Contractile Reserve and Intracellular Calcium Regulation in Mouse Myocytes From Normal and Hypertrophied Failing Hearts

Kenta Ito, Xinhua Yan, Minori Tajima, Zhi Su, William H. Barry, Beverly H. Lorell

Abstract—Mouse myocyte contractility and the changes induced by pressure overload are not fully understood. We studied contractile reserve in isolated left ventricular myocytes from mice with ascending aortic stenosis (AS) during compensatory hypertrophy (4-week AS) and the later stage of early failure (7-week AS) and from control mice. Myocyte contraction and \([Ca^{2+}]_o\), transients with fluo-3 were measured simultaneously. At baseline (0.5 Hz, 1.5 mmol/L \([Ca^{2+}]_o\), 25°C), the amplitude of myocyte shortening and peak-systolic \([Ca^{2+}]_i\), in 7-week AS were not different from those of controls, whereas contraction, relaxation, and the decline of \([Ca^{2+}]_i\), transients were slower. In response to the challenge of high \([Ca^{2+}]_o\), fractional cell shortening was severely depressed with reduced peak-systolic \([Ca^{2+}]_i\), in 7-week AS compared with controls. In response to rapid pacing stimulation, cell shortening and peak-systolic \([Ca^{2+}]_i\), increased in controls, but this response was depressed in 7-week AS. In contrast, the responses to both challenge with high \([Ca^{2+}]_o\), and rapid pacing in 4-week AS were similar to those of controls. Although protein levels of Na\(^-\)Ca\(^+\) exchanger were increased in both 4-week and 7-week AS, the ratio of SR \(Ca^{2+}\)-ATPase to phospholamban protein levels was depressed in 7-week AS compared with controls but not in 4-week AS. This was associated with an impaired capacity to increase sarcoplasmic reticulum \(Ca^{2+}\) load during high work states in 7-week AS myocytes. In hypertrophied failing mouse myocytes, depressed contractile reserve is related to an impaired augmentation of systolic \([Ca^{2+}]_i\), and SR \(Ca^{2+}\) load and simulates findings in human failing myocytes. (Circ Res. 2000;87:588-595.)

Key Words: myocytes ■ contractility ■ sarcoplasmic reticulum ■ \(Ca^{2+}\)-ATPase ■ heart failure ■ hypertrophy

The regulation of contractility is under intense investigation with the use of transgenic mice.\(^1\)\(^,\)\(^2\) The mouse cardiovascular system differs from that of humans and larger mammals, including the rapid heart rate, faster myofibrillar ATPase activity and sarcoplasmic reticulum (SR) \(Ca^{2+}\) uptake,\(^2\)\(^,\)\(^3\) and higher mechanical performance per unit ventricular mass.\(^4\) To interpret findings in genetically manipulated mice, it is critical to understand the properties of normal mouse myocyte contractility and the changes induced by clinically relevant stimuli such as chronic load. We reported that mice with ascending aortic stenosis (AS) develop compensated hypertrophy (4-week AS) and the later stage of early heart failure (7-week AS).\(^4\)\(^,\)\(^5\) The aim of the present study was to examine contractile reserve in myocytes from AS mice in transition from hypertrophy to early heart failure. We measured myocyte contraction and \([Ca^{2+}]_i\), transients in left ventricular (LV) myocytes from normal, 4-week AS, and 7-week AS mice in response to the challenge of stepped increases in \([Ca^{2+}]_o\). In separate experiments, we examined the response to rapid pacing stimulation. Although only subtle abnormalities in the time course of contraction and the \([Ca^{2+}]_i\), transients are present at baseline, contractile reserve is depressed in myocytes from 7-week AS mice at the stage of transition to early failure. This is related to a deficit in the capacity to increase SR \(Ca^{2+}\) load at high work states.

Materials and Methods

Animal Model of Pressure Overload

Ascending AS surgery was performed in male FVB/n mice as described previously.\(^4\)\(^,\)\(^6\) Mice were studied at either 4 weeks (4-week AS) or 7 weeks (7-week AS) after the surgery, and age-matched animals served as controls (n=30 to 35 per group).

Simultaneous Measurement of \([Ca^{2+}]_i\), and Cell Motion

Myocyte contraction and \([Ca^{2+}]_i\), measured with fluo-3 were monitored simultaneously in LV myocytes. The calibration procedure of \([Ca^{2+}]_i\), is described in the online-only Materials and Methods (available in an online-only data supplement at http://www.circresaha.org). Myocyte cell area was calculated with NIH Image software (version 1.60, NIH) at the end-diastolic phase.
Experimental Protocols

Myocytes were superfused with HEPES-buffered solution of the following composition (in mmol/L): NaCl 137, KCl 3.7, MgCl2 0.5, HEPES (free acid) 4.0, CaCl2 1.5, glucose 5.6, and probenecid 0.5, with final pH 7.40 at 25°C. At baseline, myocytes were paced with field stimulation at 0.5 Hz with 1.5 mmol/L [Ca2+]i, at 25°C. To study contractile reserve at high work states, the [Ca2+]i was increased to 2.5 and 4.0 mmol/L. Measurements were made after 3 minutes at each level of elevated [Ca2+]i. In separate experiments, the pacing frequency was increased to 1, 2, 3, 4, and 5 Hz. Measurements were made after 1 minute at each stimulation frequency.

Assessment of SR Ca2+ Load

The Ca2+ content of the SR was assessed through the rapid application of caffeine (10 mmol/L) with a rapid solution switcher. In voltage-clamped cells, SR Ca2+ content was determined by measuring the integral of the caffeine-induced inward I_{Ca,ov} in separate experiments, in unclamped cells loaded with fluo-3, the peak of the [Ca2+]i transient induced by 0 Na+/0 Ca2+ solution with caffeine was used as an index of the SR Ca2+ load. At baseline, similar results were obtained with both methods, and the latter approach was used to measure SR Ca2+ load at high work states.

Gene Expression and Protein Levels in LV Tissue

Total RNA was extracted from LV tissue and purified with TRI Reagent (Sigma Chemical Co). Northern blots were performed as previously described to assess mRNA levels of β-myosin heavy chain, atrial natriuretic peptide, and SR Ca2+-ATPase (SERCA2).4,10 For Western blot analyses, LV tissue was frozen and stored at −80°C until use. Western blotting was performed to assess protein levels of phospholamban (PLB), the Na+/Ca2+ exchanger, and SERCA2 and were normalized to GAPDH. 10

SERCA2 Protein Levels in Myocytes

In addition, freshly dissociated LV myocytes were homogenized in buffer containing 20 mmol/L Tris-HCl, 20 mmol/L NaCl, 0.1 mmol/L EDTA, 0.1% Triton X-100, and 0.5% deoxycholate. Fifty micrograms of protein was loaded onto a 10% SDS gel and then transferred to a nitrocellulose membrane. The filter was incubated with anti-SERCA2a antibody (Affinity Bioreagents, Inc). After incubation with anti-mouse HRP (Sigma Chemical Co), blots were normalized to protein levels of cyclophilin A (Upstate Biotechnology Inc).

Statistical Analysis

Values are expressed as mean±SEM. Comparisons among the groups were analyzed by ANOVA followed by a post hoc test. Two-way ANOVA with repeated measures was used to compare the values measured in the groups in response to the stepped increases in [Ca2+]i, and the increases in pacing frequency. Statistical significance was accepted at the level of P<0.05. An expanded Materials and Methods section can be found in an online data supplement available at http://www.circresaha.org.

Results

The ratio of LV to body weight was significantly higher in AS mice than in age-matched control mice (4-week AS 5.4±0.2 mg/g, control 3.1±0.1 mg/g, P<0.0005; 7-week AS 4.7±0.2 mg/g, control 2.8±0.1 mg/g, P<0.0005, n=10 hearts per group). The Table shows the baseline characteristics of myocyte size, contraction, and the [Ca2+]i, transients from 4-week AS, 7-week AS, and control mice. Because there was no significant difference in the baseline parameters and the responses to high [Ca2+]i, and rapid pacing stimulation between 4-week control myocytes (n=16) and 7-week control myocytes (n=19), the data from all control myocytes were pooled for the analyses. Myocyte area was increased in 4-week AS (n=24) and 7-week AS (n=30) myocytes compared with controls (n=35), with similar diastolic cell length. Fractional cell shortening and levels of peak-systolic and end-diastolic [Ca2+]i were similar in all 3 groups; however, time to peak shortening, 50% relengthening, and 50% decline in [Ca2+]i, were longer in 7-week AS than in control myocytes. Signal-averaged tracings of cell motion and [Ca2+]i transients, which illustrate these subtle differences between 7-week AS and control myocytes, are shown in Figure 1.

Contractile reserve at high work state was studied by increasing [Ca2+]i, to 2.5 and 4.0 mmol/L at a constant pacing frequency of 0.5 Hz. Representative tracings from control and 7-week AS myocytes are shown in Figure 1 online (available in an online-only data supplement at http://www.circresaha.org). Diastolic cell length decreased slightly, but there were no differences among the groups (Figure 2A). In control and 4-week AS myocytes, both fractional cell shortening and peak-systolic [Ca2+]i, increased in response to the increased [Ca2+]i, (Figures 2B and 2C). In contrast, in 7-week AS myocytes, fractional cell shortening was severely depressed compared with controls (7.9±1.1% versus 12.2±0.9% at 4.0 mmol/L [Ca2+]i, P<0.001, Figure 2B) in association with reduced peak-systolic [Ca2+]i, (422±41 versus 666±56

### Baseline Characteristics of Myocyte Contraction and the [Ca2+]i Transients

<table>
<thead>
<tr>
<th></th>
<th>Control Mice (n=35)</th>
<th>4-wk AS Mice (n=24)</th>
<th>7-wk AS Mice (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocyte area, μm²</td>
<td>2826±149</td>
<td>3769±230*</td>
<td>3580±200*</td>
</tr>
<tr>
<td>Diastolic cell length, μm</td>
<td>115±3</td>
<td>122±4</td>
<td>119±4</td>
</tr>
<tr>
<td>Fractional cell shortening, %</td>
<td>4.5±0.4</td>
<td>5.1±0.4</td>
<td>5.1±0.7</td>
</tr>
<tr>
<td>Time to peak shortening, ms</td>
<td>120±4</td>
<td>128±6</td>
<td>139±6*</td>
</tr>
<tr>
<td>Time to 50% relengthening, ms</td>
<td>76±5</td>
<td>81±5</td>
<td>95±8†</td>
</tr>
<tr>
<td>Peak-systolic [Ca2+]i, nmol/L</td>
<td>367±16</td>
<td>346±15</td>
<td>351±20</td>
</tr>
<tr>
<td>End-diastolic [Ca2+]i, nmol/L</td>
<td>99±4</td>
<td>90±5</td>
<td>99±6</td>
</tr>
<tr>
<td>Amplitude of [Ca2+]i transients, nmol/L</td>
<td>268±15</td>
<td>257±17</td>
<td>252±19</td>
</tr>
<tr>
<td>Time to peak [Ca2+]i, ms</td>
<td>44±1</td>
<td>47±3</td>
<td>49±3</td>
</tr>
<tr>
<td>Time to 50% decline in [Ca2+]i, ms</td>
<td>110±3</td>
<td>115±4</td>
<td>122±6†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *P<0.01, †P<0.05 vs controls.
Because the amount of Ca\(^{2+}\) release from the SR is affected by SR Ca\(^{2+}\) content,\(^{6,11}\) SR Ca\(^{2+}\) load was measured in 7-week AS and controls (Figure 4). SR Ca\(^{2+}\) load was similar in 7-week AS and controls under baseline conditions in either voltage-clamped cells, in which the integral (nA · ms=pC) of the caffeine-induced inward \(I_{\text{Ca,v}}\), normalized by cell capacitance was measured\(^{8}\) (0.87±0.11 versus 0.89±0.07 pC/pF) or unclamped fluo-3–loaded cells, in which the peak of the [Ca\(^{2+}\)], transient induced by 0 Na\(^+\)/0 Ca\(^{2+}\) solution with caffeine was measured\(^{8}\) (627±54 versus 666±55 nmol/L). The latter method was then used to measure SR Ca\(^{2+}\) load in response to the challenge of high [Ca\(^{2+}\)], as well as rapid pacing (n=8 to 13 myocytes per group). In response to the stimulus of high [Ca\(^{2+}\)], (3.0 mmol/L), SR Ca\(^{2+}\) load was lower in 7-week AS than in controls (665±58 versus 956±52 nmol/L, \(P<0.005\)). In response to rapid pacing (3 Hz), SR Ca\(^{2+}\) load was lower in 7-week AS (761±73 versus 1003±91 nmol/L, \(P<0.05\)). These experiments suggest that the impaired augmentation of peak-systolic [Ca\(^{2+}\)], in 7-week AS myocytes is in part attributed to impaired SR Ca\(^{2+}\) loading under high work states.

LV message levels of \(\beta\)-myosin heavy chain (\(\beta\)-MHC) and atrial natriuretic peptide (ANP) are increased in both 4-week and 7-week AS mice compared with control mice, whereas mRNA levels of SERCA2 are increased in 4-week AS mice relative to control mice (1.68±0.07 versus 1.38±0.06 densitometric units, \(P<0.01\)). On the other hand, SERCA2 mRNA levels are depressed in 7-week AS mice compared with control mice (0.91±0.10 versus 1.29±0.04 densitometric units, \(P<0.001\), Figure 5A). The protein levels of SERCA2 in isolated LV myocytes are also reduced in 7-week AS mice compared with control mice (1.44±0.25 versus 2.28±0.06 densitometric units, \(P<0.05\), Figure 5B), whereas the SERCA2 protein levels in myocytes from 4-week AS mice are similar to those from control mice (data not shown).

In additional animals, Western blots of SERCA2, PLB, and Na\(^+\)/Ca\(^{2+}\) exchanger in LV tissue were performed. As shown in Figure 6, the protein levels of the Na\(^+\)/Ca\(^{2+}\) exchanger and PLB are increased both in 4-week and 7-week AS compared with age-matched controls. The SERCA2-to-PLB protein ratio is depressed in 7-week AS compared with controls (0.49±0.02 versus 1.04±0.08, \(P<0.0005\)), whereas the ratio is similar in 4-week AS and controls (1.06±0.28 versus 1.10±0.17, Figure 6C).

Discussion

This is the first report that characterizes normal mouse myocyte contractility and [Ca\(^{2+}\)], transients in compensated hypertrophy and the later stage of early heart failure. In hypertrophied myocytes from 7-week AS mice with early failure, contractile reserve is depressed in response to the elevation of [Ca\(^{2+}\)], as well as the increase in pacing frequency. This depressed contractile reserve is related to impaired augmentation of peak-systolic [Ca\(^{2+}\)], and SR Ca\(^{2+}\) load during these high work states. This is associated with a reduced SERCA2-to-PLB ratio, which is not observed at an earlier stage of hypertrophy.
Contractility in Normal Mouse Myocytes

Normal mouse myocytes exhibit a positive frequency-shortening relationship at >1 Hz, as well as a robust increase in contractility in response to the elevation of [Ca^{2+}]_i. Using mouse trabeculae, Gao et al.\textsuperscript{12} observed a similar response to the elevation of [Ca^{2+}]_o and a positive force-frequency relationship in the range of 0.2 to 4 Hz at room temperature. Our observations are also consistent with the biphasic shortening-frequency response reported by Borzak et al.\textsuperscript{13} who observed a negative staircase at <1 Hz and a positive staircase at >1 Hz in rat myocytes. This biphasic frequency response is not usually observed in muscle preparations of larger mammals, including humans, and may be related in part to species differences in the relative contribution of SERCA2 and Na^{+}-Ca^{2+} exchanger for Ca^{2+} homeostasis.\textsuperscript{14,15}

Contractile Reserve in Hypertrophied and Failing Mouse Myocytes

We characterized the mouse model of chronic pressure overload due to ascending AS and observed that both 4-week and 7-week AS mice develop severe concentric hypertrophy relative to control mice. However, 4-week AS mice exhibit normal indices of systolic performance in vivo, whereas 7-week AS mice show a depression of LV endocardial and midwall shortening and LV systolic pressure development.\textsuperscript{4,5} Human hemodynamic studies have shown that changes in LV geometry with concentric remodeling may partially mask intrinsic changes in contractile function.\textsuperscript{16} We also observed abnormal cell–cell and cell–matrix connectivity in 7-week AS mice.\textsuperscript{5} These factors could confound an assessment of contractile function in vivo or in isolated muscle preparations. Therefore, we examined contractile function in isolated LV hypertrophied myocytes from this model.

In contemporary studies of mouse myocyte contractility, it is common for conditions of low frequency stimulation and room temperature in isolated myocytes\textsuperscript{8} and muscle preparations\textsuperscript{12} to enhance stability. Increases in temperature accelerate all Ca^{2+} transport systems, although the relative contributions remain the same.\textsuperscript{17,18} Under the baseline conditions of low workload in the present experiments, only subtle differences in the kinetics of contraction between normal and hypertrophied mouse myocytes are present. However, under a high work state induced by the increase in perfusate Ca^{2+} concentration, myocyte shortening is severely depressed in 7-week AS myocytes but not in 4-week AS myocytes, in association with the impaired augmentation of peak-systolic [Ca^{2+}]_i. Reduced levels of peak-systolic [Ca^{2+}]_i, as well as reduced rates of myocyte shortening and relengthening, have also been observed in myocytes from several animal models of heart failure,\textsuperscript{19–22} as well as in muscle and myocyte

Figure 2. Ca^{2+}-dependent contractile reserve. A, Relationships between [Ca^{2+}]_o and diastolic cell length expressed as percent of baseline value. B, Relationships between [Ca^{2+}]_o and fractional cell shortening. C, Relationships between [Ca^{2+}]_o and peak-systolic [Ca^{2+}]_i. D, Relationships between peak-systolic [Ca^{2+}]_i and fractional cell shortening. The 7-week AS myocytes fail to augment peak-systolic [Ca^{2+}]_i in response to the elevation of perfusate Ca^{2+} compared with controls.
preparations from patients with end-stage dilated cardiomyopathy. However, no data are yet available that characterize Ca\textsuperscript{2+}-dependent contractile reserve from myocytes or muscle preparations from humans with adaptive hypertrophy or early heart failure. In the present study, myofilament sensitivity to Ca\textsuperscript{2+} was not measured. However, over the range of [Ca\textsuperscript{2+}]o used in our experiments, the relationship between myocyte shortening and peak-systolic [Ca\textsuperscript{2+}], in hypertrophied myocytes is similar to that in controls (Figure 2D), implying that impaired contractile reserve is not predominantly related to an alteration in the responsiveness to Ca\textsuperscript{2+}.

Frequency-dependent contractile reserve is also depressed in 7-week AS myocytes, but not in 4-week AS myocytes, compared with control myocytes. In 7-week AS myocytes, fractional cell shortening is depressed at 5 Hz compared with 0.5 Hz, whereas peak-systolic [Ca\textsuperscript{2+}] tends to be higher at 5 Hz than at 0.5 Hz. This suggests that an alteration in myofilament responsiveness to Ca\textsuperscript{2+}, as well as impaired augmentation of peak-systolic [Ca\textsuperscript{2+}], may contribute to the depressed frequency-dependent contractile reserve in 7-week AS myocytes. Pieske et al\textsuperscript{23} reported that both peak tension and peak-systolic [Ca\textsuperscript{2+}], increase in muscle preparations from nonfailing hearts in response to increases in pacing frequency, whereas both parameters decrease in muscle preparations from end-stage failing dilated cardiomyopathic hearts. Thus, our observations partially simulate the abnormal systolic force-frequency relationship in failing human cardiac muscle preparations.

Factors That Contribute to Impaired Contractile Reserve
The upregulation of \( \beta \)-MHC isoform modifies the extent of shortening and force development as well as its kinetics,\textsuperscript{25,26} and ANP has the potential to depress contractility via the activation of cGMP.\textsuperscript{27} However, these mechanisms are unlikely to underlie our findings, because both \( \beta \)-MHC and ANP are markedly upregulated in both 4-week and 7-week AS. Protein levels of PLB and Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger are also upregulated in both 4-week and 7-week AS. In contrast, SERCA2 protein levels are increased only in 4-week AS mice, not in 7-week AS mice, in comparison with age-matched control mice. Moreover, the SERCA2-to-PLB ratio, which is a major determinant of cardiac contractility and SR function,\textsuperscript{1,28} is decreased only in the 7-week AS mice. These data support the hypothesis that the upregulation of PLB with its inhibitory effects on SR Ca\textsuperscript{2+} uptake, without the concom-
tant increase in SERCA2 protein levels, contributes to the impaired contractile reserve in 7-week AS mouse myocytes at the stage of early failure. However, the effects of PLB on SR Ca$^{2+}$ load cannot be inferred from measurements of message or protein levels, because dynamic alterations in the phosphorylation state modify the effects on the affinity of SERCA2 for Ca$^{2+}$.29–32

Reduced SR Ca$^{2+}$ Loading

Therefore, we measured SR Ca$^{2+}$ load at baseline and during an increase in work state in response to an increase in perfusate Ca$^{2+}$, as well as an increase in stimulation frequency, in 7-week AS and control myocytes. SR Ca$^{2+}$ loading is depressed in 7-week AS myocytes under high work states, although there is no difference under baseline conditions compared with controls. These results are consistent with the report by Pieske et al.,33 who observed an impaired augmentation of SR Ca$^{2+}$ loading only at high pacing frequency in human heart muscle.

In human cardiomyopathy, the upregulation of the Na$^{+}$-Ca$^{2+}$ exchanger partially compensates for downregulation of SERCA2 and protects against the frequency-dependent rise in diastolic force and slowing of force decay.34 In comparison with humans and large mammals, rodents have a higher dependence on SERCA2 relative to Na$^{+}$-Ca$^{2+}$ exchanger in competition for cytosolic Ca$^{2+}$.14,15 In the mouse, relatively high resting intracellular Na$^{+}$ levels also favor SR Ca$^{2+}$ uptake rather than forward Na$^{+}$-Ca$^{2+}$ exchange for Ca$^{2+}$ removal.8 However, in 7-week AS myocytes, where the SERCA2-to-PLB ratio is reduced by 50% and the Na$^{+}$-Ca$^{2+}$ exchanger protein levels are increased by 2-fold, the calcium ions released from the SR could be preferentially removed by the Na$^{+}$-Ca$^{2+}$ exchanger. The increased Ca$^{2+}$ extrusion by the Na$^{+}$-Ca$^{2+}$ exchanger may protect hypertrophied myocytes from diastolic dysfunction; however, the increased Ca$^{2+}$ removal may contribute to the reduction in SR Ca$^{2+}$ load and impaired augmentation of systolic [Ca$^{2+}]_{i}$, during high work states.

These findings do not exclude a contribution of other mechanisms to the reduced levels of systolic [Ca$^{2+}]_{i}$ in the 7-week AS myocytes, including decreased efficacy of Ca$^{2+}$-induced Ca$^{2+}$ release35,36 due to impaired ryanodine channel responsiveness,37 differences in sarcolemmal subspace [Ca$^{2+}]_{s}$,38 or changes in proteins involved in the SR Ca$^{2+}$ release cascade.9,39 SR Ca$^{2+}$ release is sensitive to I$_{Ca,L}$ and SR Ca$^{2+}$ load.6,11 A depressed “gain” in the coupling of SR Ca$^{2+}$ release in response to I$_{Ca,L}$ has been observed in failing rat
myocytes, which has the potential to modify peak-systolic [Ca^{2+}] levels.\textsuperscript{35} Shannon and Bers\textsuperscript{40} reported that fractional SR Ca^{2+} release increases in a nonlinear manner, with the relationship becoming much steeper at higher SR Ca^{2+} content. Thus, any change in SR Ca^{2+} content itself during high work states has the potential to modulate the gain function and the amplitude of the [Ca^{2+}] transient.\textsuperscript{11,36}

**SR Ca^{2+} Load and Hypertrophy**

The effects of pressure overload hypertrophy on SR Ca^{2+} load appear to depend on both the experimental study conditions and the stage of hypertrophy. Shorofsky et al\textsuperscript{41} reported an enhanced SR Ca^{2+} release without alterations in I_{Ca,	ext{L}} density or kinetics, SR function, or the expression of Ca^{2+} cycling proteins in rats with compensatory hypertrophy. Delbridge et al\textsuperscript{42} examined hypertrophied rat myocytes under steady-state voltage-clamped conditions and observed no change in SR Ca^{2+} load or amount of Ca^{2+} released per twitch. Using voltage-clamped myocytes from the same model after field stimulation to load the SR, McCall et al\textsuperscript{48} observed no difference in myocyte SR Ca^{2+} load during a caffeine-induced transient; in this study, fractional SR Ca^{2+} release in response to a given I_{Ca,	ext{L}} trigger was the same under baseline conditions but depressed under a low [Ca^{2+}], of 0.5 mmol/L. In contrast, in this same rat model, SR Ca^{2+} content estimated by rapid cooling contractures was depressed at higher pacing frequency and after long rest intervals.\textsuperscript{43} The present study in normal and hypertrophied mouse myocytes illustrates the importance of an assessment of myocyte contractile function and Ca^{2+} regulation under variable work conditions and at differing stages in the natural history of hypertrophy.

**Acknowledgments**

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**References**


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Materials and Methods

Simultaneous Measurement of [Ca^{2+}]_i and Cell Motion

The LV myocytes were dissociated with the modified method of Wolska et al.\textsuperscript{1} Myocyte contraction and [Ca^{2+}]_i measured with fluo-3 were monitored simultaneously, and the optical systems for fluorescence signal detection and cell motion monitoring are described in detail elsewhere.\textsuperscript{2-4} The calibration of [Ca^{2+}]_i was done as previously reported from our laboratory in rats \textsuperscript{2} with some modification. Briefly, to estimate calibrated levels of the [Ca^{2+}]_i transients, immediately after each experiment the myocyte was superfused with the same buffer supplemented with 30 mmol/L 2,3-butanedione monoxime and 10 μmol/L calcium ionophore ionomycin in the presence of 1 mmol/L calcium. Then a 1-mol/L MnCl\textsubscript{2} stock solution was added to the buffer to yield a final concentration of 10 mmol/L. The cell was abruptly superfused with Mn\textsuperscript{2+} for saturation of fluo-3. After the fluorescence intensity with Mn\textsuperscript{2+} (F\textsubscript{Mn}) was recorded, the intensity of the fluorescence from the field (F\textsubscript{BKG}) was measured by blowing the myocyte away from the field with pipette. In the preliminary study, the autofluorescence of unloaded myocytes (F\textsubscript{AUTO}) was very small compared to the fluo-3 signal and was negligible. After measurement of F\textsubscript{Mn} and F\textsubscript{BKG}, the values of F\textsubscript{max}, F\textsubscript{min}, and estimated [Ca^{2+}]_i were calculated with the following formula reported by Kao et al.\textsuperscript{5}

\begin{align*}
F\textsubscript{max} &= (F\textsubscript{Mn} - F\textsubscript{BKG})/0.2 + F\textsubscript{BKG} \\
F\textsubscript{min} &= (F\textsubscript{max} - F\textsubscript{BKG})/40 + F\textsubscript{BKG} \\
[Ca^{2+}]_i &= K_d x (F - F\textsubscript{min})/(F\textsubscript{max} - F)
\end{align*}
where F is the measured fluorescence intensity, and Kd is the dissociation constant for fluo-3. The Kd of fluo-3 is known to be temperature dependent and is reported to be 400 and 864 nmol/L at 22 °C and 37 °C, respectively. Therefore, we used 493 nmol/L as the Kd at 25 °C, assuming a linear relationship between Kd and temperature. In the present study, baseline values of systolic and diastolic [Ca^{2+}]i and myocyte shortening in normal mouse myocytes were similar to those previously reported in adult mouse myocytes using fluo-3.8-10

References


6. Merritt JE, McCarthy SA, Davies MP, Moores KE. Use of fluo-3 to measure cytosolic Ca^{2+} in platelets and neutrophils. Loading cells with the dye, calibration of traces,


Online-Only Supplementary Information

Figure Legends

Figure 1 online. Representative recordings of myocytes from (A) control mice and (B) 7-week AS mice in response to elevation of perfusate Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]). In the cell motion traces, systolic shortening is shown as an upward deflection.

Figure 2 online. Representative recordings of myocytes from (A) control mice and (B) 7-week AS mice in response to increases in pacing frequency. In the cell motion traces, systolic shortening is shown as an upward deflection.
Figure 1 online
(A) Control

(B) 7-week AS

Figure 2 online