the cell isolation buffer containing 2,3-butanedione monoxime and without Ca$^{2+}$ (not shown). Thus, effects of contraction did not play a role in the longer diastolic sarcomere length of RV versus LV cells.

The systolic sarcomere length of RV cells was 37 nm longer than LV cells (1.763 ± 0.008 μm, n = 31, vs. 1.726 ± 0.007 μm, n = 28, P < 0.01). Consistent with smaller Ca$^{2+}$ transients in RV cells, there was a trend for the mean contraction amplitude (difference between diastolic and systolic sarcomere lengths) for RV cells to be smaller (80%) compared with LV cells, but the difference did not reach statistical significance (P = 0.09).

Quantitation of the relaxation phase used the time constant estimated for the exponential decline of signals for Ca$^{2+}$ concentration and sarcomere length. Compared with LV cells, RV cells had slower Ca$^{2+}$ transient decline (greater time constant value; Fig. 1C). Furthermore, RV cells had a slower time course of relaxation than LV cells, consistent with a previous report (15). The slower Ca$^{2+}$ transient decline might have contributed to the slower relaxation in RV cells compared with LV cells.

In summary, compared with LV myocytes, RV myocytes have smaller Ca$^{2+}$ transients with slower Ca$^{2+}$ decline and somewhat smaller contractions with slower relaxation.

**Cellular heterogeneity of α1-AR inotropic responses.** Previously, we found that α1-AR stimulation resulted in a NIE in the RV myocardium but a PIE in the LV myocardium (27, 28). Interestingly, in this study, when examined at the cellular level, considerable heterogeneity of α1-AR inotropic responses was noted. Figure 2 shows that α1-AR stimulation manifested either a NIE or PIE in cells from the RV free wall (A and B) or cells from the LV free wall (C and D). For all cells studied, there was no relation between baseline contractility and the direction of the inotropic response. For example, the cells shown in Fig. 2, C and D, had similar baseline contraction amplitudes but directionally opposite inotropic responses.

For RV cells, α1-AR stimulation with PE elicited a NIE in the majority (66%) of the 21 hearts from which at least 2 cells were studied, α1-AR stimulation with PE elicited both PIE and NIE types of the cellular response. This indicates that the observed cellular heterogeneity of α1-AR responses existed within hearts rather than arising from variability between animals.

**Cellular heterogeneity of α1A-AR subtype inotropic responses.** There are two predominant α1-AR subtypes on cardiac myocytes (α1A and α1B), which may have different roles in cardiac inotropy (6, 9, 11, 16, 22). Therefore, in a subgroup of cells, we tested the role of a single α1-AR subtype (α1A-ARs) in cellular inotropic responses. For both RV and LV myocytes, we found that the highly selective α1A-AR agonist A-61603 meditated either a PIE or a NIE (Fig. 3B). Thus, the single α1A-AR subtype could mediate fundamentally different inotropic responses, suggesting that the type of inotropic response (PIE or NIE) for a particular cell depends on events downstream from the receptor. Similar to PE, A-61606 elicited a PIE in the majority (82%) of LV cells (14 of 17 cells).

**Cellular heterogeneity of α1-AR effects on Ca$^{2+}$ handling.** Contraction and relaxation are triggered by the rise and fall of activator Ca$^{2+}$. Therefore, we determined the role of activator Ca$^{2+}$ in the heterogeneous α1-AR inotropic responses of LV and RV myocytes.

Figure 4 shows that fura-2-induced Ca$^{2+}$ transients and sarcomere length transients measured in LV and RV myocytes before and after α1-AR stimulation with PE elicited either a NIE (A and C) or a PIE (B and D). Evidently, the α1-AR-mediated NIE was associated with a marked decrease of the Ca$^{2+}$ transient, whereas the PIE was associated with an increase of the Ca$^{2+}$ transient. Although changes in Ca$^{2+}$ and sarcomere length are not in a dynamic equilibrium during myocyte contraction, nevertheless there is a positive correlation between the size of the Ca$^{2+}$ transient and the corresponding magnitude of contraction (10). The pooled data shown in Fig. 5 demonstrate the relationship between cell contraction amplitude and the corresponding Ca$^{2+}$ transient amplitude. For LV myocytes, there was a linear relationship between contraction amplitude and Ca$^{2+}$ transient amplitude for cells before and after α1-AR stimulation with PE. This relationship suggests that the NIE or PIE of α1-AR stimulation was appreciably driven by corresponding decreases or increases of Ca$^{2+}$ transient amplitude. Figure 5 shows that RV myocytes also manifested a relationship between cell contraction and Ca$^{2+}$ transient amplitude. This relationship appeared steeper for RV myocytes than for LV myocytes, suggesting that for a PIE elicited by PE, the myofilament Ca$^{2+}$ responsiveness of RV myocytes was greater than for LV myocytes.

Numerous factors contribute to the amplitude of the Ca$^{2+}$ transient. We investigated if α1-AR effects on Ca$^{2+}$ transients were linked to changes in Ca$^{2+}$ loading of the SR. SR Ca$^{2+}$ loading was assessed by a rapid application of 20 mM caffeine, which causes rapid unloading of SR Ca$^{2+}$. Figure 6A shows that caffeine caused a rapid rise of intracellular Ca$^{2+}$ concentration followed by a slow decline of Ca$^{2+}$ concentration. Figure 6A shows that for cells manifesting an α1-AR-induced NIE, the peak of the caffeine-induced Ca$^{2+}$ transient was reduced compared with cells without α1-AR stimulation by PE. In contrast, cells manifesting an α1-AR-induced PIE did not have an increase in the peak of the caffeine-induced Ca$^{2+}$ transient compared with cells without α1-AR stimulation.
The pooled data (Fig. 6B) demonstrate that for both RV and LV cells, a 1-AR-induced NIE was associated with a reduced caffeine-induced Ca\textsuperscript{2+} transient. In contrast, a 1-AR-induced PIE was not associated with a change in the caffeine-induced Ca\textsuperscript{2+} transient compared with cells without 1-AR stimulation. This suggests that for LV and RV cells, the 1-AR-induced NIE may have been contributed to by a reduction in SR Ca\textsuperscript{2+} loading. In contrast, for LV and RV cells, the 1-AR-induced PIE did not involve increased SR Ca\textsuperscript{2+} loading.

The decline of Ca\textsuperscript{2+} concentration in the presence of caffeine reflects Ca\textsuperscript{2+} transport from the cytosol by other Ca\textsuperscript{2+}-handling systems besides sarco(endo)plasmic reticulum Ca\textsuperscript{2+}-ATPase, principally, Ca\textsuperscript{2+} export from the cell by the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange (1). Figure 6C shows that for both LV and RV cells, 1-AR stimulation tended to increase the rate of Ca\textsuperscript{2+} decline by these other Ca\textsuperscript{2+} transport systems. After 1-AR stimulation, faster Ca\textsuperscript{2+} export from the cell via Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange could contribute to decreased SR Ca\textsuperscript{2+} load and a NIE. In contrast, faster Ca\textsuperscript{2+} export from the cell would not contribute to an 1-AR-induced PIE.

**DISCUSSION**

The major finding of this study was that the free walls of both the RV and LV are composed of myocytes that manifest considerable cell to cell heterogeneity in their 1-AR handling and contractile responses to stimulation of 1-ARs. This finding reveals a new aspect of biological heterogeneity among cardiac myocytes that might encompass other regulatory systems. Moreover, this study illuminates a complexity to the

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**Fig. 2.** Slow time-based recordings of electrically stimulated contractions before and after stimulation of 1-adrenergic receptors (1-ARs) with 10 μM phenylephrine (PE) plus the β-blocker timolol (10 μM). A: addition of PE (arrow) elicited a negative inotropic effect (NIE) in some RV cells. B: PE elicited a positive inotropic effect (PIE) in other RV cells. C and D: LV cells also manifest a NIE or PIE after 1-AR stimulation. E: there was in no inotropic response to treatment with the vehicle (Veh) control.
effects of $\alpha_1$-ARs on Ca$^{2+}$ signaling and contraction that was not suspected from previous studies of the intact myocardium.

Cellular heterogeneity in the heart. The heart is known to manifest regional heterogeneity, with reports of transmural differences between the endocardium and epicardium in voltage-gated K$^+$ currents (2), action potential duration (14), excitation-contraction coupling (7), myosin light chain kinase abundance (5), and myofilament properties (3, 4, 25).

Fig. 3. Summary of $\alpha_1$-AR inotropic responses of RV and LV myocytes (expressed as the percent change from the baseline contraction level for each cell). Heterogeneity of inotropic responses was observed using the nonsubtype-selective $\alpha_1$-AR agonist PE (A) and also using the $\alpha_1A$-AR subtype-selective agonist A-61603 (B).

Fig. 4. Examples of a NIE and PIE elicited by $\alpha_1$-ARs in LV and RV myocytes. Records of fura-2-induced Ca$^{2+}$ transients and cell contractions (assessed from sarcomere length measures) are shown. Compared with before the addition of PE, $\alpha_1$-AR stimulation caused decreased Ca$^{2+}$ transients and decreased contractions in some cells (A and C) or increased Ca$^{2+}$ transients and contraction in other cells (B and D).

HETEROGENEITY OF CELLULAR RESPONSES TO $\alpha_1$-AR STIMULATION

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We have previously reported that \( \alpha \)-ARs mediated fundamentally different inotropic responses in the RV versus LV myocardium (27, 28). This difference was associated with different effects of \( \alpha \)-AR stimulation on myofilament Ca\(^{2+} \) sensitivity of the LV versus RV myocardium (28). However, \( \alpha \)-ARs also influence Ca\(^{2+} \) handling. Moreover, differences in Ca\(^{2+} \) handling between the RV and LV have been reported (15). Therefore, we investigated the effects of \( \alpha \)-AR stimulation on Ca\(^{2+} \) handling in RV and LV myocytes.

**Cellular heterogeneity of Ca\(^{2+} \) signaling and inotropic responses to \( \alpha \)-adrenergic stimulation.** At the cellular level, the effects of \( \alpha \)-AR stimulation were complex. We found considerable cell-to-cell variability in \( \alpha \)-AR effects on Ca\(^{2+} \) signaling and contraction. For cells from the RV or LV free wall, \( \alpha \)-ARs elicited a NIE in some cells but a PIE in other cells. The \( \alpha \)-AR-induced NIE was associated with a decreased Ca\(^{2+} \) transient, and the \( \alpha \)-AR-induced PIE was associated with an increased Ca\(^{2+} \) transient. These findings suggest that \( \alpha \)-AR-induced changes in the Ca\(^{2+} \) transient played a key role in the inotropic response.

We found that cells with an \( \alpha \)-AR-induced NIE and reduced Ca\(^{2+} \) transient manifested a reduction in SR Ca\(^{2+} \) loading (as assessed from the caffeine-induced Ca\(^{2+} \) transient). Reduced SR Ca\(^{2+} \) load induced by \( \alpha \)-ARs might contribute to the observed reduction of Ca\(^{2+} \) transients and the NIE. Moreover, with transport of Ca\(^{2+} \) to the SR prevented in the presence of caffeine, the decay of the caffeine-induced Ca\(^{2+} \) transient is thought to reflect other Ca\(^{2+} \) transport systems, predominantly the Na\(^{+}/\)Ca\(^{2+} \) exchanger, which exports Ca\(^{2+} \) out of the cell (1). We found that \( \alpha \)-AR stimulation accelerated the decay of the caffeine-induced Ca\(^{2+} \) transient. This is consistent with \( \alpha \)-AR-induced stimulation of Na\(^{+}/\)Ca\(^{2+} \) exchange (19). Faster export of Ca\(^{2+} \) from the cell could contribute to a decreased SR Ca\(^{2+} \) load and the NIE elicited by \( \alpha \)-AR stimulation.

In contrast, cells with an \( \alpha \)-AR-induced PIE and increased Ca\(^{2+} \) transients did not manifest an increased SR Ca\(^{2+} \) load, suggesting that other mechanisms contributed to the increased Ca\(^{2+} \) transient [e.g., prolongation of the action potential (8), increased Ca\(^{2+} \) current, and increased gain of Ca\(^{2+} \)-induced Ca\(^{2+} \) release from the SR].

**Cellular heterogeneity of \( \alpha \)-AR inotropic responses in the RV versus LV.** The inotropic response to \( \alpha \)-AR stimulation has remained uncertain because it has varied considerably among...
studies and may be influenced by experimental conditions (23, 28). The results of the present study demonstrate that cellular heterogeneity within both the RV and LV is another complexity to α1-AR inotropic responses. Moreover, the fraction of cells manifesting an α1-AR-mediated NIE was significantly greater in the RV (68%) than in the LV (36%). This trend appears consistent with a previous report (28) of α1-ARs mediating a NIE in the RV myocardium. Thus, both intraventricular and interventricular factors may play a role in α1-AR inotropy.

Previous studies (6, 9, 11, 16, 22) have suggested that α1-AR subtypes may have different roles in cardiac inotropy, with the α1A-AR subtype mediating positive inotropy and the α1B-AR playing a negative modulatory role. However, in myocytes, we found that the α1A-AR subtype could mediate either a positive or negative inotropic response. This finding is consistent with our previous report (28), which showed that the α1A-AR agonist A-61603 mediated negative inotropy in the RV myocardium but positive inotropy in the LV myocardium, again indicating that the α1A-AR subtype can mediate a PIE or a NIE. Together, these findings suggest that events downstream from the α1A-AR subtype determine whether a PIE or NIE is elicited. The present study identifies that differential effects of α1-ARs on Ca2⁺ handling play a critical role.

Limitations. The clinical disease relevance of the presence of heterogeneous α1-AR responses is unclear. Potentially, the balance of cells manifesting a PIE versus a NIE elicited by α1-ARs may shift in disease and thereby impact the myocardial α1-AR inotropic response. For example, we (27) have previously reported a shift in the nonfailing RV myocardium from a NIE in the failing RV.

This study indicates that effects of α1-ARs on Ca2⁺ handling contribute to the heterogeneous inotropic responses of myocytes. Further studies will be required to identify the specific mechanisms downstream from α1-ARs that determine the inotropic response.

Like most studies of myocytes, only a small number of myocytes per heart were studied. However, the myocytes that survive the isolation process may not be truly representative of the total myocyte populations of the RV or LV. Cells were studied under artificial in vivo conditions, and thus it is not clear how well the in vitro cell behavior reflects that of cells in vivo. We studied mouse cells, which are similar to humans in cardiac α1-AR abundance (24). However, it will be important to study cells from other species. As we studied only a single class of receptors, it will be interesting to determine if there is cellular heterogeneity in responses to other receptor classes.

Conclusions. This study demonstrates the intraventricular and interventricular heterogeneity of cellular inotropic responses to stimulation of α1-ARs. A single α1-AR subtype (α1A) can manifest either a PIE or a NIE. Multiple mechanisms, including effects on Ca2⁺ transients, SR Ca2⁺ loading, and export of Ca2⁺ from the cell, are involved. This study reveals a new aspect of biological heterogeneity among cardiac myocytes in the regulation of contraction.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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