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Increasing Cardiac Contractility After Myocardial Infarction Exacerbates Cardiac Injury and Pump Dysfunction

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Abstract

Rationale—Myocardial infarction (MI) leads to heart failure (HF) and premature death. The respective roles of myocyte death and depressed myocyte contractility in the induction of HF after MI have not been clearly defined and are the focus of this study.

Objectives—We developed a mouse model in which we could prevent depressed myocyte contractility after MI and used it to test the idea that preventing depression of myocyte Ca²⁺-handling defects could avert post-MI cardiac pump dysfunction.

Methods and Results—MI was produced in mice with inducible, cardiac-specific expression of the $\beta 2a$ subunit of the L-type Ca²⁺ channel. Myocyte and cardiac function were compared in control and $\beta 2a$ animals before and after MI. $\beta 2a$ myocytes had increased Ca²⁺ current; sarcoplasmic reticulum Ca²⁺ load, contraction and Ca²⁺ transients (versus controls), and $\beta 2a$ hearts had increased performance before MI. After MI, cardiac function decreased. However, ventricular dilation, myocyte hypertrophy and death, and depressed cardiac pump function were greater in $\beta 2a$ versus control hearts after MI. $\beta 2a$ animals also had poorer survival after MI. Myocytes isolated from $\beta 2a$ hearts after MI did not develop depressed Ca²⁺ handling, and Ca²⁺ current, contractions, and Ca²⁺ transients were still above control levels (before MI).

Conclusions—Maintaining myocyte contractility after MI, by increasing Ca²⁺ influx, depresses rather than improves cardiac pump function after MI by reducing myocyte number.

Keywords

myocardial infarction; cardiac contractility; heart failure; Ca²⁺ handling

Vascular disease can lead to interruption of blood flow to the heart (myocardial infarction [MI]). The resulting loss of contractile mass causes an acute reduction in cardiac pump function. Cardiac output and blood pressure are initially maintained by activation of sympathetic reflex responses that increase myocyte contractility in regions of the heart where blood flow is maintained. Soon after MI, the ventricles begin to remodel (dilate),

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which further increases the work demands (systolic wall stress) on the surviving myocardium.

Myocyte contractility is increased after MI through activation of adrenergic signaling pathways that increase Ca^{2+} influx and sarcoplasmic reticulum (SR) uptake, storage, and release. The increased myocyte Ca^{2+} transients that enhance contractile function after MI are also thought to induce myocyte hypertrophy,^{1–3} increasing contractile mass and partially normalizing the increased systolic wall stress. However, persistent pathological stress in the remodeled, post-MI heart is associated with abnormal myocyte contractile properties^{4,5} and increases in the rate of myocyte death.^{6–8} These two changes develop with congestive heart failure (CHF) and increase during its progression.^{6,9} The respective contributions of myocyte contractile abnormalities (weak myocytes) and myocyte death (not enough myocytes) in the induction and progression of HF after MI is still not clearly defined. We studied this issue by preventing depression of myocyte contractile function in genetically modified mice subjected to MI.

Persistent pathological stress (MI and hypertension) induces abnormalities in myocyte Ca^{2+} handling^{5,10} and sympathetic signaling cascades.¹¹ SR Ca^{2+} uptake rates are slowed; Ca^{2+} transient and action potential durations are prolonged^{5,12}; and inotropic responses to sympathetic agonists are blunted.¹³ These changes are centrally involved in the slowing of contraction and relaxation rates, prolongation of contractile duration, and depressed contractility reserve.^{14,15} What is still not clear is whether these Ca^{2+} handling alterations induce cardiac decompensation and cause its progression or whether they are secondary to the ever increasing demand for enhanced myocyte function in the pathological heart.

The depressed myocyte contractility hypothesis predicts that HF therapies that increase myocyte contractility will improve cardiac pump function, HF symptoms, and survival. Unfortunately, most inotropic therapies that have been tested in patients over the past few decades have either had no effect on survival¹⁶ or have increased death rates.^{17,18} Therapies¹⁹ that have clinical benefit in HF, inhibitors of excess renin–angiotensin and β -adrenergic signaling, reduce rather than enhance myocyte contractility,^{19,20} suggesting that myocyte contractile abnormalities in HF might be the consequence of the excessive contractility demands rather than the cause of HF per se.

HF induction and progression is also associated with an increased rate of myocyte death from apoptosis and necrosis.⁸ Excessive activation of sympathetic and renin–angiotensin signaling pathways in HF,²¹ as well as oxidative stress, cytokine accumulation,²² and persistent activation of Ca^{2+} signaling,^{3,23,24} are all thought to contribute.^{25,26} Our working hypothesis is that excessive demands for contractility in HF increase cell death and it is cell death rather than reduced myocyte contractile function that causes HF progression.

The objective of our present study was to determine whether preventing depressed myocyte contractility after MI, without activating the sympathetic nervous system, improves cardiac pump function and slows CHF progression. To explore this topic we developed a genetically modified mouse in which we could increase Ca^{2+} influx by conditional, cardiac specific overexpression of the $\beta 2\alpha$ subunit of the L-type Ca^{2+} channel (termed $\beta 2\alpha$ mice).⁸ This approach increases Ca^{2+} influx and myocyte contractility^{26,27} without requiring sympathetic nerve activity. If depressed myocyte contractility causes abnormal pump function and CHF after MI, then we would expect better cardiac pump function in our $\beta 2\alpha$ mice in which depressed myocyte contractility is prevented. If on the other hand the remodeling of myocyte Ca^{2+} handling after MI is a response to the excessive demands for enhanced function, and in part this remodeling protects the myocyte from Ca^{2+} -mediated damage, then

maintaining high Ca^{2+} might increase myocyte death and exacerbate the depression of cardiac pump function.

Methods

All methods have been described in detail in previous reports and are described in detail in the Online Data Supplement, available <http://circres.ahajournals.org>. Briefly, myocardial infarction was induced in wild-type and $\beta 2a$ transgenic mice by permanent occlusion of the left anterior descending coronary artery. Cardiac function was measured with echocardiography. At euthanasia, hearts were removed and used for functional studies (isovolumic hearts and isolated single ventricular myocytes [VMs]), tissue was used for Western or biochemical analyses, or hearts were fixed and processed for histological studies.

Results

$\beta 2a$ -LTCC Hearts Are Hypercontractile

Mice with conditional, cardiac-specific, low-level expression of Cav1.2 $\beta 2a$ were used (Figure 1A).⁸ A modified α -myosin heavy chain promoter was used for cardiac-specific expression of the $\beta 2a$ gene, and mice containing both tTA (tetracycline-controlled transactivator) and $\beta 2a$ transgenes (double transgenic) allowed for doxycycline (Dox) suppression of $\beta 2a$ expression (Dox-off system) during development. Mice were taken off Dox-containing chow after weaning (at 21 days) and used at 4 to 6 months of age, because at this age, the $\beta 2a$ gene was fully expressed and mice showed enhanced cardiac performance (Figure 1B), with no signs of cardiomyopathy.⁸ $\beta 2a$ mice had increased ejection fraction (EF) (Figure 1C) and fractional shortening (FS) (Figure 1D) versus controls. Posterior wall thickness (PWT) and septal wall thickness were greater and left ventricular (LV) internal diameter was smaller (LVID) in $\beta 2a$ animals (Figure 1E, 1F, and 1G). These data show that $\beta 2a$ hearts were hypercontractile with modest LV hypertrophy (Figure 1H).

Infarct Size Is Increased in $\beta 2a$ Hearts Because of Increased Myocyte Death

MI was induced by interruption of blood flow to the LV via left anterior descending coronary artery (LAD). The area of the heart with interrupted blood flow was identical in control and $\beta 2a$ hearts (area at risk [AAR]; control versus $\beta 2a$: $45.1 \pm 3.2\%$ versus $50.4 \pm 6.8\%$; Figure 2A; Online Figure I, A). However, myocardial infarct size (as a % of AAR) was significantly larger in $\beta 2a$ mice than in controls (control versus $\beta 2a$: $45.3 \pm 6.1\%$ versus $83.8 \pm 14.4\%$) (Figure 2B; Online Figure I, B). Infarction length measured 6 weeks after MI was also significantly greater in $\beta 2a$ than in control mice (control versus $\beta 2a$: $21.7 \pm 4.9\%$ versus $45.8 \pm 6.8\%$) (Figure 2C; Online Figure I, C). These data suggest that more myocytes in the AAR die in $\beta 2a$ mice. We used the TUNEL assay to measure apoptotic cell death in the MI border and in remote zones. TUNEL + myocyte number was significantly greater in $\beta 2a$ hearts 3 days ($\beta 2a$ versus WT: 59 015 versus 16 600 in border and 3407 versus 1984 labeled/ 10^6 nuclei in the remote zone) and 3-week after MI (959 versus 647 in border and 1415 versus 709 labeled/ 10^6 nuclei in remote zone) (Figure 2D through 2F; and Online Figure II, A and B). Caspase 3 activity was also greater in $\beta 2a$ hearts (remote zone) 3 weeks after MI; indicative of cells undergoing apoptosis (Figure 2G). Myocyte death is associated with replacement fibrosis²⁸ and the collagen content of $\beta 2a$ hearts was significantly greater than in controls and increased to a greater extent with time after MI (Figure 2H). Ki67 (proliferative marker) labeling was greater in $\beta 2a$ versus WT hearts after acute (4223 versus 2621 labeled/ 10^6) or 3 weeks MI (1974 versus 1542 labeled/ 10^6) (Figure 2I; Online Figure II, C). Collectively these results show that there is an increased rate of

death of $\beta_2\alpha$ myocytes after MI stress, both at the infarct border zone (larger infarct size) and in remote areas of the heart.

$\beta_2\alpha$ Hearts Have Depressed Function After Ischemia/Reperfusion Stress

Our MI experiments suggest that the $\beta_2\alpha$ heart is more prone to acute ischemic injury. To more directly test this idea we compared the effects of in vitro ischemia-reperfusion (IR) stress on ventricular performance in isovolumic hearts subjected to 15 minutes of global no flow ischemia, followed by 30 minutes of reperfusion. Before IR, LV developed pressure (LVDP) and $\pm dP/dT$ (mm Hg/s) in $\beta_2\alpha$ hearts were significantly greater than in controls (Figure 3A; Online Figure III, A and B). During the ischemic period, end diastolic pressure (EDP) became significantly greater in $\beta_2\alpha$ hearts versus control, suggesting they develop Ca^{2+} overload-related increases in diastolic pressure (Figure 3B through 3C). $\beta_2\alpha$ hearts also had greater disruption of contractile function after reperfusion (Figure 3B). Recovery of LVDP was significantly reduced in $\beta_2\alpha$ versus control hearts (Figure 3B) and the EDP was significantly greater in $\beta_2\alpha$ hearts than in control hearts (Figure 3D). The increased EDP and reduced LVDP are consistent with enhanced Ca^{2+} stress in the $\beta_2\alpha$ that results in SR Ca^{2+} overload.²⁶ These results document that $\beta_2\alpha$ hearts are prone to ischemia-related injury, without the neurohormonal stressors present in the post-MI heart.

More $\beta_2\alpha$ Animals Die After MI and Survivors Have More Pathological Hypertrophy

Animals were studied over a 6 week period after MI. Representative examples of heart and lung morphology in control and $\beta_2\alpha$ mice after MI surgery are shown in Online Figure I (D). $\beta_2\alpha$ animals were significantly more likely to die after MI than were controls (Figure 4A), with only 19% alive after 6 weeks, versus 60% of controls.

Lung weight was significantly increased in $\beta_2\alpha$ animals early after MI, documenting more severe acute HF, and returned toward normal in those animals that survived for longer times (Figure 4B). Both groups of animals had increased heart weight/body weight (HW/BW) ratio, but in $\beta_2\alpha$ mice, HW/BW was increased more than in controls (Figure 4C).

To define myocyte hypertrophy, we measured indices of myocyte size in tissue sections of animals euthanized 6 weeks after MI. Myocyte hypertrophy was significantly greater in $\beta_2\alpha$ than in control hearts after MI (Figure 4D through 4G). These data show that $\beta_2\alpha$ animals have exacerbated pathological responses to MI, with enhanced acute heart failure and increased pathological myocyte hypertrophy.

Cardiac Pump Function After MI Was Depressed More in $\beta_2\alpha$ Hearts Than in Controls

Echocardiography was used to measure changes in LV function and chamber dimensions after MI (see examples in Online Figure IV, A and B). In sham animals, LV contractile function was greater in $\beta_2\alpha$ versus controls (Figures 1 and 5). Importantly, cardiac structure and function were stable in sham $\beta_2\alpha$ mice throughout the time period of this study (Figure 5). After MI, all animals had reduced pump function and significant enlargement in LV chamber dimensions (dilation) (Figure 5). LV FS and EF were significantly decreased 1 week after MI in control mice (FS, pre-MI versus after MI: 37.4% versus 17.6%; EF, 67.7% versus 38.2%) and $\beta_2\alpha$ mice (FS, pre-MI versus after MI: 41.1% versus 19.9%; EF, 72.7% versus 40.8%), and there were now no differences in these functional parameters between $\beta_2\alpha$ and control hearts. Over the next few weeks, FS and EF continued to decrease in $\beta_2\alpha$ hearts (FS, after MI 2, 4, 6 weeks: 15.5%, 11.5%, 13.2%; EF, 32.7%, 24.7%, 25.4%), whereas in control mice, these functional parameters were stable (FS, after MI 2, 4, 6 weeks: 20.6%, 21.2%, 19.6%; EF: 41.9%, 42.6%, 40.4%) (Figure 5). At the end of 6-week study interval, cardiac function was significantly more depressed in $\beta_2\alpha$ versus control hearts.

There were significant pathological changes in ventricular geometry and wall thickness after MI (Online Figure IV, A and B). The magnitude of these changes was greater in $\beta 2a$ than in control hearts. LV wall thickness was greater in $\beta 2a$ versus control hearts before MI. After MI, PWT was decreased in both $\beta 2a$ and control hearts (control pre-MI versus post-MI: 1.08 versus 1.00 mm; $\beta 2a$: 1.21 versus 1.06 mm). Over the next 3 weeks, PWT returned to values near pre-MI levels in control hearts, whereas PWT continued to decrease in $\beta 2a$ hearts (Figure 5D). All hearts showed some evidence of dilation after MI; however, LVID increased significantly more in $\beta 2a$ than in control hearts in the first week after MI (control pre-MI versus post-MI: 3.7 versus 4.0 mm; $\beta 2a$: 3.4 versus 4.0 mm). By 4 weeks after MI, LVID was significantly more dilated in $\beta 2a$ hearts than in controls (4.2 versus 5.2 mm, Figure 5C). These results show that MI causes significantly greater deterioration of cardiac function and structure (lower EF, FS, enlarger chamber) in $\beta 2a$ mice.

LTCC Current ($I_{Ca,L}$) Remains Increased in $\beta 2a$ Mice After MI

We measured LTCC currents, cell contractions, and Ca^{2+} transients in myocytes from control and $\beta 2a$ hearts with or without MI. $I_{Ca,L}$ density was significantly larger in sham $\beta 2a$ versus control myocytes (peak $I_{Ca,L}$ in $\beta 2a$ versus control: -24.5 ± 1.7 [n=13] versus -11.98 ± 3.1 pA/pF [n=11], $P < 0.05$) (Figure 6A through 6C). The voltage dependence of $I_{Ca,L}$ activation was shifted to negative potentials in $\beta 2a$ myocytes, consistent with the known function of this subunit^{29,30} and with what we have shown previously.⁸ After MI, $I_{Ca,L}$ density was decreased in all myocytes but remained significantly larger in $\beta 2a$ than in control myocytes ($\beta 2a$ versus control: -17.6 ± 1.4 n=15 versus -10.4 ± 0.8 pA/pF n=12 $P < 0.05$) (Figure 6A through 6C). $I_{Ca,L}$ density in $\beta 2a$ after MI was significantly greater than in control myocytes before MI.

$I_{Ca,L}$ is increased by protein kinase A-mediated phosphorylation in normal myocytes.³¹ This regulation is altered in myocytes from diseased hearts.^{32,33} We measured the effects of isoproterenol (Iso) on $I_{Ca,L}$ in myocytes from sham and post-MI hearts. In control (sham) myocytes, Iso increased $I_{Ca,L}$ density (pre-Iso versus after Iso: -13.3 ± 1.95 to -17.9 ± 3.9 pA/pF, n=4, $P < 0.05$) and caused a significant negative shift in the voltage dependence of $I_{Ca,L}$ activation. Iso had no significant effect on $I_{Ca,L}$ in $\beta 2a$ (sham) myocytes (-22.9 ± 2.7 versus -20.2 ± 0.9 pA/pF, n=7) (Figure 7A, 7C, and 7E; and Online Figure V [A, B, and E]). After MI, baseline $I_{Ca,L}$ density was reduced in both control and $\beta 2a$ myocytes (Figure 6). In control MI myocytes Iso increased $I_{Ca,L}$ but less so than under control conditions (control MI pre-Iso versus after Iso: -9.4 ± 1.8 versus -12.6 ± 2.7 pA/pF, n=6, $P < 0.05$) and Iso shifted the voltage dependence of activation to more negative potential (Figure 7B, 7D, and 7F; and Online Figure V [C, D, and F]). Surprisingly, Iso caused a significant increase in $I_{Ca,L}$ in $\beta 2a$ MI myocytes (pre-Iso versus after Iso: -17.0 ± 1.3 versus -23.9 ± 2.4 pA/pF, n=5, $P < 0.05$) (Figure 7 B, 7D, and 7F; and Online Figure V [C, D, and F]), increasing $I_{Ca,L}$ to values measured in control $\beta 2a$ myocytes. These results show that $I_{Ca,L}$ in $\beta 2a$ myocytes remains significantly greater than in controls after MI.

Contractions and $[Ca^{2+}]_i$ Transients in $\beta 2a$ Myocytes After MI

We confirmed⁸ that $\beta 2a$ myocytes have larger contractions and Ca^{2+} transients than controls (Figure 8). FS of $\beta 2a$ was significantly greater than control (sham) myocytes ($\beta 2a$ versus control: $12.1 \pm 1.2\%$ n=14 versus $7.8 \pm 1.0\%$ n=19 $P < 0.05$). After MI, contractions remained significantly greater in $\beta 2a$ than in control myocytes ($\beta 2a$ versus control: $12.3 \pm 0.7\%$ n=28 versus $8.2 \pm 0.8\%$ n=12 $P < 0.05$).

Myocyte contractions in both control and $\beta 2a$ increased with Iso (control sham pre-Iso versus after Iso: $7.8 \pm 0.97\%$ versus $10.7 \pm 0.70\%$, n=19, $P < 0.05$; $\beta 2a$ sham: $12.1 \pm 1.2\%$ versus $14.5 \pm 0.9\%$, n=9, $P < 0.05$), with contractions in $\beta 2a$ being greater than in controls.

Contractions of MI myocytes were increased by Iso, and again β 2a myocytes had greater contractions than controls (post-MI control with or without Iso: $8.2 \pm 0.8\%$ versus $10.9 \pm 0.95\%$ $n=9$ $P<0.05$; post-MI β 2a with or without Iso: $12.3 \pm 0.7\%$ versus $14.9 \pm 0.6\%$ $n=21$ $P<0.05$) (Figure 8A; Online Figure VI, A through D).

$[Ca^{2+}]_i$ transients and SR Ca^{2+} content in β 2a (sham) myocytes were significantly greater than in controls (Figure 8B through 8F), consistent with our previous results.⁸ After MI, $[Ca^{2+}]_i$ transients in control myocytes were decreased, whereas those in β 2a myocyte were similar to what was measured under basal conditions (F/F_0 ratio in β 2a sham versus control sham: 4.3 ± 0.6 versus 3.4 ± 0.2 ; post-MI β 2a versus control: 4.3 ± 0.3 versus 2.9 ± 0.7) (Figure 8B; Online Figure VI, E through H). After MI, Iso increased Ca^{2+} transient amplitude in control and β 2a myocytes, with Ca^{2+} transient amplitude being significantly greater in β 2a myocytes (control [sham]) pre-Iso versus post-Iso: 3.4 ± 0.2 versus 4.7 ± 0.4 $n=8$ $P<0.05$; β 2a [sham]: 4.3 ± 0.6 versus 5.8 ± 0.9 , $n=9$, $P<0.05$; post-MI control: 2.9 ± 0.7 versus 3.6 ± 0.5 , $n=5$, $P<0.05$; post-MI β 2a: 4.3 ± 0.3 versus 5.1 ± 0.4 , $n=9$, $P<0.05$).

Collectively, these data show that myocytes from β 2a MI hearts with cardiac pump function that was worse than in control MI hearts remained hypercontractile.

Discussion

In the present study, we tested the hypothesis that preventing depression of myocyte contractility after MI improves cardiac pump function and slows heart failure progression. The major findings of this study are that increasing Ca^{2+} influx through the L-type Ca^{2+} channel after MI prevents depressed myocyte contractility but increases the risk of ischemic injury, precipitates sudden death, and exacerbates depressed cardiac pump function.

β 2a Hearts Have Depressed Pump Function After Ischemia/Reperfusion

Our studies in isolated hearts show that hypercontractile β 2a hearts have more depressed contractile function after IR (Figure 3). EDP was elevated during ischemia and after reperfusion in β 2a hearts, and systolic function after reperfusion was more depressed than in controls. These results suggest that the higher Ca^{2+} influx in β 2a myocytes precipitates SR Ca^{2+} overload²⁶ resulting in diastolic and systolic dysfunction and Ca^{2+} -mediated injury.^{34,35}

β 2a Hearts Have Larger Infarcts

The AAR after myocardial infarction was not different in control and β 2a mice; however, infarct size (as a % of AAR) was significantly greater in β 2a hearts than in controls (Figure 2). Not all portions of the AAR die after MI because the border of the ischemic region receives some blood flow and some myocytes in this region can survive. Our results show that a greater number of myocytes in the border zone died in the β 2a hearts. The mechanism of enhanced myocyte death is not entirely clear, but we did show an increase in TUNEL⁺ myocytes in the β 2a infarct border and remote zone, suggesting that apoptotic cell death is increased. We cannot rule out the possibility that necrotic cell death is also increased in β 2a hearts subjected to MI.⁸

The factors that influence myocyte death in the MI border zone are complex and involve ischemia and systemic sympathetic reflex responses. After MI, cardiac pump function decreases and this elicits a reflex sympathetic nervous system response to maintain cardiac output and systemic blood pressure. Because β 2a hearts have enhanced basal contractility, less sympathetic reflex response might be expected after MI, and this could reduce any damaging effects of sympathetic stimulation on myocyte survival. Because we found larger

infarcts in $\beta 2a$ hearts we conclude that this primarily results from excess myocyte Ca^{2+} rather than excessive sympathetic activity.

Cardiac Dysfunction, Wall Thinning, and Chamber Dilation Are Enhanced in $\beta 2a$ Hearts After MI

MI causes cardiac structural remodeling (wall thinning and dilation).³⁶ These changes in ventricular size and shape increase systolic wall stress, further increasing the contractile demands on a heart with a reduced number of working myocardial cells. This persistent elevation in wall stress causes progressive decay of myocardial performance and induces heart failure and premature death.³⁶ Our results show that $\beta 2a$ hearts develop more dilation and poorer pump function than controls after MI (Figure 5) and that this was associated with increased rates of myocyte death in the remote zone of the $\beta 2a$ heart (Figure 2F). The most important finding in our study is that these detrimental changes take place in hearts in which we prevented depressed myocyte contractility (Figures 6 and 7), documenting that depressed myocyte contractile function is not a prerequisite for poor cardiac pump function after MI.

Why Is Cardiac Pump Function Depressed in the Dilated Heart?

In patients with heart failure, the dilated heart has a reduced EF, and blood is expelled slowly.³⁷ The afterload (systolic wall stress) in the failing heart is significantly increased and correlates closely with heart failure severity and prognosis.³⁸ Many groups,⁴ including ours,^{8,39} have shown that Ca^{2+} handling is deranged in failing myocytes and this depresses myocyte contractility reserve.⁵ Pathologically high systolic wall stress and depressed myocyte contractility both reduce the pump function of these hearts. However, it is still not clear whether either or both mechanisms represent the primary cause(s) of heart failure progression. Our study shows that heart failure progresses at a faster rate in mice in which myocyte contractility is maintained after MI (Figure 5).

Heart failure progression is also associated with an increased rate of myocyte death.^{8,25,40,41} Our experiments showed that apoptotic myocyte death was increased in remote zones of the $\beta 2a$ heart (versus control) after MI (Figure 2 and Online Figure II). We conclude that MI-induced heart failure progression in $\beta 2a$ mice results from a vicious cycle of myocyte loss, ventricular dilation, increased systolic wall stress (and Ca^{2+} signaling cascades; Online Figure VII), increased sympathetic responses (which preserves myocyte contractility at a very high level in $\beta 2a$ myocytes; Figures 6 through 8), and myocyte death mediated by excessive Ca^{2+} (and/or sympathetic nervous system) stress.

Could Depressed Myocyte Contractility Be the Effect of Heart Failure?

Ca^{2+} handling is deranged in heart failure.⁵ L-type Ca^{2+} channel density and phosphorylation are altered to limit Ca^{2+} influx.³³ SERCA abundance is reduced and phospholamban phosphorylation is reduced to slow the rate of SR Ca^{2+} uptake and reduce SR Ca^{2+} loading.^{12,42} The phosphorylation of the SR Ca^{2+} release channel (ryanodine receptor) is abnormal and this leads to arrhythmias and SR Ca^{2+} leak.⁴³ Collectively, these defects produce a failing heart that must develop high systolic stress with a reduced ability to increase Ca^{2+} influx and SR Ca^{2+} uptake and release.

These changes in Ca^{2+} handling have led some to conclude that myocyte force development in the failing heart is less than in the normal heart. This is clearly not the case, because systolic wall stress (force being generated per unit myocardium) is significantly elevated.³⁸ Although contractility reserve is reduced in these myocytes, the activity of neuroendocrine regulatory mechanisms must provide the stimulus to induce high force generation in vivo. Our present hypothesis is that the persistent need to develop high wall stress (requiring high Ca^{2+}) induces the deranged Ca^{2+} handling that reduces contractility reserve.⁵ Therefore,

deranged Ca^{2+} handling may be the effect of rather than the cause of heart failure progression. Our data suggest that downregulation of Ca^{2+} handling in the diseased heart might provide some protection from high Ca^{2+} -related injury.

Relevance of the Study

Heart failure is a major health problem in our society. Available therapies provide only modest prolongation of life⁴⁴; therefore, novel therapies are clearly needed. Importantly, those therapies⁴⁴ that show clinical utility are negative inotropic agents (β -adrenergic antagonists and angiotensin antagonists). Conversely, those therapies that increase contractility have actually increased death rates of heart failure patients.^{17,18} Therefore, increasing Ca^{2+} stress (contractility) in failing hearts may not be the best strategy for long-term improvement in cardiac pump function. However, we forced Ca^{2+} handling to be increased by augmenting the most proximal portion of the Ca^{2+} -handling cascade, Ca^{2+} influx. Approaches that improve Ca^{2+} handling by manipulation of more distal elements, such as increasing SERCa²⁺⁴⁵ or blocking SR Ca^{2+} leak⁴⁶ might rebalance cardiac contractility without predisposing the cell to Ca^{2+} overload and cell death.

Novelty and Significance

What Is Known?

- Persistent hemodynamic stress (from high blood pressure or after a myocardial infarction [MI]) requires an increase in the contractile strength of cardiac myocytes.
- Increased contractile demand induces a sympathetic response that increases cell Ca^{2+} and contractility.
- With the development of heart failure, Ca^{2+} handling becomes abnormal with a reduced ability to maintain high levels of contractile strength (muscle weakening), and there is an increase in myocyte death rate.
- Most pharmacological approaches that increase contractility in human heart failure have not been improved patient outcome.

What New Information Does This Article Contribute?

- Expressing the $\beta_2\alpha$ subunit of the L-type Ca^{2+} channel prevents depression of myocyte contractility after myocardial infarction.
- Maintaining high levels of contractility after MI was associated with an increase in infarction size.
- Maintaining high levels of contractility with $\beta_2\alpha$ expression was associated with exacerbation of depressed cardiac pump function and cardiac dilation. This study showed that MI-induced heart failure could be seen in hearts with hypercontractile myocytes.
- Maintaining high Ca^{2+} and contractility after MI was associated with increased myocyte apoptosis and ventricular dilation.

Myocardial infarction results in a heart with fewer myocytes that must increase their force development to maintain systemic blood pressure. Over time, the heart dilates and fails. Although myocytes in the dilated heart must develop high stress *in vivo*, they develop contractile defects that limit their peak contractile performance. These changes may also protect myocytes from negative aspects (activation of cell death signaling) of sustained high Ca^{2+} states. This study tested whether preventing depression of myocyte contractility after MI improves cardiac performance or exacerbates MI related cardiac

injury. Our results show that animals with genetically induced increases in myocyte contractility were more prone to stress related injury and death. Maintaining high contractility caused expansion of infarct size and exacerbated cardiac dilation and depressed pump function, even though the β_2 myocytes remained hypercontractile. These studies suggest that positive inotropic therapy should be performed cautiously because in addition to the increased arrhythmogenic risk of this type of therapy (if it leads to enhanced myocyte Ca^{2+}), it has the potential to induce myocyte death, further reducing myocyte number to exacerbate depressed cardiac pump function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Non-standard Abbreviations and Acronyms

AAR	area at risk
β2a	β 2a subunit of the L-type Ca^{2+} channel
BW	body weight
CHF	congestive heart failure
Dox	doxycycline
EDP	end diastolic pressure
EF	ejection fraction
FS	fractional shortening
HF	heart failure
HW	heart weight
Iso	isoproterenol
LAD	left anterior descending coronary artery
LV	left ventricular

LVDP	left ventricular developed pressure
LVID	left ventricle internal diameter
MI	myocardial infarction
PWT	posterior wall thickness
SR	sarcoplasmic reticulum
VM	ventricular myocyte

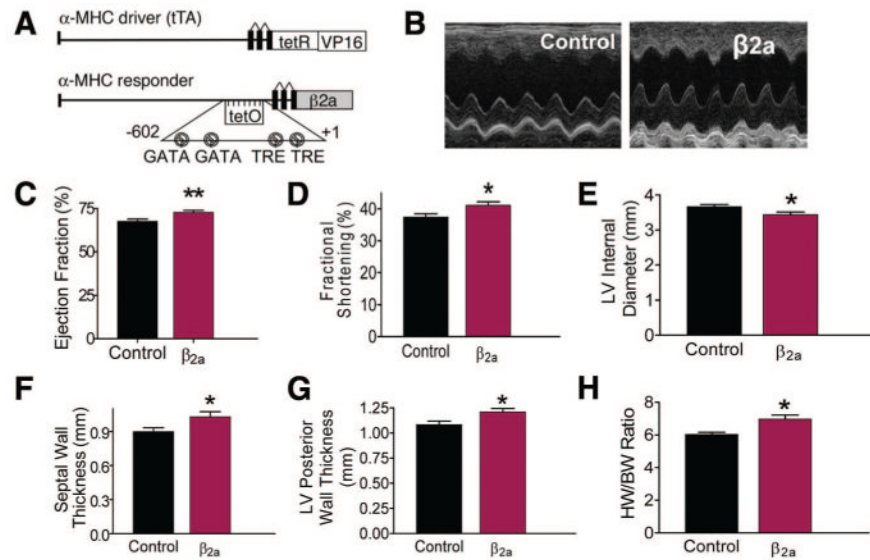


Figure 1. β 2a hearts are hypercontractile with mild hypertrophy

A, Bitransgenic inducible expression system to induce β 2a expression. tTA is the tetracycline-controlled transactivator. **B**, Representative echocardiography M-mode image in control and β 2a hearts. **C through G**, EF, FS, PWT, and septal wall thickness were greater, and left ventricle internal diameter (LVID) was smaller in β 2a vs control mice (control, n=46; β 2a, n=36). **H**, HW/BW ratio in β 2a hearts (n=51) was greater than in controls (n=18). * P <0.05; ** P <0.01.

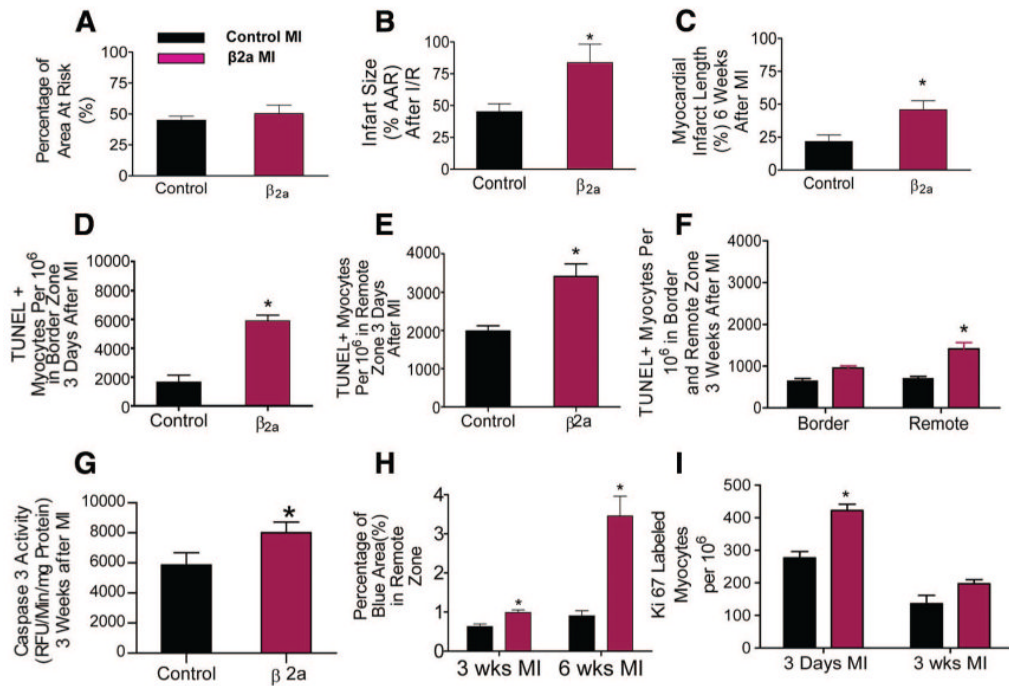


Figure 2. MI and ischemia/reperfusion (I/R) causes more cell death in β 2a hearts

A, AAR was not different in control (n=15) and β 2a hearts (n=6). **B**, Infarct size (% of AAR) in hearts with 30 minutes of ischemia followed by 24 hours of reperfusion (I/R) was greater in β 2a (n=6) than in control hearts (n=8). **C**, Infarct length after permanent occlusion was greater in β 2a (n=6) than in controls (n=7) and. **D through F**, Myocytes per 10^6 undergoing apoptosis in the border and remote zones of control (n=3) and β 2a hearts (n=4). **G**, Caspase 3 activity in remote zone tissues (control, n=9; β 2a, n=10). **H**, Fibrotic area (blue) in remote zones of trichrome-stained cardiac histological sections from control (n=3) and β 2a hearts (n=3). **I**, Ki67⁺ myocytes per 10^6 in control (n=3) and β 2a hearts (n=4) after MI. * P <0.05; ** P <0.01.

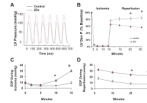


Figure 3. Ischemia/reperfusion depresses cardiac function in β_2a hearts
A, Representative recordings of LV pressure in control and β_2a isolated hearts. **B through D**, LVDP and LV end diastolic pressure (EDP) during and after ischemia/reperfusion in control (n=8) and β_2a (n=5) isolated hearts. * $P < 0.05$.

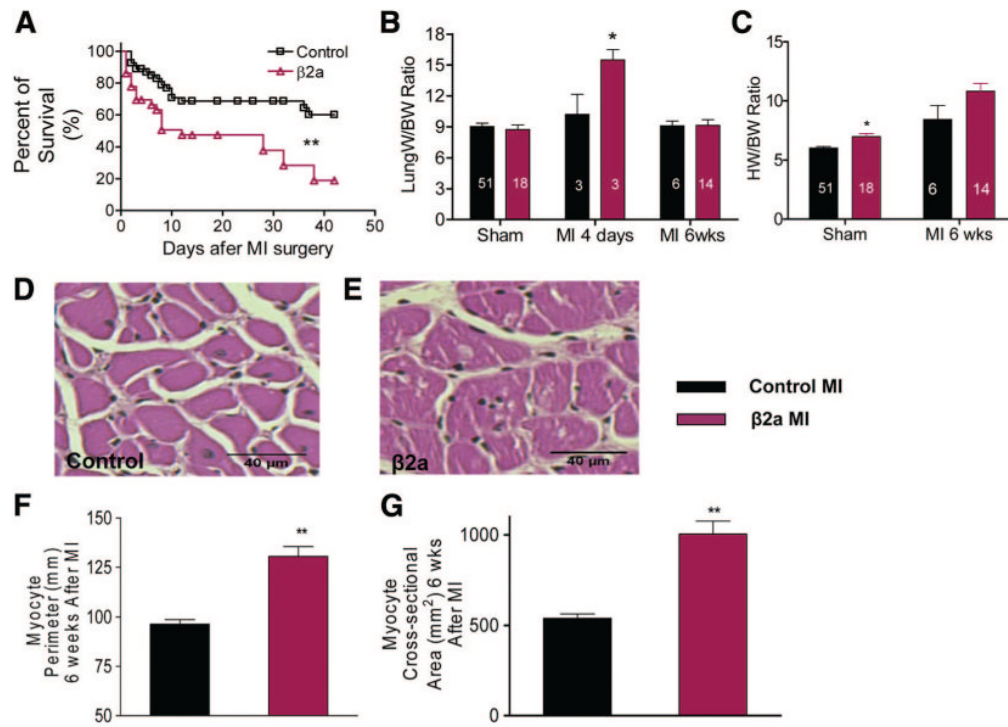


Figure 4. Mortality and cardiac remodeling is increased in $\beta 2a$ mice after MI

A, Kaplan–Meier survival curves during 6 weeks after MI in control (n=54) and $\beta 2a$ (n=36) mice. **B and C**, HW and lung weight (Lung W) were normalized to BW in control and $\beta 2a$ mice. **D and E**, Representative hematoxylin/eosin-stained sections from control and $\beta 2a$ hearts. **F and G**, Myocyte cross sectional area and perimeter in control (n=3) and $\beta 2a$ hearts (n=3). **Numbers in the bars** are the numbers of animals examined. ** $P < 0.01$; * $P < 0.05$.

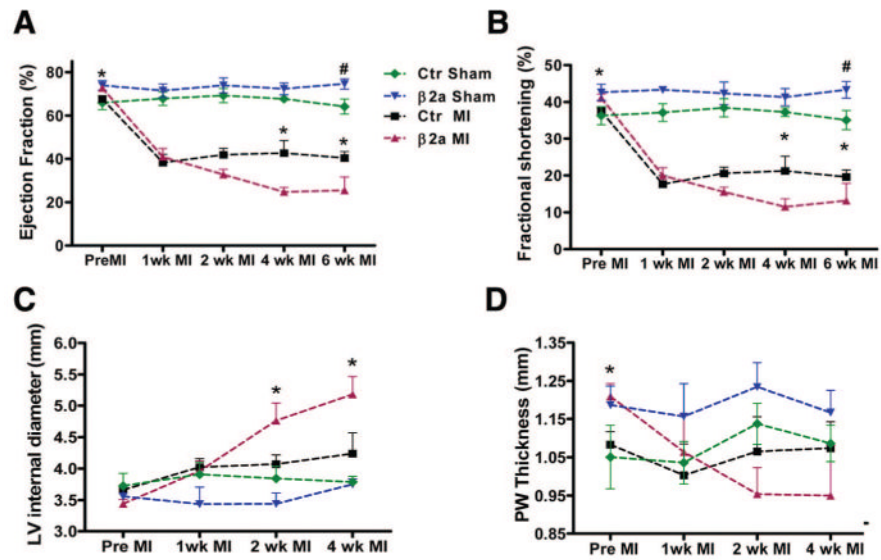


Figure 5. More severe cardiac failure in $\beta 2a$ mice after MI

Average cardiac EF (A), FS (B), LV internal diameter (C), PWT (D) were measured in control (n=46) and $\beta 2a$ (n=36) mice before and 1, 2, 4, or 6 weeks after MI and in sham-operated mice ($\beta 2a$, n=11; control, n=11). * $P < 0.05$ control MI vs $\beta 2a$ MI; # $P < 0.05$ control sham vs $\beta 2a$ sham.

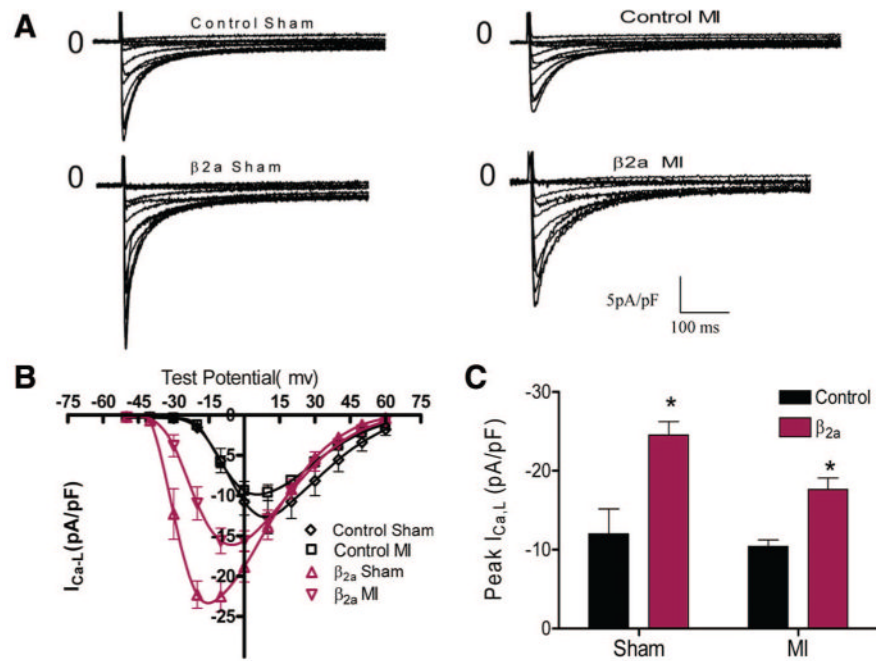


Figure 6. $I_{Ca,L}$ is greater in β_2a VMs from sham or MI hearts

A, Representative $I_{Ca,L}$ recordings in control and β_2a VMs \pm MI. **B**, Average voltage-current relationship of $I_{Ca,L}$ in control (sham, n=11; MI, n=12) and β_2a VMs (sham, n=13; MI, n=15). **C**, Peak $I_{Ca,L}$ was significantly greater in β_2a than control myocytes before and after MI. * $P < 0.05$.

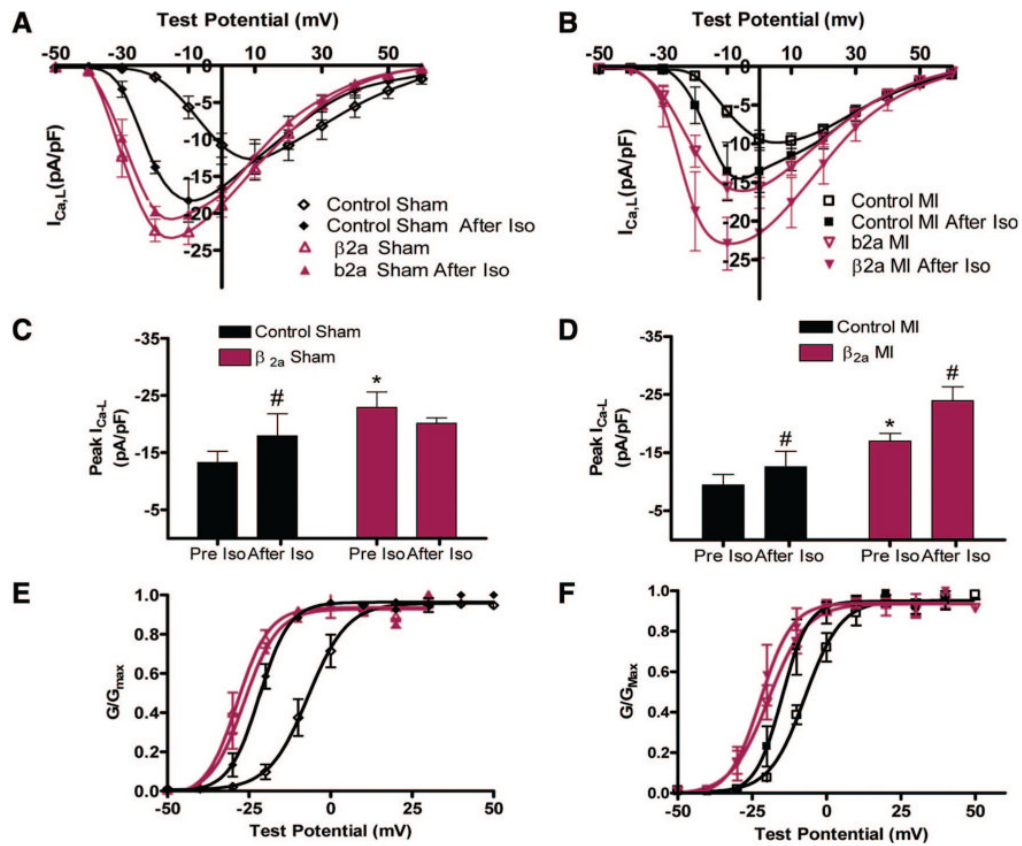


Figure 7. Effects of Iso (1 μ mol/L) on $I_{Ca,L}$ in sham or post-MI myocytes

A and B, Current–voltage relationships in sham and MI (β_{2a} and controls) with or without Iso. **C**, Iso increased peak $I_{Ca,L}$ in control (n=4) but not in β_{2a} ventricular myocytes (VMs) (n=7). **D**, Iso increased $I_{Ca,L}$ in both control-MI (n=6) and β_{2a} -MI VMs (n=5). **E and F**, Voltage dependence of $I_{Ca,L}$ activation in sham or post-MI VMs with or without Iso.

* $P < 0.05$ between control and β_{2a} VMs; # $P < 0.05$ with or without Iso.

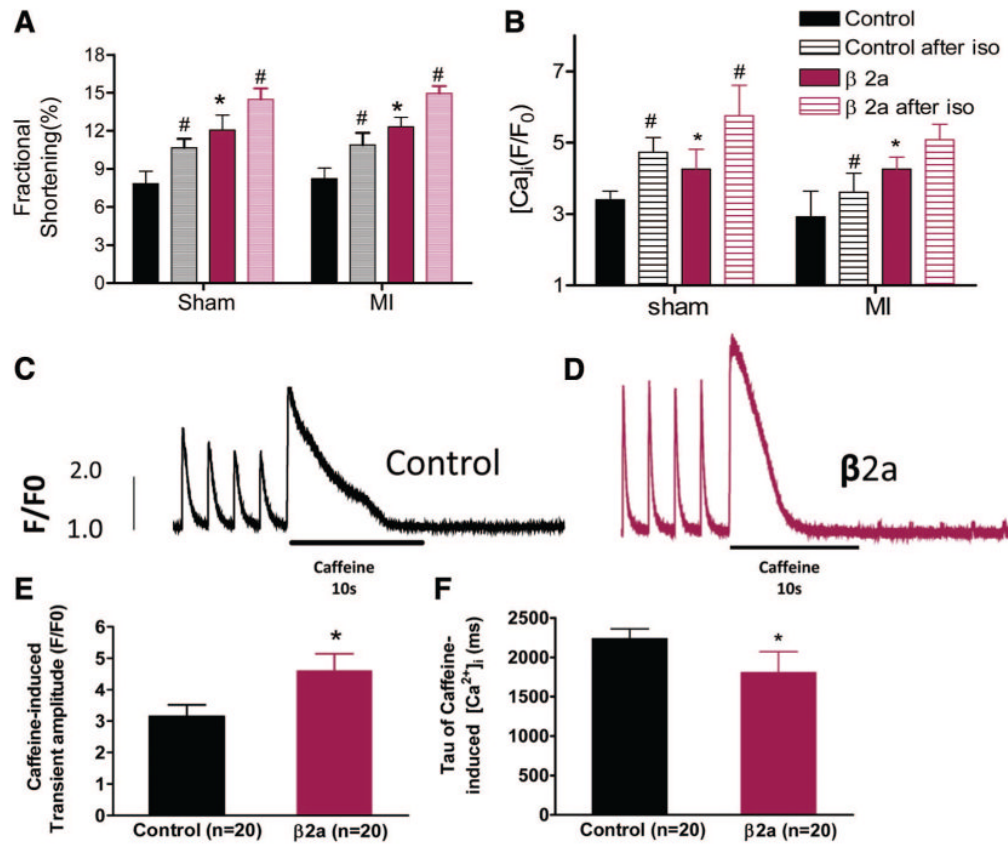


Figure 8. Contractions, $[Ca^{2+}]_i$ transients, and SR load with or without Iso

A, Comparison of FS between control and $\beta 2a$ in sham or post-MI myocytes with or without Iso (sham control, n=19 and $\beta 2a$, n=9; post-MI control, n=9 and $\beta 2a$, n=21). **B**, Comparison of $[Ca^{2+}]_i$ transients between control and $\beta 2a$ in sham or post-MI myocytes with or without Iso (sham control, n=8 and $\beta 2a$, n=9; post-MI control, n=5 and $\beta 2a$, n=9). **C and D**, Representative example of caffeine-induced $[Ca^{2+}]_i$ transients in control and $\beta 2a$ myocytes. **E and F**, Average data of peak caffeine-induced $[Ca^{2+}]_i$ transients and rate of decay in control and $\beta 2a$ myocytes. * $P < 0.05$ between control and $\beta 2a$ VMs; # $P < 0.05$ with or without Iso.