Enhanced Myocardial Contractility and Increased Ca\textsuperscript{2+}
Transport Function in Transgenic Hearts Expressing the
Fast-Twitch Skeletal Muscle Sarcoplasmic Reticulum Ca\textsuperscript{2+}-ATPase

Evgeny Loukianov, Yong Ji, Ingrid L. Grupp, Darryl L. Kirkpatrick, Debra L. Baker, Tanya Loukianova, Gunter Grupp, Jonathan Lytton, Richard A. Walsh, Muthu Periasamy

Abstract—In this study, we investigated whether the fast-twitch skeletal muscle sarcoplasmic reticulum Ca\textsuperscript{2+} transport pump (SERCA1a) can functionally substitute the cardiac SERCA2a isoform and how its overexpression affects cardiac contractility. For this purpose, we generated transgenic (TG) mice that specifically overexpress SERCA1a in the heart, using the cardiac-specific α-myosin heavy chain promoter. Ectopic expression of SERCA1a resulted in a 2.5-fold increase in the amount of total SERCA protein. At the same time, the level of the endogenous SERCA2a protein was decreased by 50%, whereas the level of other muscle proteins, including calsequestrin, phospholamban, actin, and tropomyosin, remained unchanged. The steady-state level of SERCA phosphoenzyme intermediate was increased 2.5-fold, and the maximal velocity of Ca\textsuperscript{2+} uptake was increased 1.7-fold in TG hearts, demonstrating that the overexpressed protein is functional. Although the basal cytosolic calcium signal was decreased by 38% in TG cardiomyocytes, the amplitude of cytosolic calcium signal was increased by 71.8%. The rate of calcium reserequestration was also increased in TG myocytes, which was reflected by a 51.6% decrease in the normalized time to 80% decay of calcium signal. This resulted in considerably increased peak rates of myocyte shortening and lengthening (50.0% and 66.6%, respectively). Cardiac functional analysis using isolated work-performing heart preparations revealed significantly faster rates of contraction and relaxation in TG hearts (41.9% and 39.5%, respectively). The time to peak pressure and the time to half-relaxation were shorter (29.1% and 32.7%, respectively). In conclusion, our study demonstrates that the SERCA1a pump can functionally substitute endogenous SERCA2a, and its overexpression significantly enhances Ca\textsuperscript{2+} transport and contractile function of the myocardium. These results also demonstrate that the SERCA pump level is a critical determinant of cardiac contractility. (Circ Res. 1998;83:889-897.)

Key Words: SERCA1a ■ overexpression ■ transgenic mouse ■ cardiac contractility

In cardiac and skeletal muscle, contraction and relaxation are regulated by cyclic release and removal of Ca\textsuperscript{2+} by the sarcoplasmic reticulum (SR). Release of Ca\textsuperscript{2+} from the SR initiates muscle contraction, whereas reuptake of Ca\textsuperscript{2+} into the SR results in muscle relaxation. The rate of muscle relaxation is determined largely by the Ca\textsuperscript{2+} uptake function of the SR Ca\textsuperscript{2+}/Mg\textsuperscript{2+}-ATPase (SERCA). This enzyme is a transmembrane protein of ~110 kDa and belongs to a family of highly conserved proteins. Three different genes, SERCA1, SERCA2, and SERCA3, have been identified. The SERCA1 gene encodes 2 isoforms, SERCA1a and SERCA1b, expressed in adult and neonatal fast-twitch skeletal muscle, respectively, but never in the heart. The SERCA2 gene encodes 2 different isoforms, SERCA2a and SERCA2b. SERCA2a is the primary cardiac isoform but also is expressed in slow-twitch skeletal muscle. The SERCA2b isoform is expressed in most smooth muscle and nonmuscle tissues, whereas the SERCA3 isoform is expressed primarily in epithelial and endothelial cells. The differential expression of SERCA1a and SERCA2a isoforms in skeletal and cardiac muscles suggests that they may have distinct Ca\textsuperscript{2+} uptake properties. However, it is unclear whether the differential isoform expression is purely an evolutionary coincidence or provides for unique muscle contractile characteristics.

Studies from our laboratory and others have shown that the SR Ca\textsuperscript{2+}-ATPase pump level is altered significantly during cardiac adaptation to pressure overload and changes in thyroid hormone level. Using tissue samples from failing human hearts, we and others have found that the expression level of SR Ca\textsuperscript{2+}-ATPase was decreased both at the mRNA and the protein levels in end-stage heart failure. Intracellular Ca\textsuperscript{2+} measurements showed that Ca\textsuperscript{2+} transients...
SERCA1a Overexpression in the Heart

in muscle samples from failing human hearts were prolonged markedly in both Ca\(^{2+}\) release and uptake phases.\(^{21,22}\) The decrease in the levels of SR Ca\(^{2+}\)-ATPase can be correlated closely with a decreased myocardial function.\(^{20,23}\)

Recently, adenovirus-mediated gene transfer of SERCA2a into failing cardiomyocytes has been shown to reconstitute depressed pump level and shorten prolonged Ca\(^{2+}\) transients, paving the way for potential gene therapy to treat heart failure.\(^{24}\) However, this approach should not be restricted to the use of cardiac isoform SERCA2a only. In a recent study, Inesi et al\(^{25}\) used adenovirus-mediated gene transfer overexpressed SERCA1 isoform in chicken embryonic cardiac myocytes. They showed that SERCA1 overexpression resulted in a 4-fold increase in the rates of Ca\(^{2+}\) transport activity. Cytosolic Ca\(^{2+}\) transients and tension development of transfected myocytes also were enhanced by SERCA1 overexpression, suggesting that SERCA1 can be functional in cardiac myocytes. The goal of this study was to develop a transgenic (TG) mouse model to explore how SERCA1a overexpression affects cardiac contractility and Ca\(^{2+}\) transport function in the intact heart. The use of a TG mouse model overcomes certain limitations of in vitro systems and provides us with an in vivo system to study the properties of different isoforms in cardiac environment and the various aspects of excitation-contraction coupling and Ca\(^{2+}\) handling in response to SERCA pump overexpression.

To determine whether SERCA1a functionally can substitute SERCA2a in the heart, we created a TG mouse model in which SERCA1a protein is expressed in the heart using the cardiac \(\alpha\)-myosin heavy chain (MHC) promoter. Ectopic expression of SERCA1a in the heart resulted in a 2.5-fold increase in the total SERCA protein but caused a 50% decrease in the level of endogenous SERCA2a protein. As a result, the Ca\(^{2+}\) transport function in TG hearts was considerably increased, and TG hearts demonstrated significantly enhanced rates of contraction and relaxation, suggesting that the SERCA pump level is a critical determinant of myocardial contractility.

Materials and Methods

Generation of SERCA1a TG Mice

The complete coding and 3′-untranslated region of rat SERCA1a cDNA\(^{26}\) was ligated into the Sau3A site of plasmid pCI26 downstream of 5.5 kb mouse cardiac \(\alpha\)-MHC promoter.\(^{27}\) SERCA1a cDNA 5′-untranslated sequence was removed, and the sequence preceding the \(\alpha\)-MHC promoter was confirmed by restriction mapping and nucleotide sequencing. A linear 9.6-kb DNA fragment containing the entire cardiac \(\alpha\)-MHC promoter, the complete rat SERCA1a cDNA open reading frame, and 3′-untranslated region followed by human growth hormone (Hgh) polyadenylation signal was purified from plasmid sequences. The DNA was used for microinjection of fertilized mouse eggs and production of TG mice.\(^{28}\) Polymerase chain reaction was used to screen 3-week-old pups for expression of the transgene. Primers cardiac \(\alpha\)-MHC (5′-GCCCAACACGAAATGACAG-3′) complementary to cardiac \(\alpha\)-MHC promoter and PR3 (5′-TCGATGCGATCTGATGCTCC-3′) complementary to rat SERCA1a cDNA were used. Stable TG lines were raised by breeding the founder mice with nontransgenic (NTG) cohorts. All animal care and procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee and complied with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-213, 1985).

Genomic Southern and Dot Blotting

Genomic DNA was isolated from tail clips and digested with BamHI to yield a unique 2-kb fragment, characteristic for the transgene. The DNA was electrophoresed in 0.8% agarose gel and transferred on nylon membranes. The copy number of the transgene in each line was determined by quantitative DNA dot blot analysis.\(^{29}\) Samples of BamHI-digested DNA (10 \(\mu\)g) were applied on nylon membranes, and a serial dilution of purified \(\alpha\)-MHC/SERCA1a/Hgh fragment was used as a standard. The membranes were probed with \(^{32}\)P-labeled 0.9-kb XhoI-NdeI Hgh-fragment. Quantitation was carried out on PhosphorImager using ImageQuant V 3.0 analysis system (Molecular Dynamics).

Northern Blot Analysis

Total RNA extraction from whole TG and NTG mouse hearts, rat hearts, and rat fast-twitch skeletal muscle, size-fractionation in formaldehyde agarose gel (10 \(\mu\)g/lane), transfer on nylon membranes, and hybridization were performed using standard protocols.\(^{30}\) In TG microsomes only, the SERCA band (110 kDa) was enhanced, compared with NTG microsomes. The identity of SERCA band was confirmed by Western blot analysis. After SDS-PAGE, the proteins were transferred onto nitrocellulose filters and stained transiently with Ponceau S (Sigma). The position of the 110-kDa band was marked, and after washing out of the stain, the filters were probed with a SERCA2a-specific polyclonal antibody\(^{32}\) or a monoclonal SERCA1a-specific antibody, A-52.\(^{33}\) The SERCA2a antibody cross-reacted with the 110-kDa band in both TG and NTG samples, whereas the SERCA1a antibody cross-reacted with the 110-kDa band only in TG samples, but not in NTG samples. This demonstrated that the 110-kDa band indeed corresponds to SERCA (SERCA2a in NTG microsomes and SERCA1a+SERCA2a in TG microsomes).

To accurately determine the total amount of SERCA protein in TG hearts, a quantitative SDS-PAGE with Western blot analysis as follows. SR-enriched microsomes were prepared from the whole TG and NTG mouse hearts as previously described.\(^{31}\) Equal amounts of TG and NTG microsomal proteins were separated in SDS-PAGE and stained with Coomassie blue according to standard protocols.\(^{30}\) In TG microsomes only, the SERCA band (110 kDa) was enhanced, compared with NTG microsomes. The identity of SERCA band was confirmed by Western blot analysis. After SDS-PAGE, the proteins were transferred onto nitrocellulose filters and stained transiently with Ponceau S (Sigma). The position of the 110-kDa band was marked, and after washing out of the stain, the filters were probed with a SERCA2a-specific polyclonal antibody\(^{32}\) or a monoclonal SERCA1a-specific antibody, A-52.\(^{33}\) The SERCA2a antibody cross-reacted with the 110-kDa band in both TG and NTG samples, whereas the SERCA1a antibody cross-reacted with the 110-kDa band only in TG samples, but not in NTG samples. This demonstrated that the 110-kDa band indeed corresponds to SERCA (SERCA2a in NTG microsomes and SERCA1a+SERCA2a in TG microsomes).

Protein and Western Blot Analysis

To determine the level of total SERCA protein in TG hearts, we used a combination of quantitative SDS-PAGE with Western blot analysis as follows. SR-enriched microsomes were prepared from the whole TG and NTG mouse hearts as previously described.\(^{31}\) Equal amounts of TG and NTG microsomal proteins were separated in SDS-PAGE and stained with Coomassie blue according to standard protocols.\(^{30}\) In TG microsomes only, the SERCA band (110 kDa) was enhanced, compared with NTG microsomes. The identity of SERCA band was confirmed by Western blot analysis. After SDS-PAGE, the proteins were transferred onto nitrocellulose filters and stained transiently with Ponceau S (Sigma). The position of the 110-kDa band was marked, and after washing out of the stain, the filters were probed with a SERCA2a-specific polyclonal antibody\(^{32}\) or a monoclonal SERCA1a-specific antibody, A-52.\(^{33}\) The SERCA2a antibody cross-reacted with the 110-kDa band in both TG and NTG samples, whereas the SERCA1a antibody cross-reacted with the 110-kDa band only in TG samples, but not in NTG samples. This demonstrated that the 110-kDa band indeed corresponds to SERCA (SERCA2a in NTG microsomes and SERCA1a+SERCA2a in TG microsomes).

Quantitative immunoblotting with SERCA1a-specific antibody, A-52,\(^{32}\) SERCA2a-specific polyclonal antibody,\(^{34}\) monoclonal anti-phospholamban antibody (Affinity Bioreagents, Inc.), a monoclonal anti-\(\alpha\)-sarcomeric actin antibody 5CS (Sigma), a monoclonal anti-tropomyosin antibody, CH1 (Developmental Studies Hybridoma Bank), and a polyclonal anti-calsequestrin antibody, CSH6 (Swant) was performed using pooled whole cardiac homogenates as previously described.\(^{31,35-36}\) After incubation with primary antibodies and washing, the membranes were incubated with the appropriate (antimouse or anti-rabbit IgG)\(^{32}\) \(^{38}\)-labeled secondary antibody (Amer- sham Inc) at a specific activity of 1 \(\mu\)Ci/10 mL of blocking buffer.
The signal was quantitated using PhosphorImager and ImageQuant software.

**Ca^{2+} Uptake Assay**

Ca^{2+} uptake measurements in whole mouse hearts were performed as described in previous studies.31,36,39,40 Briefly, frozen cardiac powder was homogenized in 50 mmol/L KPi (pH 7.0), 10 mmol/L NaF, 1 mmol/L EDTA, 0.3 mol/L sucrose, 0.3 mmol/L phenylmethylsulfonyl fluoride, and 0.5 mmol/L diithiothreitol. Ca^{2+} uptake in tissue homogenates (0.1 mg/mL) was measured by the Millipore filtration technique. The reaction mixture contained in (mmol/L): imidazole 40 (pH 7.0), KCl 100, MgCl2 5, NaN3 5, potassium oxalate 5, EGTA 0.5 and also included various concentrations of CaCl2 to yield 0.03 to 3 μmol/L free Ca^{2+} (containing 1 μCi/μmol ^{45}Ca^{2+}) as determined by the computer program. The reaction was initiated by the addition of 5 mmol/L ATP. The rates of Ca^{2+} uptake were calculated by least squares linear regression analysis of uptake values at 30, 60, and 90 s. The results were analyzed using MicroCal Origin Software.

**Quantitation of SERCA Phosphoenzyme Intermediate**

The steady-state level of SERCA phosphoenzyme intermediate (E-P) was measured as described in a recent study.39 Whole cardiac homogenate (0.2 mg/mL), prepared as mentioned above except 50 mmol/L KPi was replaced by 20 mmol/L imidazole; pH 7.0) was added into 0.1 mL reaction mixture containing 20 mmol/L imidazole (pH 7.0), 100 mmol/L KCl, 5 mmol/L NaN3, and either 100 μmol/L free Ca^{2+} or 1 mmol/L EGTA. The reaction was started by the addition of 2 μmol/L [γ-32P]ATP (specific activity, 10 μCi/μmol) at 0°C. The reaction was terminated after 30 s by the addition of 1 mL ice-cold stop solution (6% trichloroacetic acid, 0.3 mmol/L ATP, 5 mmol/L Pi). The samples were placed on ice for 5 minutes and then were vacuum filtered through the Millipore filter membranes. The filters were processed for scintillation counting. The SERCA E-P level was calculated as the difference between ^32P incorporation into protein in the presence and absence of added Ca^{2+}.

**Measurements of Ca^{2+} Transients and Contractile Parameters in Isolated Ventricular Myocytes**

Ventricular cardiomyocytes were isolated as previously described.31,36,39,40 The isolated cells were washed and resuspended in a physiological buffer [132 mmol/L NaCl, 4.8 mmol/L KCl, 1.2 mmol/L MgCl2, 5 mmol/L glucose, 10 mmol/L HEPES (pH 7.4)] supplemented with 1.8 mmol/L Ca^{2+}. Half of the cells from each heart then were used for mechanical studies and the other half were used for measurements of intracellular free Ca^{2+} transients.

Intracellular free Ca^{2+} transients were measured as described.31,36,39,40 Briefly, myocytes were loaded with 7.5 μmol/L Fura-2 AM (emission wavelength, 510 nm) using a photo scan dual spectrophotometer (Photon Tech, Inc) coupled with an Olympus IMT-2 UV fluorescent microscope with UV transparent optics.

Morphological and mechanical properties of myocytes were measured as described.31,36,39,40 Briefly, cells were placed in a well on the stage of an inverted microscope and were perfused continuously with oxygenated physiological buffer. Myocytes were field stimulated at 0.5 Hz, 5-ms pulse duration, for at least 40 s per pacing rate. Cell images were videotaped, and myocyte length, percentage of shortening, and peak rates of shortening (+dL/dt and −dL/dt) were quantitated by comparison with a calibrated micrometer on the microscope stage.

At least 5 cells were examined per mouse, and the values were averaged for mechanical parameters and Ca^{2+} kinetics. Statistical analysis was based on the number of animals rather than the number of cells.

**Measurements of Cardiac Contractile Parameters in Work-Performing Heart Preparations**

The work-performing heart preparations were described in detail previously.41–43 Age-matched TG and NTG mice (12 to 16 weeks old) were used. ECG and heart rates were recorded before opening of the chest and removal of the heart. The optimal venous return and the afterload required to maintain constant function of the hearts were 5 mL/min cardiac output and 50 mm Hg aortic pressure, respectively, providing a basal workload of 250 mL×mm Hg/min. Heart rate, aortic pressure, left intraventricular pressure (IVP; systolic, diastolic, and end-diastolic), and atrial pressure were monitored continuously. The first derivative of IVP (+dP/dt and −dP/dt), the time to peak pressure (TPP)/mm Hg, and the time to half-relaxation (RT_{1/2})/mm Hg were calculated with a custom-designed computer program. Venous return (equal to cardiac output) and aortic flows were measured continuously with a Dual Channel Transonic Flowmeter. Coronary flow was computer calculated as venous return−aortic flow.

**Statistical Analysis**

Statistical significance was determined by unpaired Student t test or ANOVA followed by the Newman-Keuls test as appropriate. If data were not distributed normally or failed equal variance tests after log10 transformations, they were analyzed by nonparametric statistics (ie, Kruskal-Wallis for ANOVA designs or Mann-Whitney rank sum test for comparison between 2 groups of data). Values represent mean±SEM of at least 3 determinations (n=No. of mice). A P value <0.05 was set as the criteria for statistical significance.

**Results**

**Generation and Characterization of SERCA1a TG Mice**

To direct cardiac-specific expression of the fast-twitch skeletal muscle, SR Ca^{2+}-ATPase, the rat SERCA1a cDNA was linked to the mouse cardiac α-MHC promoter (Figure 1A). TG mice were generated, and 5 founder mice (Nos. 33, 38, 40, 48, and 50) were identified as carriers of the transgene by polymerase chain reaction analysis with specific primers. They were bred with FVB/N wild-type mice to establish germ-line transmission. All founder mice passed the transgene to their offspring. The SERCA1a TG mice cannot be distinguished phenotypically from NTG mice and reproduce well. The heart weight to–body weight ratio was not different between TG and NTG animals. Histological examination of TG hearts (6 months old) did not reveal any evidence of cardiac pathology (data not shown).

Genomic Southern and dot blot analysis revealed that the SERCA1a transgene copy number ranged from 2 to 9 copies in different lines. Transgene expression was assessed by Northern blot analysis using total cardiac RNA from the F1, heterozygotes and the rat SERCA1a cDNA 3′-noncoding region as a probe. The 3.5-kb SERCA1a mRNA was detected in TG lines 33, 38, 40, and 48. Line 38 carrying 9 copies of the transgene had the highest level of SERCA1a mRNA in the heart, whereas line 33 carrying 2 copies had the lowest SERCA1a mRNA level (Figure 1B). In this article, we report detailed characterization of the TG line 38 expressing the highest SERCA1a protein level. Heterozygous mice (12 to 16 weeks old) were used for biochemical, myocyte, and cardiac functional analysis.
Ectopic Expression of SERCA1a in TG Hearts

Results in a 2.5-Fold Increase in the Total Amount of SERCA Protein

To detect the SERCA1a protein expression in TG hearts, Western blot analysis was performed using cardiac homogenates. SERCA1a antibody33 detected a specific band only in TG hearts, but not in NTG hearts (see Figure 2A and 2B). Coomassie blue staining of SDS-PAGE separated proteins revealed that only SERCA band (110 kDa) was enhanced in TG cardiac microsomes compared with NTG microsomes (Figure 2A). Western blot analysis with specific antibodies confirmed that this band indeed corresponded to SERCA (SERCA2a and SERCA1a+SERCA2a in NTG and TG hearts, respectively; see Methods and Figure 2A). Quantitative SDS-PAGE analysis showed that this band contain 2.5-fold more protein in TG microsomes compared with NTG microsomes (Figure 2A). These data suggest that ectopic expression of SERCA1a in mouse hearts resulted in a 2.5-fold increase in the total SERCA protein level.

To determine whether SERCA1a overexpression altered the endogenous SERCA2a protein level in the heart, quantitative immunoblotting34 was performed with cardiac homogenates using a SERCA2a-specific antibody.22 The SERCA2a protein level was decreased by 50% in TG hearts compared with NTG hearts (Figure 2B). Inesi et al25 also reported that SERCA1 overexpression in embryonic chick cardiac myocytes down-regulated (30% to 60%) the endogenous SERCA2a pump. In TG hearts, the total amount of SERCA (SERCA1a+SERCA2a) was increased 2.5-fold (250%) compared with NTG hearts, whereas the level of SERCA2a protein was decreased to 50%. Therefore, the SERCA1a level=(total SERCA)−(SERCA2a)=250%−50%=200%. Based on this, SERCA1a:SERCA2a ratio is 4:1 (200%:50%). This means that 80% of total SERCA pumps in TG hearts are represented by SERCA1a.

In addition, we addressed whether SERCA1a expression in the heart alters the expression levels of other proteins involved in calcium transport and contractile function. The quantitative immunoblotting using specific antibodies revealed no difference in the levels of actin, tropomyosin, calsequestrin, and phospholamban between TG and NTG hearts (Figure 2B). These data suggest that the ectopic expression of SERCA1a did not affect the expression level of other proteins in TG hearts.

The Steady-State Level of SERCA E-P and Maximal Velocity of SR Ca2+ Uptake Are Increased Significantly in SERCA1a TG Hearts

Ca2+ translocation by SERCA pump is coupled tightly with the formation of E-P,44 which under specific conditions could be trapped to quantitate the amount of active enzyme.45 Therefore, we measured the steady-state level of E-P in TG and NTG cardiac homogenates. The steady-state level of E-P was 2.5-fold higher in TG hearts than in NTG hearts (154.33±4.47 and 60.57±3.96 pmol/mg, respectively; n=6; P<0.01), indicating that the level of active SERCA enzyme is 2.5-fold higher in TG hearts. These data are consistent with a 2.5-fold increase in the amount of total SERCA protein in TG hearts, demonstrating that the overexpressed SERCA1a pumps are functional.

To study the effects of SERCA1a overexpression on cardiac SR calcium transport function, the initial rates of ATP-dependent, oxalate-facilitated SR Ca2+ uptake were measured. As shown in Figure 3, Ca2+ uptake rates were increased significantly in TG hearts compared with NTG hearts over the whole range of free Ca2+ concentrations (0.03 to 3 μmol/L). The maximal velocity of Ca2+ uptake was 1.7-fold higher in TG hearts compared with NTG hearts (165.87±8.05 and 97.90±0.51 nmol/mg per minute, respectively; n=4; P<0.01; Figure 3).
Cardiomyocytes Isolated from SERCA1a TG Hearts Demonstrate Faster Ca^{2+} Transients and Higher Contractility

To examine whether SERCA1a pump overexpression alters intracellular free Ca^{2+} kinetics, left ventricular myocytes isolated from TG and NTG hearts were loaded with Fura-2 AM, and the phasic Ca^{2+} signals during electrical pacing at 0.5 Hz (30 bpm) were examined (Figure 4). The baseline cytosolic calcium signal was decreased by 38.1% in SERCA1a TG myocytes (Table 1), suggesting a higher degree of Ca^{2+} sequestration by SR. As a result, the amplitude of calcium signal was increased by 71.8% in TG myocytes. Although the 18% decrease in the absolute time for 80% decay of Ca^{2+} signal (T_{80}) in TG myocytes was not statistically significant (P=0.146), TG myocytes showed a 51.6% decrease (P<0.05) in T_{80} normalized to the amplitude of calcium signal (T_{80,nor}), suggesting considerably faster Ca^{2+} removal from the cytosol (Table 1). The normalization was performed, because T_{80} is a linear function of the calcium signal (which is increased) and the rate of decay of the signal (which is accelerated).46

To determine how altered Ca^{2+} kinetics affect contractility, mechanical properties of isolated cardiomyocytes were ex-
amined. Although TG and NTG myocytes showed no significant difference in the cell length and the extent of cell shortening, \( \frac{1}{dL/dt} \) and \( \frac{2}{dL/dt} \) were increased in TG myocytes by 50.0% and 66.6%, respectively (Table 1).

**SERCA1a TG Hearts Demonstrate Significantly Increased Contractility**

To determine whether elevated \( \mathrm{Ca}^{2+} \) transport function in SERCA1a TG hearts would affect contractile performance, we implemented the isolated work-performing mouse heart preparations\(^{41–43} \). This allowed us to compare TG and NTG hearts at similar loading conditions without the influence of neural or hormonal input. At 50 mm Hg mean aortic pressure (afterload) and 5 mL/min venous return (an approximation of preload), TG hearts showed similar heart rates and left ventricular minute work (Table 2). At the same time, the systolic pressure in TG hearts was increased by 17 mm Hg, and the diastolic pressure was decreased by 4 mm Hg, without a significant change in the end-diastolic pressure (Table 2). The maximal rates of pressure development for contraction (+dP/dt) and relaxation (−dP/dt) were increased by 41.9% and 39.5%, respectively, and the TPP and the RT\(_{1/2}\) decreased by 29.1% and 32.7%, respectively, in TG hearts (Figure 5; Table 2).

**Discussion**

The data presented in this article clearly demonstrate that overexpression of the fast-twitch skeletal muscle SR \( \mathrm{Ca}^{2+} \)-ATPase in the mammalian heart results in significantly elevated \( \mathrm{Ca}^{2+} \) transport function and cardiac contractile performance. This is the first demonstration that SERCA1a can substitute functionally for SERCA2a in the intact heart. In addition, our studies suggest that the SERCA pump level is a critical determinant of myocardial contractility.

The work-performing heart preparations used in the present study enabled us to make definitive functional comparisons between TG mice and their wild-type NTG littermates. Although the SERCA1a TG mice and NTG mice had similar heart rates, body weights, and heart weight–to–body weight ratios, TG hearts revealed significantly altered cardiac contractile parameters: higher systolic and lower diastolic IVP, increased rates of pressure development for contraction (+dP/dt) and relaxation (−dP/dt), and shorter TPP and RT\(_{1/2}\).
TABLE 1. Calcium Transients and Contractile Parameters of Isolated Cardiomyocytes

<table>
<thead>
<tr>
<th>Calcium transients</th>
<th>NTG (n=6)</th>
<th>TG (n=6)</th>
<th>(p) (Student t Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC, 340 nm/380 nm U</td>
<td>1.065±0.0429</td>
<td>0.659±0.0545</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CA, 340 nm/380 nm U</td>
<td>0.390±0.0499</td>
<td>0.670±0.0657</td>
<td>0.007</td>
</tr>
<tr>
<td>T80, s</td>
<td>0.888±0.0692</td>
<td>0.726±0.0751</td>
<td>0.146</td>
</tr>
<tr>
<td>T80/nor, s</td>
<td>2.774±0.483</td>
<td>1.342±0.243</td>
<td>0.035</td>
</tr>
</tbody>
</table>

Contractile parameters

| CL, \(\mu m\) | 115.28±5.20 | 111.38±2.58 | 0.51 |
| PC, % | 4.92±0.61 | 6.46±1.09 | 0.246 |
| \(+dL/dt, \mu m/s\) | 180.93±11.03 | 267.77±31.57 | <0.05 |
| \(-dL/dt, \mu m/s\) | 148.47±12.68 | 247.36±36.78 | <0.05 |

n indicates No. of animals; BC, base calcium; CA, calcium amplitude; CL, cardiomyocyte length; and PC, % of cell shortening. Left ventricular myocytes were paced at 0.5 Hz (30 bpm). Values represent mean±SEM.

The cardiac myocyte data displayed in Figure 4 and Table 1 provide a mechanism for the enhanced rates of pressure development and decay that were observed in the isolated work-performing preparations of SERCA1a TG hearts. Analysis of \(Ca^{2+}\) transients using Fura-2 AM indicated that the peak cytosolic calcium signal was not significantly different between TG and NTG myocytes. However, TG myocytes showed a considerably decreased basal cytosolic calcium signal. As a result, the amplitude of calcium cycling produced more rapid rates of myocyte shortening and relengthening in the absence of a significant increase in the extent of shortening. It is important to emphasize that Fura-2 fluorescence dye indicators permit a qualitative assessment of change in free cytosolic calcium over time. This and other calcium-sensitive microelectrodes permits accurate measurement of mean free cytosolic calcium concentration but does not permit a dynamic assessment of calcium cycling. Although it is possible to provide an estimate of absolute calcium concentration using fluorescent indicators, we and others have avoided this approach because of variable and unpredictable subcompartmentalization of calcium within the mitochondria.

In this study, we demonstrated that by ectopic expression of SERCA1a, we can achieve a 2.5-fold increase in the total amount of SERCA protein, with 80% represented by SERCA1a. The unaltered expression pattern of other proteins suggested that SR volume was not changed, but the SERCA pump density was increased. The corresponding 2.5-fold increase in the steady-state level of E-P revealed that the expressed SERCA1a pump is functional. Indeed, TG hearts demonstrated significantly higher rates of \(Ca^{2+}\) uptake, suggesting that the SERCA1a pump can substitute functionally the endogenous SERCA2a isofrom. Recent attempts to overexpress the SERCA2a cardiac isoform in the heart, however, resulted only in a modest increase in the total pump level. In our laboratory, \(\alpha\)-MHC–driven SERCA2a overexpression resulted in a 4-fold increase in SERCA2a mRNA level but only in a 30% increase of total SERCA protein in mouse TG hearts. In a separate study, He et al. reported a 20% increase in the total pump level, achieved using the human cytomegalovirus (enhancer)–\(\beta\)-actin promoter. Similar results were reported for adenovirus-mediated gene transfer into cardiac myocytes. The adenoviral transfer of rat SERCA2a into rat ventricular neonatal myocytes resulted in a 1.1-fold increase, whereas the transfer of rabbit SERCA2a resulted in a 1.5-fold increase in the total SERCA protein. It should also be noted that the amount of SERCA protein is significantly lower in neonatal cardiac SR compared with adult cardiac SR.

Recently, Inesi et al. using adenovirus-mediated gene transfer, showed that the SERCA1 pump can be overex-

TABLE 2. Functional Parameters of SERCA1a TG Hearts

<table>
<thead>
<tr>
<th></th>
<th>NTG (n=8)</th>
<th>TG (n=7)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>337±3.7</td>
<td>351±7.2</td>
<td>0.096</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg) (afterload)</td>
<td>50.0±0.05</td>
<td>50.2±0.04</td>
<td>Set</td>
</tr>
<tr>
<td>Venous return (mL/min)</td>
<td>5.1±0.01</td>
<td>5.1±0.02</td>
<td>Set</td>
</tr>
<tr>
<td>LV minute work (mm Hg×mL per min)</td>
<td>255.5±2.8</td>
<td>252±22</td>
<td>Set</td>
</tr>
<tr>
<td>Intraventricular pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>100±0.5</td>
<td>117±1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic</td>
<td>-6±0.3</td>
<td>-10±0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>End-diastolic</td>
<td>8.1±0.3</td>
<td>7±0.6</td>
<td>0.112</td>
</tr>
<tr>
<td>Contraction properties</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+dP/dt, mm Hg/s)</td>
<td>4185±35.9</td>
<td>5937±72.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TPP (ms/mm Hg)</td>
<td>0.406±0.0034</td>
<td>0.288±0.0035</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Relaxation properties</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-dP/dt, mm Hg/s)</td>
<td>-3430±46.1</td>
<td>-4785±82.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RT1/2 (ms/mm Hg)</td>
<td>0.456±0.0067</td>
<td>0.307±0.0053</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
pressed in chick embryonic cardiac myocyte. SERCA1 overexpression down-regulated SERCA2a but resulted in a 4-fold increase in ATP-dependent calcium uptake. This study and our results suggest that the SERCA1 pump can be expressed at a much higher level in cardiac myocytes. The higher level of SERCA1a expression in TG hearts may be due to unique properties of the SERCA1a pump, including more efficient integration sites. Ectopic expression in the heart of some other muscle proteins, including β-tropomyosin and fast-twitch skeletal muscle myosin light chain isoform MLC2, also resulted in functional replacement of the endogenous SERCA2a isoform in the heart without producing any cardiac pathology. Furthermore, we have shown that the amount of SERCA protein in the heart can be increased up to 2.5-fold by overexpression, suggesting that the cardiac SR can accommodate more SERCA protein. Most importantly, we have shown that SERCA1a overexpression leads to significantly elevated contractile performance, demonstrating that the SR Ca\(^{2+}\)-ATPase level is a critical factor in determining cardiac contractility. Our studies pave the way for the potential use of the SERCA1a pump for gene therapy to enhance myocardial performance in failing hearts. In addition, the SERCA1a TG model is an excellent system to use when studying how changes in the level of SR Ca\(^{2+}\)-ATPase can influence overall intracellular Ca\(^{2+}\) homeostasis and cardiac muscle contractility.

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