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

Hongyu Zhang,* Xiongwen Chen,* Erhe Gao,* Scott M. MacDonnell, Wei Wang, Mikhail Kolpakov, Hiroyuki Nakayama, Xiaoying Zhang, Naser Jaleel, David M. Harris, Yingxin Li, Mingxin Tang, Remus Berretta, Annarosa Leri, Jan Kajstura, Abdelkarim Sabri, Walter J. Koch, Jeffery D. Molkentin, Steven R. Houser

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 β2a subunit of the L-type Ca2+ channel. Myocyte and cardiac function were compared in control and β2a animals before and after MI. β2a myocytes had increased Ca2+ current; sarcoplasmic reticulum Ca2+ load, contraction and Ca2+ transients (versus controls), and β2a 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 β2a versus control hearts after MI. β2a animals also had poorer survival after MI. Myocytes isolated from β2a 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. (Circ Res. 2010;107:00-00.)

Key Words: 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), 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 Ca2+ influx and sarcoplasmic reticulum (SR) uptake, storage, and release. The increased myocyte Ca2+ 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 properties4,5 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 Ca2+ handling5,10 and sympathetic signaling cascades.11 SR Ca2+ uptake rates are slowed; Ca2+ transient and action potential durations are prolonged5,12; and inotropic responses to sympathetic agonists are blunted.13 These changes are centrally involved in

Original received February 19, 2010; revision received July 15, 2010; accepted July 19, 2010. In June 2010, the average time from submission to first decision for all original research papers submitted to Circulation Research was 14.5 days.

From the Temple University School of Medicine (H.Z., X.C., W.W., M.K., X.Z., N.J., Y.L., M.T., R.B., A.S., S.R.H.), Philadelphia, Pa; Thomas Jefferson University (E.G., W.J.K.), Philadelphia, Pa; Boehringer Ingelheim Pharmaceuticals Inc (S.M.M.), Ridgefield, Conn; Children’s Hospital Medical Center (H.N., J.D.M.), Cincinnati, Ohio; Drexel University (D.M.H.), Philadelphia, Pa; and Brigham and Women’s Hospital (A.L., J.K.), Boston, Mass.

This manuscript was sent to Donald Bers, Consulting Editor, for review by expert referees, editorial decision, and final disposition.

*These authors contribute equally to this work.

Correspondence to Steven R. Houser, PhD, Temple University, School of Medicine, 3500 N Broad St, MERB 1041, Philadelphia, PA 19140. E-mail srhouser@temple.edu

© 2010 American Heart Association, Inc.

Circulation Research is available at http://circres.ahajournals.org

DOI: 10.1161/CIRCRESAHA.110.219220
Increased Myocyte Death

Increased myocyte death from apoptosis and necrosis.8 HF per se.

Whether preventing depressed myocyte contractility after MI, the excessive contractility demands rather than the cause of CHF progression. To explore this topic we developed a genetically modified mouse without activating the sympathetic nervous system, improves cardiac pump function and slows CHF progression. 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 β2a subunit of the L-type Ca$^{2+}$ channel (termed β2a mice).8 This approach increases Ca$^{2+}$ influx and myocyte contractility26-27 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 β2a 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 β2a 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

β2a-LTCC Hearts Are Hypercontractile

Mice with conditional, cardiac-specific, low-level expression of Cav1.2 β2a were used (Figure 1A). A modified α-myosin heavy chain promoter was used for cardiac-specific expression of the β2a gene, and mice containing both tTA (tetracycline-controlled transactivator) and β2a transgenes (double transgenic) allowed for doxycycline (Dox) suppression of β2a 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 β2a gene was fully expressed and mice showed enhanced cardiac performance (Figure 1B), with no signs of cardiomyopathy.8 β2a 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 β2a animals (Figure 1E, 1F, and 1G). These data show that β2a hearts were hypercontractile with modest LV hypertrophy (Figure 1H).

Infarct Size Is Increased in β2a 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 β2a hearts (area at risk [AAR]; control versus β2a: 45.1±3.2% versus 50.4±6.8%; Figure 2A; Online Figure I, A). However, myocardial infarct size (as a % of AAR) was significantly larger in β2a mice than in controls (control versus β2a: 45.3±6.1% versus 83.8±14.4%) (Figure 2B; Online Figure I, B). Infarction length measured 6 weeks after MI was also significantly greater in β2a than in control mice (control versus β2a: 21.7±4.9% versus 45.8±6.8%) (Figure

Non-standard Abbreviations and Acronyms

| AAR | area at risk |
| β2a | β2a 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 |

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, 

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,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 β2a subunit of the L-type Ca$^{2+}$ channel (termed β2a mice).8 This approach increases Ca$^{2+}$ influx and myocyte contractility26-27 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 β2a 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.
2C; Online Figure I, C). These data suggest that more myocytes in the AAR die in β2a 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 β2a hearts 3 days (β2a versus WT: 59,015 versus 16,600 in border and 3407 versus 1984 labeled/106 nuclei in the remote zone) and 3-week after MI (959 versus 647 in border and 1415 versus 709 labeled/106 nuclei in remote zone) (Figure 2D through 2F; and Online Figure II, A and B). Caspase 3 activity was also greater in β2a 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 β2a 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 β2a versus WT hearts (Figure 1).

Figure 2. MI and ischemia/reperfusion (I/R) causes more cell death in β2a hearts. A, AAR was not different in control (n=15) and β2a 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 β2a (n=6) than in control hearts (n=8). C, Infarct length after permanent occlusion was greater in β2a (n=6) than in controls (n=7) and. D through F, Myocytes per 10⁶ undergoing apoptosis in the border and remote zones of control (n=3) and β2a hearts (n=4). G, Caspase 3 activity in remote zone tissues from control (n=9; β2a, n=10). H, Fibrotic area (blue) in remote zones of trichrome-stained cardiac histological sections from control (n=3) and β2a hearts (n=3). I, Ki67+ myocytes per 10⁶ in control (n=3) and β2a hearts (n=4) after MI. *P<0.05; **P<0.01.
after acute (4223 versus 2621 labeled/10^6) or 3 weeks MI (1974 versus 1542 labeled/10^6) (Figure 2f; Online Figure II, C). Collectively these results show that there is an increased rate of death of β2a myocytes after MI stress, both at the infarct border zone (larger infarct size) and in remote areas of the heart.

**β2a Hearts Have Depressed Function After Ischemia/Reperfusion Stress**

Our MI experiments suggest that the β2a 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 dP/dT (mm Hg/s) in β2a 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 β2a hearts versus control, suggesting they develop Ca^{2+} overload-related increases in diastolic pressure (Figure 3B through 3C). β2a hearts also had greater disruption of contractile function after reperfusion (Figure 3B). Recovery of LVDP was significantly reduced in β2a versus control hearts (Figure 3B) and the EDP was significantly greater in β2a hearts than in control hearts (Figure 3D). The increased EDP and reduced LVDP are consistent with enhanced Ca^{2+} stress in the β2a that results in SR Ca^{2+} overload.26 These results document that β2a hearts are prone to ischemia-related injury, without the neurohormonal stressors present in the post-MI heart.

**More β2a 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 β2a mice after MI surgery are shown in Online Figure I (D). β2a 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 β2a 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 β2a 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 β2a than in control hearts after MI (Figure 4D through 4G). These data show that β2a 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 β2a 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 β2a versus controls (Figures 1 and 5). Importantly, cardiac structure and function were stable in sham β2a 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 β2a 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 β2a and control hearts. Over the next few weeks, FS and EF continued to decrease in β2a 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 \( \beta_{2a} \) 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.

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.
LTCC Current ($I_{\text{Ca,L}}$) Remains Increased in β2a Mice After MI

We measured LTCC currents, cell contractions, and Ca$^{2+}$ transients in myocytes from control and β2a hearts with or without MI. $I_{\text{Ca,L}}$ density was significantly larger in sham β2a versus control myocytes (peak $I_{\text{Ca,L}}$ in β2a 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 $I_{\text{Ca,L}}$ activation was shifted to negative potentials in β2a myocytes, consistent with the known function of this subunit E22.9,30 and with what we have shown previously.8 After MI, $I_{\text{Ca,L}}$ density was decreased in all myocytes but remained significantly larger in β2a than in control myocytes (β2a versus control: $-17.6±1.4$ n=15 versus $-10.4±0.8$ pA/pF n=12 $P<0.05$) (Figure 6A through 6C). $I_{\text{Ca,L}}$ density in β2a after MI was significantly greater than in control myocytes before MI.

$I_{\text{Ca,L}}$ 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 $I_{\text{Ca,L}}$ in myocytes from sham and post-MI hearts. In control (sham) myocytes, Iso increased $I_{\text{Ca,L}}$ density (pre-Iso versus after Iso: $-13.3±1.9$ to $-17.9±3.9$ pA/pF, n=4, $P<0.05$) and caused a significant negative shift in the voltage dependence of $I_{\text{Ca,L}}$ activation. Iso had no significant effect on $I_{\text{Ca,L}}$ in β2a (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 $I_{\text{Ca,L}}$ density was reduced in both control and β2a myocytes (Figure 6). In control MI myocytes Iso increased $I_{\text{Ca,L}}$ 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 $I_{\text{Ca,L}}$ in β2a MI myocytes (pre-Iso versus after Iso: $-17.0±1.3$ versus $-23.9±2.4$ pA/pF, n=5, $P<0.05$) (Figure 7B, 7D, and 7F; and Online Figure V [C, D, and F]), increasing $I_{\text{Ca,L}}$ to values measured in control β2a myocytes. These results show that $I_{\text{Ca,L}}$ in β2a myocytes remains significantly greater than in controls after MI.

Contractions and [Ca$^{2+}$], Transients in β2a Myocytes After MI

We confirmed8 that β2a myocytes have larger contractions and Ca$^{2+}$ transients than controls (Figure 8). FS of β2a was significantly greater than control (sham) myocytes (β2a versus control: 12.1±1.2% n=14 versus 7.8±1.0% n=19 $P<0.05$). After MI, contractions remained significantly greater in β2a than in control myocytes (β2a versus control: 12.3±0.7% n=28 versus 8.2±0.8% n=12 $P<0.05$).

Myocyte contractions in both control and β2a increased with Iso (control sham pre-Iso versus after Iso: 7.8±0.97% versus 10.7±0.70%, n=19, $P<0.05$; β2a sham: 12.1±1.2% versus 14.5±0.9%, n=9, $P<0.05$), with contractions in β2a being greater than in controls. Contractions of MI myocytes were increased by Iso, and again β2a myocytes had greater contractions than controls (post-MI control with or without Iso: 8.2±0.8% versus 10.9±0.95% n=9 $P<0.05$; post-MI β2a with or without Iso: 12.3±0.7% versus 14.9±0.6% n=21 $P<0.05$) (Figure 8A; Online Figure VI, A through D).

[Ca$^{2+}$], transients and SR Ca$^{2+}$ content in β2a (sham) myocytes were significantly greater than in controls (Figure 8B through 8F), consistent with our previous results.8 After MI, [Ca$^{2+}$], transients in control myocytes were decreased, whereas those in β2a myocyte were similar to what was measured under basal conditions (F/F$_0$ ratio in β2a sham versus control sham: 4.3±0.6 versus 3.4±0.2; post-MI β2a 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 β2a myocytes, with Ca$^{2+}$ transient amplitude being significantly greater in β2a myo-
cytes (control [sham]) pre-Iso versus post-Iso: 3.4 versus 4.7 n, P<0.05; post-MI control: 2.9 versus 3.6, n=5, P<0.05; post-MI β2a: 4.3 versus 5.1, n=9, P<0.05).

Collectively, these data show that myocytes from β2a 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 SR Ca\(^{2+}\) overload\(^{26}\) resulting in diastolic and systolic dysfunction and Ca\(^{2+}\)-mediated injury.\(^{34,35}\)

β2a Hearts Have Larger Infarcts

The AAR after myocardial infarction was not different in control and β2a mice; however, infarct size (as a % of AAR) was significantly greater in β2a 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 β2a hearts. The mechanism of enhanced myocyte death is not entirely clear, but we did show an increase in TUNEL\(^+\) myocytes in the β2a 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 β2a 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

**Figure 7. Effects of Iso (1 μmol/L) on I\(_{\text{Ca,L}}\) in sham or post-MI myocytes.** A and B, Current–voltage relationships in sham and MI (β2a and controls) with or without Iso. C, Iso increased peak I\(_{\text{Ca,L}}\) in control (n=4) but not in β2a ventricular myocytes (VMs) (n=7). D, Iso increased I\(_{\text{Ca,L}}\) in both control-MI (n=6) and β2a-MI VMs (n=5). E and F, Voltage dependence of I\(_{\text{Ca,L}}\) activation in sham or post-MI VMs with or without Iso. *P<0.05 between control and β2a VMs; #P<0.05 with or without Iso.
response to maintain cardiac output and systemic blood pressure. Because β2a 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 β2a 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 β2a Hearts After MI**

MI causes cardiac structural remodeling (wall thinning and dilation). 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. Our results show that β2a 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 β2a 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. The afterload (systolic wall stress) in the failing heart is significantly increased and correlates closely with heart failure severity and prognosis. Many groups, including ours, have shown that Ca\(^{2+}\) handling is deranged in failing myocytes and this depresses myocyte contractility reserve. 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. Our experiments showed that apoptotic myocyte death was increased in remote zones of the β2a heart (versus control) after MI (Figure 2 and Online Figure II). We conclude that MI-induced heart failure...
progression in β2a mice results from a vicious cycle of myocyte loss, ventricular dilatation, increased systolic wall stress (and Ca2+ signaling cascades; Online Figure VII), increased sympathetic responses (which preserves myocyte contractility at a very high level in β2a myocytes; Figures 6 through 8), and myocyte death mediated by excessive Ca2+ (and/or sympathetic nervous system) stress.

Could Depressed Myocyte Contractility Be the Effect of Heart Failure?

Ca2+ handling is deranged in heart failure.5 L-type Ca2+ channel density and phosphorylation are altered to limit Ca2+ influx.38 SERCA abundance is reduced and phospholamban phosphorylation is reduced to slow the rate of SR Ca2+ uptake and reduce SR Ca2+ loading.12,42 The phosphorylation of the SR Ca2+ release channel (ryanodine receptor) is abnormal and this leads to arrhythmias and SR Ca2+ leak.43 Collectively, these defects produce a failing heart that must develop high systolic stress with a reduced ability to increase Ca2+ influx and SR Ca2+ uptake and release.

These changes in Ca2+ 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.18 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 Ca2+) induces the deranged Ca2+ handling that reduces contractility reserve.4 Therefore, deranged Ca2+ handling may be the effect of rather than the cause of heart failure progression. Our data suggest that downregulation of Ca2+ handling in the diseased heart might provide some protection from high Ca2+-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, these 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 failure44; therefore, novel therapies are clearly needed. Important, these 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 failure44; therefore, novel therapies are clearly needed.

Sources of Funding

Supported by NIH grants HL089312, HL033921, and HL091799 (to S.R.H.) and HL088243 (to X.C.).

Disclosures

None.

References

Maintaining high Ca\(^{2+}\) and contractility after MI was associated with increased myocyte apoptosis and ventricular dilatation.

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 dilatation and depressed pump function, even though the \(\beta_2\) 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.

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 \(\beta_2\) 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 infarct size.
- Maintaining high levels of contractility with \(\beta_2\) expression was associated with exacerbation of depressed cardiac pump function and cardiac dilatation. This study showed that MI-induced heart failure could be seen in hearts with hypercontractile myocytes.
Increasing Cardiac Contractility After Myocardial Infarction Exacerbates Cardiac Injury and Pump Dysfunction

Hongyu Zhang, Xiongwen Chen, Erhe Gao, Scott M. MacDonnell, Wei Wang, Mikhail Kolpakov, Hiroyuki Nakayama, Xiaoying Zhang, Naser Jaleel, David M. Harris, Yingxin Li, Mingxin Tang, Remus Berretta, Annarosa Leri, Jan Kajstura, Abdelkarim Sabri, Walter J. Koch, Jeffery D. Molkentin and Steven R. Houser

Circ Res. published online July 29, 2010;
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/early/2010/07/29/CIRCRESAHA.110.219220.citation

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2010/07/22/CIRCRESAHA.110.219220.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/
Online Figure I. Area at risk (AAR) and infarction size in hearts subject to I/R or MI. (A). Representative images of area at risk (AAR) in cardiac tissue sections stained with Evans blue. (B). Infarction area measurement in tissue sections stained with TTC from hearts after 30 minutes ischemia and 24 hours reperfusion. (C). Infarct length measurement in cardiac tissue sections stained with TTC from hearts at 6 weeks after MI. (D). Representative hearts and lungs from control and β 2a mice at 6 weeks after MI.
Online Figure II. TUNEL and Ki67 staining in cardiac tissue sections after MI. **A-B.** Representative images of TUNEL+ nuclei in border (A) and remote zone (B) of myocardium in control and β2a hearts. TdT is white, alpha-sarcomeric actin is red and DAPI is blue. (C). Representative Ki67 staining (shown as green) in tissue sections from control and β2a hearts. Scale bar = 10um.
Online Figure III. Baseline level of LVDP (A) and ±dp/dt (B) were greater in β2a isolated hearts (n=5) versus control (n=8). * p< 0.05
**Online Figure IV.** (A). Representative LV B-mode echocardiographic images in a β2a mouse before and 2, 4 weeks after MI. The area between the red arrow heads indicate myocardial infarction zone. (B). Representative LV M-mode echocardiographic images in control and β2a mice before and 2, 4 weeks after MI.
Online Figure V.  (A-B). Representative recordings of ICa,L before and after the application of Iso in sham control and β 2a VMs. (C-D). Representative recordings of ICa,L before and after application of Iso in post-MI control and β 2a VMs. (E). Average half-activation potential (V₀.₅) in sham VMs before and after Iso treatment. (F). Average half-activation potential (V₀.₅) in post-MI VMs before and after Iso treatment.
Online Figure VI. (A-D). Representative recordings of myocyte shortening before and after Iso in sham or post-MI myocytes from control and β 2a hearts. (E-H). Representative recordings of [Ca\(^{2+}\)]_i transients before and after Iso in sham or post-MI VMs from control and β 2a hearts.
**Online Figure VII.** Western Blot analysis of PLBt and PLB<sub>p</sub>Thr17 protein levels in control and β 2a hearts after 3 weeks MI. (A). Representative western blot of PLBt and PLB<sub>p</sub>Thr17 protein from control and β 2a hearts. (B). Analysis of abundance of PLBt and PLB<sub>p</sub>Thr17 protein levels normalized to GAPDH from control (n=3) and β 2a hearts (n=3). *p < 0.05.
**Detailed Methods:**

**Animals and Coronary Artery Ligation:** A transgenic mouse line with cardiac specific, conditional, low level expression of the β2a subunit of the LTCC was used\(^1\). Experiments were performed following the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all procedures were approved by the Institutional Animal Care and Use Committee at Temple University. Myocardial infarction (MI) surgeries were carried out in mice at the age of 4 months when the β2a gene is fully expressed\(^1\). Mice were anesthetized with inhalation of 2% isoflurane. A skin incision was made over the left thorax, and the pectoral muscles were retracted to expose the ribs. At the level of the fifth intercostal space, the heart was exposed. A permanent knot or a slipknot (to allow for reperfusion) was made around the left anterior descending coronary artery (LAD) 2–3 mm from its origin with a 6-0 silk suture. After the knot was tied the lung were expanded and muscle and the skin layers were closed. Sham-operated animals were subjected to the same surgical procedures except that the suture was passed under the LAD but was not tied\(^2\). To induce ischemia/reperfusion injury and determine infarction size the LAD was occluded for 30 min and then the heart was reperfused for 24 hours.

**Determination of LV area at risk and infarct size after I/R or MI:** Area at risk (AAR) was measured by injecting 0.2 ml of 2% Evans blue dye into the right ventricle before the heart was excised. Areas of the heart with normal blood flow stained blue. The stained heart was quickly removed and frozen and placed on dry ice. Then the heart was cut into eight 1.0-mm-thick sections perpendicular to the long axis of the heart. The sections were incubated in PBS at room temperature for 15 min and then digitally photographed. The blue areas (area not at risk, ANAR), and non blue areas (AAR) were measured with the NIH ImageJ software in at least 7 hearts of each group and the percentage of AAR was calculated (AAR/(AAR+ANAR)).

To evaluate infarction size, viable tissue was stained in frozen hearts excised after 24 hours of reperfusion. The sections were incubated in PBS containing 2% triphenyltetrazolium chloride (TTC; Sigma) at room temperature for 15 min and digitally photographed. TTC-negative staining area (infarcted myocardium) was measured and normalized by total myocardial section area. Myocardial infarct size was calculated using normalized infarcted area (TTC-negative staining area) divided by the average AAR determined in other hearts from the same group.

In permanent ischemia studies, only TTC was used to stain viable tissue, and non-stained scar tissue was measured as infarct length, which was expressed as the percentage of total circumference of LV tissue sections.

**In-vivo functional analysis (Echocardiography, ECHO):** ECHO was performed (VisualSonics Vevo 770) as described in our previous studies\(^3\). Mice were anesthetized with 2% isoflurane initially and then 1% during the ECHO procedure to maintain the heart rate between 400 and 450 bpm. Hearts were viewed in the short-axis between the two papillary muscles and analyzed in M-mode. Parameters (Vevo software) including end-diastolic diameter (EDD), end-systolic diameter (ESD), posterior wall thickness (PWT), and septal wall thickness (SWT) were measured to determine changes in cardiac morphology and function (ejection fraction (EF) and fractional shortening (FS)).

**Ex-vivo functional analysis before and after ischemia (Langendorff):** Hearts were placed on a Langendorff apparatus (ML785B2, ADInstruments, Colorado Springs, Colo), paced at 8.0 Hz, and perfused at a constant pressure of 80 mm Hg (STH pump controller ML175, ADInstruments) with a modified Krebs–Henseleit buffer solution containing (in mmol/L): 2.0 CaCl\(_2\), 130 NaCl, 5.4 KCl, 11 glucose, 2 pyruvate, 0.5 MgCl\(_2\), 0.5 NaH\(_2\)PO\(_4\), and 25 NaHCO\(_3\) and aerated with 95% oxygen and 5% carbon dioxide, pH 7.35 to 7.4. The temperature was maintained at 37°C by immersing the heart in a water-heated glassware reservoir containing
preheated KHB. A water-filled balloon was inserted into the left ventricle and adjusted to achieve a left ventricular end-diastolic pressure (LVEDP) of 10 mm Hg. After a 20-min equilibration period, hearts are subjected to 15 min of no flow global ischemia, followed by 30 min of reperfusion. The LVEDP, left ventricular developed pressure (LVDP) (peak systolic pressure minus LVEDP), maximum rate of contraction (+dP/dt), and maximum rate of relaxation (−dP/dt) were recorded continuously by a data acquisition system (Powerlab/8SP, ADInstruments).

**Histology:** Animals were anesthetized with sodium pentobarbital (120mg/kg BW, intraperitoneal injection) and heparinized intravenously. Hearts were excised, trimmed of excess tissue, weighed, rinsed and perfused with Ca²⁺-free KHB to to remove blood from blood vessels and then perfused with 10% buffered formalin. The fixed heart tissues were dehydrated, embedded in paraffin, sectioned at 5-μm thickness, and stained with hematoxylin/eosin for cross-sectional area measurement. Myocyte cross-sectional area was measured from sections of non-infarct zone obtained mid-distance between the base and apex. Suitable cross-sections were defined as having nearly circular-to-oval myocyte contour. The perimeter of 100–200 myocytes was traced in sections from 5 regions of the LV of each animal, using NIH Image J software system. The mean area was calculated for all the regions measured in control and β2a heart tissue sections. Masson’s trichrome staining was used to evaluate gross morphology, fiber integrity and interstitial fibrosis. Images were acquired using SPOTINSIGHT software (Diagnostic Instruments Ins.). 3 fields were studied from each tissue section per heart and blue area (interstitial fibrosis) were analyzed using Metamorph 6.1 software (Universal Image Corp.). Average blue area percentage was calculated in at least 3 different hearts.

Apoptotic cell death was detected in situ by TUNEL on paraffin sections of mouse hearts. After the TUNEL procedure, DAPI was used for the staining of all nuclei and slides were observed under a fluorescence microscope. The mean number of positive nuclei and DAPI-stained nuclei per 40X field in LV regions was determined by manual counting. A minimum of 5 sections from 5 LV regions (ischemic border zone and remote region of the LV) of each heart were examined for TUNEL-positive cells. Approach from Dr. kajstura lab for TUNEL assay: sections were incubated in a solution containing 5 U TdT, 2.5 mM CoCl₂, 0.2 M cacodylate, 25 mM Tris-HCl, 0.25% BSA, and 0.5 nM of biotin-16-dUTP (Roche Biochemicals). dUTP was detected by exposing samples to 5 μg/ml of FITC-avidin dissolved in 4X concentrated saline-sodium citrate buffer containing 0.1% Triton X-100. Myocyte cytoplasm was identified by α-sarcomeric actin antibody. Nuclei were stained with 10 μg/ml of propidium iodide (PI) 6-7. *Ki67 labeling:* Sections were incubated with Ki67 rabbit polyclonal antibody (Beckman Coulter) and FITC-conjugated anti-rabbit IgG (Sigma). Myocyte cytoplasm was recognized by α-sarcomeric actin antibody and nuclei were identified by propidium iodide. Sections were evaluated by confocal microscopy.

**Western Blot analysis:** Western blotting: Cytoplasmic and membrane protein were isolated from ventricular tissue using PBS lysis buffer containing: 0.5% Triton X-100, 5 mM EDTA (pH7.4), phosphatase inhibitors (10 mM NaF and 0.1 mM NaVO₄), proteinase inhibitors (10 μg/ml aprotinin, 10 μg/ml leupeptin, 1mM phenylmethylsulfonyl fluoride, 5 μg/ml pepstatin A, 8 μg/ml calpain inhibitor I & II, and 200 μg/ml benzamidine). Cardiac actin was isolated from resulting pellet using PBS lysis buffer containing 2% sodium dodecyl sulfate, SDS (FisherBiotech), 1% IGEPAL CA-630 (Sigma), 0.5 % deoxycholate (Sigma), 5 mM EDTA (pH 7.4), and proteinase inhibitors. Protein abundance and phosphorylation levels in isolated protein were analyzed with Western blot analysis as described previously 8. Target antigens were probed with the following antibodies: phospholamban (PLB) (Upstate Biotechnology), GAPDH (Serotec) and PT17-PLB (Badrilla).
**Caspase Assay:** Caspase activity was measured with Caspase assay system (Promega, Madison, WI). In brief, LV lysates were prepared by dounce homogenization in lysis buffer provided with the kit. The lysates were centrifuged at 15,000g for 20 minutes at 4°C, and the supernatants containing 100μg protein were used for caspase activity assay using specific fluorogenic conjugated substrate MCA-Val-Asp-Gln-Met-Asp-Gly-Trp-Lys-(DNP)-NH2.

**Cellular functional analysis:** Myocytes were isolated from hearts 2-3 weeks after MI to measure cellular fractional shortening (FS), Ca\(^{2+}\) transients ([Ca\(^{2+}\)]), and L-type calcium current (I\(_{\text{CaL}}\)). All experiments were done at 35-37°C, in superfused myocyte chambers mounted on fluorescence-capable microscopes. All myocytes were characterized with the same series of experiments.

To isolate myocytes, mice were anesthetized with sodium pentobarbital (120mg/1 kg BW). The heart was excised, weighed, cannulated and perfused retrogradely on a constant-flow Langendorff apparatus. The heart was digested by retrograde perfusion with normal Tyrode solution containing 180 U/mL collagenase and (in mM): 0.02 CaCl\(_2\), 10 glucose, 5 HEPES, 5.4 KCl, 1.2 MgCl\(_2\), 150 NaCl, 2 sodium pyruvate, pH 7.4. When the heart was softened, left ventricular tissue was gently minced and myocytes were dissociated by gentle suction with a transfer pipette. Myocytes were filtered and equilibrated in Tyrode solution with 0.2 mM CaCl\(_2\), and 1% bovine serum albumin (BSA) at room temperature. Our initial yield was >90% rod-shaped VMs, and >80% calcium-tolerant, rod-shaped VMS survive by the end of the isolation.

**Electrophysiology:** I\(_{\text{CaL}}\) was measured in a sodium-free and potassium-free solution. Isolated myocytes were placed in a chamber mounted on an inverted microscope (Nikon Diaphot) and perfused with 1 mM Ca\(^{2+}\) Tyrode solution. Both the inflow solution and the chamber were water-heated to maintain the temperature at 36±1°C. A 4-5 MΩ pipette filled with a Cs\(^{+}\)-containing solution [composition in mM: 130 Cs-aspartate, 10 N-methyl-D-glucamine (NMDG), 20 tetraethylammonium chloride, 10 HEPES, 2.5 Tris-ATP, 1 MgCl\(_2\), and 10 EGTA, pH 7.2] was used to obtain gigaseals. Once a gigaseal was formed, the patch was ruptured and the cell was dialyzed for 10 min. The extracellular bath was then changed to a 2 mM Ca\(^{2+}\)-containing Cs\(^{+}\) substitution bath solution (composition in mM: 2 4-aminopyridine, 2 CaCl\(_2\), 5.4 CsCl, 10 glucose, 5 HEPES, 1.2 MgCl\(_2\), and 150 NMDG, pH 7.4 with CsOH). Membrane voltage was controlled by an Axopatch 2B voltage-clamp amplifier and the current was digitized by Digidata 1200 using pClamp8 software (Molecular Devices). Once the signal was converted to digital format, it was stored on a personal computer for off-line analysis with Clampfit 9 (Axon Instruments).

**Contraction and Ca\(^{2+}\) transients:** Fractional shortening was measured in myocytes incubated with Tyrode solution containing 1 mM Ca\(^{2+}\) at rates of 0.5 Hz stimulation with edge detection. [Ca\(^{2+}\)] were measured in paced myocytes loaded with Fluo-4AM as described in previous studies.

**Statistics:** Data were reported as mean±SEM. When appropriate, paired and unpaired T-test, ANOVA or ANOVA for repeated measures were used to detect significance with SAS 9.0 (SAS Institute Inc.). A p value of ≤0.05 was considered significant.
References:


3. Jaleel N, Nakayama H, Chen X, Kubo H, MacDonnell S, Zhang H, Berretta R, Robbins J, Cribbs L, Molkentin JD, Houser SR. Ca2+ influx through t- and l-type Ca2+ channels have different effects on myocyte contractility and induce unique cardiac phenotypes. *Circ Res.* 2008;103:1109-1119


