Transgenic Expression of Sarcoplasmic Reticulum Ca\(^{2+}\) ATPase Modifies the Transition From Hypertrophy to Early Heart Failure

Kenta Ito, Xinhua Yan, Xin Feng, Warren J. Manning, Wolfgang H. Dillmann, Beverly H. Lorell

Abstract—To examine the contribution of sarcoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA2a) to early heart failure, we subjected transgenic (TG) mice expressing SERCA2a gene and wild-type (WT) mice to aortic stenosis (AS) for 7 weeks. At an early stage of hypertrophy (4-week AS), in vivo hemodynamic and echocardiographic indices were similar in TG and WT mice. By 7 weeks of AS, which is the stage of early failure in this model, TG mice with AS had lower mortality than WT mice with AS (6.7% versus 29%). The magnitude of left ventricular (LV) hypertrophy was similar in WT and TG 7-week AS mice. In vivo LV systolic function was higher in TG than in WT 7-week AS mice. In LV myocytes loaded with fluo-3, fractional cell shortening and the amplitude of the [Ca\(^{2+}\)]\(_i\) transients were higher in TG than in WT 7-week AS mice under baseline conditions (0.5 Hz, 1.5 mmol/L [Ca\(^{2+}\)]\(_o\), 25°C). The rates of relengthening and decay in [Ca\(^{2+}\)]\(_i\) were faster in TG than in WT 7-week AS myocytes. In myocytes from WT 7-week AS compared with sham-operated WT mice, contractile reserve in response to rapid pacing was depressed with impaired augmentation of both peak-systolic [Ca\(^{2+}\)] and the SR Ca\(^{2+}\) load. In contrast, contractile reserve and the capacity to augment SR Ca\(^{2+}\) load were maintained in TG 7-week AS mice. SERCA2a protein levels were depressed in WT 7-week AS mice, but were preserved in TG 7-week AS mice. These data suggest that defective SR Ca\(^{2+}\) loading contributes to the onset of contractile failure in animals with chronic pressure overload. (Circ Res. 2001;89:422-429.)

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

Sarcoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA2a) plays a pivotal role in intracellular Ca\(^{2+}\) handling in cardiac myocytes, and its expression is decreased in many models of heart failure. We and others have reported that overexpression of SERCA2a using an adenoviral gene transfer technique transiently enhances cardiac contractile function and SR Ca\(^{2+}\) uptake. Enhanced contractility has also been reported in otherwise normal TG mice not subjected to pathological stimuli. However, many short-term interventions that improve cardiac contractility, such as adrenergic stimulation, are associated with adverse long-term effects on survival and progression of heart failure. Therefore, it is controversial whether chronic upregulation of SERCA2a in vivo will favorably modify the development of early heart failure in animals with biomechanical load.

To delineate the contribution of SERCA2a to contractile dysfunction during transition from compensatory hypertrophy to early failure, we subjected TG mice overexpressing the SERCA2a transgene to chronic left ventricular (LV) pressure overload caused by ascending aortic stenosis (AS). We recently reported that mice with AS develop compensated hypertrophy (4-week AS) and the later stage of early heart failure (7-week AS). In vivo LV systolic pressure generation is increased and contractility is preserved in 4-week AS mice, but they are depressed in 7-week AS mice concomitant with a reduction in SERCA2a protein levels. In LV myocytes, contractile reserve and the capacity to increase sarcoplasmic reticulum (SR) Ca\(^{2+}\) load are depressed in 7-week AS mice, but not in 4-week AS mice. In the present study, we show that TG expression of SERCA2a in AS mice, which prevents the fall in SERCA2a levels observed by 7-week AS in wild-type (WT) mice, maintains contractile function and the capacity to increase SR Ca\(^{2+}\) load at high work states. The magnitude of hypertrophy itself is not modified by TG SERCA2a expression. These data suggest that a defective SR Ca\(^{2+}\) loading plays a critical role in the onset of early heart failure in animals with biomechanical overload.

Materials and Methods

Animal Preparation

TG mice overexpressing rat SERCA2a transgene were produced as described previously. AS surgery was performed in the ascending...
aorta of TG and WT mice (weight 18 to 22 g, both sexes) as described previously (n=40 per group). Mice were studied at 4 weeks (TG and WT 4-week AS) or 7 weeks (TG and WT 7-week AS) after surgery. Age-matched animals (TG and WT) underwent a sham operation to serve as controls (n=25 per group).

In Vivo Hemodynamic and Echocardiographic Assessment
In vivo LV pressure hemodynamics were recorded by direct LV catheterization as described by us.11,12 The presence of the ascending aortic constriction precludes LV catheterization by a carotid approach in this model. Two-dimensional guided M-mode echocardiography was performed with conscious sedation on a warming pad after intraperitoneal chloral hydrate (200 mg/kg) injection as described in earlier reports from our laboratory.12,14 In addition to standard analyses of LV wall thickness, cavity size, and endocardial fractional shortening, we used midwall fractional shortening as an index to estimate LV systolic function because endocardial edge dynamics overestimate function in the presence of hypertrophy.14,15

Myocyte Function
Contraction and \([\text{Ca}^{2+}]_{i}\) measured with fluo-3 were monitored simultaneously in LV myocytes as described below. The calibration procedure of \([\text{Ca}^{2+}]_{i}\), is described in the online Materials and Methods (available at http://www.circresaha.org). Myocyte cell area was calculated with NIH Image software (version 1.62, NIH) at the end-diastolic phase. Under baseline conditions, myocytes were paced with field stimulation at 0.5 Hz with 1.5 mmol/L \([\text{Ca}^{2+}]_{i}\)] at 25°C. We chose this temperature because some isolated mouse myocytes exhibit aftercontractions at 37°C that are not observed at 25°C. To study contractile reserve at high work states, the pacing frequency was increased to 1, 2, 3, 4, and 5 Hz with constant \([\text{Ca}^{2+}]_{i}\) of 1.5 mmol/L (n=12 to 17 experiments per group). Measurements were made after 1 minute at each pacing frequency. In separate experiments, the \([\text{Ca}^{2+}]_{i}\) load of the SR was assessed in LV myocytes loaded with fluo-3 by the rapid application of caffeine with a rapid solution switcher (n=8 to 14 experiments per group). Myocytes were paced at 0.5 Hz for 5 minutes for stabilization. After 30 seconds at each frequency of electrical stimulation, myocytes were abruptly exposed to 0 Na⁺/0 Ca²⁺ solution with caffeine (10 mmol/L). The peak of the \([\text{Ca}^{2+}]_{i}\) transient induced by caffeine was used as an index of the SR Ca²⁺ load.16

Protein Levels in LV Myocytes and Tissue
Western blots were performed to assess protein levels of SERCA2a in LV myocytes and protein levels of phospholamban and Na⁺-Ca²⁺ exchanger in LV tissue using anti-SERCA2 antibody, anti-phospholamban antibody, and anti-Na⁺-Ca²⁺ exchanger antibody (Affinity Bioreagents, Inc), and were normalized to GAPDH (n=3 to 4 animals per group).13,17

Statistical Analysis
Values are expressed as mean±SEM. Comparisons among the groups were analyzed using ANOVA followed by a post hoc test using the Dunnett multiple-comparisons test. Two-way ANOVA with repeated measures was used to compare the values measured in the groups in response to the increase in pacing frequency. Statistical significance was accepted at the level of \(P<0.05\).

An expanded Materials and Methods section can be found in the online data supplement available at http://www.circresaha.org.

Results
Mortality and LV Hypertrophy
After AS surgery, the LV-to-body weight ratio was increased similarly both in WT and in TG mice at the 4- and 7-week stages of AS compared with sham-operated animals (Figure 1). Thus, the magnitude of LV hypertrophy was similar in WT and TG mice with AS at both 4 and 7 weeks of pressure overload. However, TG mice exhibited lower mortality than WT during chronic pressure overload for 7 weeks (6.7% [2 of 30 mice] versus 29% [9 of 31 mice], \(P<0.05\)). All sham-operated animals survived the period of observation.

In Vivo LV Function
In vivo LV hemodynamic measurements are shown in Figure 2. Because there was no significant difference in any parameter between 4-week (n=6 for in vivo studies) and 7-week (n=9 for in vivo studies) sham-operated animals, data from all sham-operated animals were pooled and are indicated as data from controls. Figure 2 shows that there was no significant difference in the hemodynamic indices between WT and TG mice subjected to sham operation. At the early 4-week stage of AS, LV systolic pressure was increased relative to controls, and the magnitude of LV systolic pressure was similar in WT and TG 4-week AS mice (84±6 versus 82±13 mm Hg, \(P=NS\)). LV developed pressure per gram LV mass was also similar in WT and TG 4-week AS mice (818±71 versus 819±86 mm Hg/g, \(P=NS\)), suggesting
that aortic banding induced a similar initial magnitude of LV pressure overload. Consistent with prior characterization of this AS model,\textsuperscript{11,12} both LV systolic pressure and LV developed pressure per gram were depressed in WT mice at the later 7-week AS stage compared with 4-week AS. However, LV systolic pressure and LV developed pressure per gram LV mass were higher in TG than in WT 7-week AS mice (121 ± 11 versus 71 ± 12 mm Hg/g, \(P < 0.01\); 1019 ± 127 versus 552 ± 155 mm Hg/g, \(P < 0.05\), respectively). Both \(+dP/dt\) and \(-dP/dt\) were also faster in TG than in WT 7-week AS mice (134 ± 1676 versus 7536 ± 1669 mm Hg/s, \(P < 0.05\); -6016 ± 1038 versus -5518 ± 1021 mm Hg/s, \(P < 0.05\), respectively). LV end-diastolic pressure was similarly elevated in TG and WT 7-week AS mice compared with controls.

In vivo echocardiograms were obtained in all groups (Table 1). LV midwall fractional shortening was similar in TG and WT AS mice at 4 weeks AS. Consistent with prior reports of the model,\textsuperscript{11,12} midwall fractional shortening was preserved compared with controls in WT mice at 4 weeks AS, but was depressed by 7 weeks AS in the absence of LV diastolic cavity dilatation. In contrast, midwall fractional shortening was significantly higher in TG compared with WT mice at 7 weeks AS (19.0 ± 1.1% versus 15.5 ± 0.6%, \(P < 0.05\)). These data show that in vivo contractile performance, which is depressed by 7 weeks AS in WT mice, is preserved in TG mice despite similar duration of chronic pressure overload and magnitude of hypertrophy.

### Myocyte Contractility and Intracellular Ca\textsuperscript{2+} Regulation

We recently reported that contractile reserve is preserved in LV myocytes from 4-week AS but is depressed in myocytes from 7-week AS mice in this model.\textsuperscript{13} Therefore, to study the mechanism of differing in vivo contractile function in WT and TG 7-week AS mice, we studied LV myocyte function.

The baseline characteristics of myocyte contraction and the [Ca\textsuperscript{2+}] transients are shown in Table 2. Myocyte area was increased to a similar magnitude in WT and TG 7-week AS compared with sham-operated controls. In LV myocytes dissociated from sham-operated animals, fractional cell shortening (FS) and the amplitude of the [Ca\textsuperscript{2+}] transients were higher in TG than in WT, and the decay in the [Ca\textsuperscript{2+}] transients was faster. Also in myocytes from 7-week AS animals, FS and the amplitude of the [Ca\textsuperscript{2+}] transients were higher in TG than in WT mice. In WT 7-week AS myocytes, time to 50% relengthening and time to 50% decline in [Ca\textsuperscript{2+}], were prolonged compared with sham-operated WT. However, both the rate of relengthening and the rate of [Ca\textsuperscript{2+}], decay in TG 7-week AS were similar to those in sham-operated TG and were faster than in WT 7-week AS myocytes. These data show that myocyte contractility and dynamics of [Ca\textsuperscript{2+}], handling are depressed in WT 7-week AS but are preserved in TG 7-week AS myocytes despite similar magnitude of hypertrophy.

### Frequency-Dependent Contractile Reserve

We next examined frequency-dependent contractile reserve in isolated myocytes. Diastolic cell length decreased slightly as pacing frequency was increased, and there were no differences among the groups (Figure 3A). In myocytes from sham-operated WT mice, both FS and peak-systolic [Ca\textsuperscript{2+}], increased in response to the increase in pacing frequency from 0.5 to 5 Hz (5.6 ± 0.6% to 6.5 ± 0.4%, \(P < 0.05\), Figure 3B; 358 ± 23 to 552 ± 49 mmol/L, \(P < 0.05\), Figure 3C). The amplitude of the [Ca\textsuperscript{2+}], transients also increased (283 ± 25 to 387 ± 47 mmol/L, \(P < 0.05\)). On the other hand, in WT myo-

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**TABLE 1. Echocardiographic Measurements of LV Function**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>AS</th>
<th>Controls</th>
<th>AS</th>
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<tbody>
<tr>
<td></td>
<td>WT TG</td>
<td>WT TG</td>
<td>WT TG</td>
<td>WT TG</td>
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<tr>
<td>4 Weeks</td>
<td></td>
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<tr>
<td>LV diastolic dimension, mm</td>
<td>3.32±0.17</td>
<td>3.35±0.12</td>
<td>3.36±0.17</td>
<td>3.22±0.30</td>
</tr>
<tr>
<td>LV systolic dimension, mm</td>
<td>1.99±0.13</td>
<td>1.92±0.10</td>
<td>2.33±0.26</td>
<td>2.02±0.44</td>
</tr>
<tr>
<td>Endocardial FS, %</td>
<td>40.2±1.7</td>
<td>43.0±2.9</td>
<td>36.2±1.9</td>
<td>40.0±4.2</td>
</tr>
<tr>
<td>Midwall FS, %</td>
<td>20.5±1.2</td>
<td>23.1±1.3</td>
<td>18.0±1.3</td>
<td>20.4±2.0</td>
</tr>
<tr>
<td>LV wall thickness, mm</td>
<td>0.61±0.02</td>
<td>0.59±0.02</td>
<td>0.88±0.07*</td>
<td>0.88±0.05†</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>451±29</td>
<td>441±53</td>
<td>404±40</td>
<td>459±23</td>
</tr>
<tr>
<td>7 Weeks</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LV diastolic dimension, mm</td>
<td>3.53±0.28</td>
<td>3.55±0.19</td>
<td>3.89±0.32</td>
<td>3.52±0.22</td>
</tr>
<tr>
<td>LV systolic dimension, mm</td>
<td>2.33±0.17</td>
<td>2.09±0.10</td>
<td>2.81±0.36</td>
<td>2.23±0.20</td>
</tr>
<tr>
<td>Endocardial FS, %</td>
<td>38.0±2.1</td>
<td>41.0±1.3</td>
<td>31.5±1.8</td>
<td>37.2±2.4</td>
</tr>
<tr>
<td>Midwall FS, %</td>
<td>20.0±0.9</td>
<td>21.8±0.8</td>
<td>15.5±0.6‡</td>
<td>19.0±1.1</td>
</tr>
<tr>
<td>LV wall thickness, mm</td>
<td>0.74±0.03</td>
<td>0.76±0.03</td>
<td>0.85±0.03*</td>
<td>0.83±0.02*</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>462±40</td>
<td>485±12</td>
<td>472±35</td>
<td>528±13*</td>
</tr>
</tbody>
</table>

Controls are age-matched sham-operated mice studied at 4 or 7 weeks after surgery. Values are mean±SEM. n=5 to 6 animals per group.

*\(P < 0.05\), † \(P < 0.01\) vs age-matched controls.

‡ \(P < 0.05\), † ‡ \(P < 0.05\); WT vs TG in 7-week AS.
cytes from 7-week AS, FS was depressed in response to pacing from 0.5 to 5 Hz (5.6±1.0% to 4.6±0.6%, P<0.05, Figure 3B) in association with the failure to augment peak-systolic [Ca\(^{2+}\)], (345±29 to 432±42 nmol/L, P=0.10, Figure 3C) and the amplitude of the [Ca\(^{2+}\)] transients (270±29 to 283±38 nmol/L, P=0.79). In TG 7-week AS myocytes, both FS and peak-systolic [Ca\(^{2+}\)], were higher than in WT 7-week AS myocytes at all frequencies (Figures 3B and 3C). These data show that the capacity to increase contractility in AS myocytes at all frequencies (Figures 3B and 3C) and the amplitude of the [Ca\(^{2+}\)] i (345±29 to 432±42 nmol/L, P=0.10, Figure 3C) and the amplitude of the [Ca\(^{2+}\)] transients (270±29 to 283±38 nmol/L, P=0.79). In TG 7-week AS myocytes, both FS and peak-systolic [Ca\(^{2+}\)], were higher than in WT 7-week AS myocytes at all frequencies (Figures 3B and 3C). These data show that the capacity to increase contractility in response to rapid pacing was depressed in WT 7-week AS myocytes but was preserved in TG 7-week AS myocytes. The enhanced contractile reserve in TG 7-week AS myocytes was related to the capacity to augment peak-systolic [Ca\(^{2+}\)], at high work states of rapid pacing.

**SR Ca\(^{2+}\) Loading**

Because the amount of Ca\(^{2+}\) release from the SR is affected by SR Ca\(^{2+}\) content,\(^{18,19}\) SR Ca\(^{2+}\) load was measured in LV myocytes under baseline pacing frequency of 0.5 Hz and in response to rapid pacing at 3 Hz (Figure 4). In myocytes from sham-operated animals, SR Ca\(^{2+}\) load was higher in TG than in WT under baseline conditions (911±113 versus 652±44 nmol/L, P<0.05) and increased in response to rapid pacing both in TG and in WT myocytes. The data in myocytes from sham-operated mice under baseline conditions are consistent with the report by Yao et al,\(^{20}\) who measured SR Ca\(^{2+}\) content in voltage-clamped myocytes from this TG model. In myocytes from AS animals, SR Ca\(^{2+}\) load was higher in TG than in WT myocytes under baseline conditions (922±72 versus 625±92 nmol/L, P<0.05). SR Ca\(^{2+}\) load in WT 7-week AS myocytes did not increase in response to rapid pacing from 0.5 to 3 Hz (625±92 to 649±99 nmol/L, P=NS). In contrast, SR Ca\(^{2+}\) load in TG 7-week AS myocytes increased in response to rapid pacing (922±72 to 1200±91 nmol/L, P<0.05). The SR Ca\(^{2+}\) load under rapid pacing in TG 7-week AS was similar to that in sham-operated TG myocytes (1200±91 versus 1244±132 nmol/L, P=NS), and significantly higher than in WT 7-week AS myocytes (1200±91 versus 649±99 nmol/L, P<0.01). These results show that the impaired augmentation of contraction and peak-systolic [Ca\(^{2+}\)], in WT 7-week AS myocytes is, in part, attributed to an impaired capacity to increase SR Ca\(^{2+}\) load at high work states. This defect is rescued in the TG myocytes from AS mice.

**LV Levels of Ca\(^{2+}\)-Cycling Proteins**

Protein levels of SERCA2a, phospholamban, and Na\(^{+}\)-Ca\(^{2+}\) exchanger are shown in Figure 5. Protein levels are expressed as percentage of the value in age-matched sham-operated WT mice. In myocytes from sham-operated mice, protein levels of SERCA2a in TG were 20% to 30% higher than those in WT (4-week AS, 132±19% versus 100±17%; 7-week AS, 127±5% versus 100±9%). These data corroborate the report by He et al\(^{3}\) that showed a 20±11% increase in SERCA2a protein levels in TG compared with WT mice. Protein levels of SERCA2a were similar in WT and TG 4-week AS myocytes (123±13% versus 158±11%, P=NS). However, in WT 7-week AS myocytes, SERCA2a protein levels were severely depressed compared with sham-operated WT (35±7% versus 100±9%, P<0.01). On the other hand, SERCA2a protein levels in TG 7-week AS were preserved at levels similar to those of sham-operated WT (111±10% versus 100±9%, P=NS). Protein levels of phospholamban and Na\(^{+}\)-Ca\(^{2+}\) exchanger were similar in WT and TG 4-week AS (108±14% versus 104±12%, P=NS; 127±8% versus 121±6%, P=NS, respectively) and were upregulated to similar magnitude in WT and TG 7-week AS (214±21% versus 206±16%, P=NS; 364±127% versus 342±35%, P=NS, respectively).

**Discussion**

This is the first report that demonstrates a chronic protective effect of TG expression of SERCA2a on development of early heart failure in mice with pressure overload. The present study shows that chronic TG expression of SERCA2a in AS mice enhances survival and systolic performance in vivo and preserves contractile reserve and the capacity to increase SR Ca\(^{2+}\) load at high work states in isolated myocytes. In

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**TABLE 2. Baseline Characteristics of Myocyte Contraction and the [Ca\(^{2+}\)], Transients**

<table>
<thead>
<tr>
<th></th>
<th>7-Week Controls</th>
<th>7-Week AS</th>
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<tbody>
<tr>
<td></td>
<td>WT</td>
<td>TG</td>
</tr>
<tr>
<td>Myocyte area, μm(^2)</td>
<td>3120±167</td>
<td>3366±194</td>
</tr>
<tr>
<td>Diastolic cell length, μm</td>
<td>127±3</td>
<td>131±4</td>
</tr>
<tr>
<td>Fractional cell shortening, %</td>
<td>5.5±0.6</td>
<td>8.2±0.7†</td>
</tr>
<tr>
<td>Time to peak shortening, ms</td>
<td>119±5</td>
<td>104±5‡</td>
</tr>
<tr>
<td>Time to 50% relengthening, ms</td>
<td>59±4</td>
<td>48±5</td>
</tr>
<tr>
<td>Peak-systolic [Ca(^{2+})], nmol/L</td>
<td>369±19</td>
<td>489±25†</td>
</tr>
<tr>
<td>End-diastolic [Ca(^{2+})], nmol/L</td>
<td>79±4</td>
<td>63±3‡</td>
</tr>
<tr>
<td>Time to peak [Ca(^{2+})], ms</td>
<td>52±2</td>
<td>46±2‡</td>
</tr>
<tr>
<td>Time to 50% decline in [Ca(^{2+})], ms</td>
<td>110±4</td>
<td>60±4†</td>
</tr>
</tbody>
</table>

*P<0.01, ‡P<0.05 vs age-matched controls.
†P<0.01, ‡P<0.05, WT vs TG in 7-week controls or in 7-week AS.
addition, chronic TG expression of SERCA2a does not modify the development of pathologic hypertrophy itself. These data suggest that the depression of SERCA2a expression in chronic pressure overload contributes to the onset of early heart failure.

Overexpression of SERCA2a enhances contractile performance and SR Ca\(^{2+}\) uptake through transient adenoviral gene transfer\(^{3,4}\) or in TG mice not subjected to pathological stimuli.\(^{5,6}\) However, there are many clinical and experimental observations whereby short-term interventions that improved cardiac contractility were associated with adverse long-term effects on survival and progression of heart failure.\(^{7-10}\) β-Adrenergic stimulation acutely increases cardiac contractility by the activation of the SERCA2a through protein kinase A–dependent phosphorylation of phospholamban whereas overexpression of β\(_2\)-adrenergic receptors exacer-

bates the development of heart failure in mice subjected to AS.\(^{10}\) With regard to chronic TG expression of SERCA2a, it is formally possible that enhanced energy-dependent SR Ca\(^{2+}\) cycling might be deleterious in hypertrophied hearts in which the capacity to rapidly generate ATP is reported to be decreased.\(^{21,22}\) Therefore, it is controversial whether chronic TG expression of SERCA2a in vivo will attenuate or accelerate the development of early heart failure and contractile dysfunction in animals with pathologic pressure overload.

Effects of SERCA2a TG Expression on Contractile Function

In this TG model, we corroborated previous observations\(^5\) that the increase in LV SERCA2a protein levels is modest (≈27%) in sham-operated TG mice. Although we observed differences in the [Ca\(^{2+}\)] transient kinetics between WT and TG myocytes as reported by Yao et al,\(^{20}\) we did not observe a significant increase in the in vivo LV systolic pressure generation or echocardiographic systolic function indices between WT and TG mice. This is consistent with the report by He et al\(^5\) which reported no difference in the in vivo LV systolic pressure between WT and TG mice. In contrast, in 7-week AS animals in the present study, TG expression of SERCA2a improved survival and prevented the development of contractile dysfunction both in vivo and in isolated myocytes.

In this AS model, LV SERCA2a levels are preserved at 4 weeks AS but depressed by 7 weeks AS.\(^{13}\) Concomitantly, in vivo LV systolic pressure is increased in 4-week AS mice with early hypertrophy and then decreases to levels observed in control mice by 7 weeks AS despite an increase in LV mass.\(^{12}\) In the present study, in vivo LV systolic function, which was similar in WT and TG 4-week AS mice, was depressed in WT 7-week AS mice consistent with prior
observations in the model. In contrast, in vivo LV systolic function was preserved in TG 7-week AS mice. In LV myocytes, FS was higher in TG than in WT 7-week AS myocytes even during the challenge of rapid pacing stimulation. These data support a critical role of SERCA2a in the development of systolic contractile dysfunction in chronic pressure overload. In terms of diastolic function, both in vivo LV negative dp/dt and myocyte relaxation rate were higher in TG compared with WT 7-week AS, whereas in vivo LV end-diastolic pressure was similarly increased in both 7-week AS groups compared with sham-operated mice. This suggests that alterations in the kinetics of myocyte relaxation contribute less to diastolic pressure in vivo than the magnitude of hypertrophic remodeling itself (wall thickness and chamber dimensions), which were similar in the TG and WT AS groups.

Preserved SR Ca\(^{2+}\) Loading

SERCA2a protein levels were severely depressed in WT 7-week AS compared with sham-operated WT mice, but were preserved in TG 7-week AS mice. The rate of decay in \([\text{Ca}^{2+}]_i\), which was prolonged in WT 7-week AS myocytes, was maintained in TG 7-week AS myocytes despite a similar magnitude of myocyte hypertrophy. These data suggest that SR Ca\(^{2+}\) uptake function is preserved in TG mice with 7-week AS. However, net SR Ca\(^{2+}\) uptake in myocytes from failing hearts is influenced by factors in addition to levels of SERCA2a, such as the competition for cytosolic Ca\(^{2+}\) between SERCA2a and Na\(^{+}\)-Ca\(^{2+}\) exchanger,\(^{23}\) and alterations in the levels and phosphorylation state of the inhibitory protein phospholamban.\(^{24}\) In myocytes from failing hearts, other alterations in \([\text{Ca}^{2+}]_i\), regulatory mechanisms could indirectly modify Ca\(^{2+}\) accessible for SR loading including decreased efficacy of Ca\(^{2+}\)-induced Ca\(^{2+}\) release,\(^{16,25}\) hyper-phosphorylation of ryanodine receptors,\(^{26}\) and differences in sarcoplasmic reticulum (SR) Ca\(^{2+}\) levels.

Limitations of Study

In this study, we examined basal myocyte function at 0.5 Hz at room temperature (25°C) to enhance stability of myocyte contraction as reported in other studies in isolated mouse myocytes.\(^{13,20}\) Many investigators have observed that adult mouse myocytes are more unstable at higher physiological temperatures, in comparison with myocytes from larger species such as rat, ferret, or humans. Although investigators have studied otherwise normal (nonfailing) mouse myocytes

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Figure 5. LV protein levels of Ca\(^{2+}\)-cycling proteins; SERCA2a, phospholamban (PLB), and Na\(^{+}\)-Ca\(^{2+}\) exchanger (NCX). Controls are age-matched sham-operated mice. Top, Representative Western blots. Bottom, Data are expressed as percentage of value in age-matched WT controls. LV protein levels of SERCA2a are severely depressed in WT 7-week AS compared with controls; in contrast, SERCA2a protein levels are preserved in TG 7-week AS mice. In 7-week WT and TG AS mice, protein levels of both phospholamban and Na\(^{+}\)-Ca\(^{2+}\) exchanger are pathologically upregulated to similar magnitude. \(\text{WP}<0.05, \text{WP}<0.01\) vs age-matched controls.
at 37°C, we found that hypertrophied mouse myocyte performance is less stable at higher temperature, and some of the hypertrophied myocytes from AS mice exhibit aftercontractions, which are not observed at 25°C. Puglisi et al. reported that increases in temperature accelerate all Ca²⁺ transport systems, whereas the relative contribution remains the same. Therefore, we studied mouse myocyte function at 25°C to enhance the stability of myocyte contraction. In addition, this strategy permits comparison with our recently published study of normal and hypertrophied mouse myocytes. We recognize the importance of also obtaining data at physiological temperature. Thus, we performed in vivo studies under physiological body temperature and also observed enhanced in vivo contractile performance in TG compared with WT 7-week AS mice consistent with the mouse myocyte data.

This study examined the effect of TG expression of SERCA2a during an observation period that includes the transition to early failure by 7 weeks of AS. In the present study, the mortality was lower and both in vivo and myocyte contractile function were better in TG than in WT AS mice. In the AS model, we have observed that the mortality is very high in WT mice at the stage of overt heart failure 13 to 15 weeks after surgery, so that it is problematic to study in vivo function. However, our preliminary data in TG AS mice (n=3) showed maintenance of LV contractile performance even 6 months after AS surgery (endocardial fractional shortening 36.8±7.4%, and midwall fractional shortening 18.7±3.9%). It is also not known whether this TG intervention will be beneficial in other models of acute or gradual development of heart failure. Future studies in multiple models of heart failure, with large numbers of animals studied over the full natural history to senescence, will be needed to determine whether premature death and progression of heart failure are prevented or merely delayed by TG SERCA2a expression. Other interventions that modulate SERCA2a expression, including low-dose treatment with thyroid hormone or growth hormone, may merit exploration. In this regard, we and others previously reported that expressing a dominant negative mutant of phospholamban or targeted ablation of the phospholamban gene enhances cardiac contractility and SR Ca²⁺ uptake.

In conclusion, for the mouse model of AS, TG expression of SERCA2a favorably modifies the chronic natural history of progression from adaptive hypertrophy to early heart failure in vivo. This work supports a critical mechanistic role of defective SR Ca²⁺ function in the onset of early heart failure in animals with chronic pressure overload.

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Transgenic Expression of Sarcoplasmic Reticulum Ca\(^{2+}\) ATPase Modifies the Transition From Hypertrophy to Early Heart Failure
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Materials and Methods

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

The LV myocytes were dissociated with the modified method of Wolska et al.\(^1\) Myocyte contraction and \([\text{Ca}^{2+}]_i\) measured with fluo-3 were monitored simultaneously, and the optical systems for fluorescence signal detection and cell motion monitoring are described in detail elsewhere.\(^2\)-\(^4\) The calibration of \([\text{Ca}^{2+}]_i\) was done as previously reported from our laboratory.\(^5\) Briefly, to estimate calibrated levels of the \([\text{Ca}^{2+}]_i\), transients, immediately after each experiment the myocyte was superfused with the same buffer supplemented with 30 mmol/L 2,3-butanedione monoxime and 10 \(\mu\)mol/L calcium ionophore ionomycin in the presence of 1 mmol/L calcium. Then a 1-mol/L MnCl\(_2\) stock solution was added to the buffer to yield a final concentration of 10 mmol/L. The cell was abruptly superfused with Mn\(^{2+}\) for saturation of fluo-3. After the fluorescence intensity with Mn\(^{2+}\) (\(F_{\text{Mn}}\)) was recorded, the intensity of the fluorescence from the field (\(F_{\text{BKG}}\)) was measured by blowing the myocyte away from the field with pipette. In the preliminary study, the autofluorescence of unloaded myocytes (\(F_{\text{AUTO}}\)) was very small compared to the fluo-3 signal and was negligible. After measurement of \(F_{\text{Mn}}\) and \(F_{\text{BKG}}\), the values of \(F_{\text{max}}, F_{\text{min}},\) and estimated \([\text{Ca}^{2+}]_i\) were calculated with the following formula reported by Kao et al.\(^6\)

\[
F_{\text{max}} = (F_{\text{Mn}} - F_{\text{BKG}})/0.2 + F_{\text{BKG}} \\
F_{\text{min}} = (F_{\text{max}} - F_{\text{BKG}})/40 + F_{\text{BKG}} \\
[\text{Ca}^{2+}]_i = K_d \times (F - F_{\text{min}})/(F_{\text{max}} - F)
\]

where \(F\) is the measured fluorescence intensity, and \(K_d\) is the dissociation constant for fluo-3. The \(K_d\) of fluo-3 is known to be temperature dependent and is reported to be 400 and 864 nmol/L at 22 \(^\circ\)C and 37 \(^\circ\)C, respectively.\(^7, 8\) Therefore, we used 493 nmol/L as the \(K_d\) at 25 \(^\circ\)C, assuming a linear relationship between \(K_d\) and temperature. In the present study, baseline values of systolic and diastolic \([\text{Ca}^{2+}]_i\) and myocyte shortening in normal mouse myocytes were similar to those previously reported in adult mouse myocytes using fluo-3.\(^5, 9-11\)
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