Sarcoplasmic Reticulum Ca\(^{2+}\) ATPase (SERCA) 1a Structurally Substitutes for SERCA2a in the Cardiac Sarcoplasmic Reticulum and Increases Cardiac Ca\(^{2+}\) Handling Capacity

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Abstract—Ectopic expression of the sarcoplasmic reticulum (SR) Ca\(^{2+}\) ATPase (SERCA) 1a pump in the mouse heart results in a 2.5-fold increase in total SERCA pump level. SERCA1a hearts show increased rates of contraction/relaxation and enhanced Ca\(^{2+}\) transients; however, the cellular mechanisms underlying altered Ca\(^{2+}\) handling in SERCA1a transgenic (TG) hearts are unknown. In this study, using confocal microscopy, we demonstrate that SERCA1a protein traffics to the cardiac SR and structurally substitutes for the endogenous SERCA2a isoform. SR Ca\(^{2+}\) load measurements revealed that TG myocytes have significantly enhanced SR Ca\(^{2+}\) load. Confocal line-scan images of field-stimulated SR Ca\(^{2+}\) release showed an increased rate of Ca\(^{2+}\) removal in TG myocytes. On the other hand, ryanodine receptor binding activity was decreased by ≈30%. However, TG myocytes had a greater rate of spontaneous ryanodine receptor opening as measured by spark frequency. Whole-cell L-type Ca\(^{2+}\) current density was reduced by ≈50%, whereas the time course of inactivation was unchanged in TG myocytes. These studies provide important evidence that SERCA1a can substitute both structurally and functionally for SERCA2a in the heart and that SERCA1a overexpression can be used to enhance SR Ca\(^{2+}\) transport and cardiac contractility. (Circ Res. 2001;89:160-167.)

Key Words: transgenic \(\square\) contractility \(\square\) gene therapy \(\square\) Ca\(^{2+}\) load \(\square\) Ca\(^{2+}\) uptake \(\square\) sparks

The sarcoplasmic reticulum (SR) Ca\(^{2+}\)-ATPase (SERCA) is a major component of beat-to-beat Ca\(^{2+}\) cycling in the heart. Reduction of SERCA pump expression and activity has been linked to diastolic dysfunction in hypertrophied and failing hearts. Previous studies from our laboratory and others have shown that overexpression of both SERCA1a and SERCA2a in the mouse heart leads to enhanced Ca\(^{2+}\) transport with a concomitant boost in contractility. Similarly, adenosine-mediated gene transfer of SERCA gene into adult myocytes results in increased contractility, with an increased rate of Ca\(^{2+}\) uptake and release. A recent study by Miyamoto et al. shows that adenoviral gene transfer of SERCA2a improves cardiac function in an aortic-banded rat heart failure model. Taken together, these in vivo and in vitro studies suggest that increased expression is feasible and results in enhanced Ca\(^{2+}\) transport and contractility.

SERCA1a has been shown to have faster Ca\(^{2+}\) transport kinetics, and it is associated with faster rates of contraction and relaxation. To investigate whether SERCA1a expression in the heart leads to faster Ca\(^{2+}\) cycling and increased contractility, we generated SERCA1a transgenic (TG) mice. SERCA1a overexpression results in a 2.5-fold increase in total SERCA pump levels in TG heart and a ≈2-fold increase in SR Ca\(^{2+}\) uptake function. SERCA1a overexpression levels are consistently maintained at 2.5-fold above controls in successive generations, indicating that expression levels do not diminish with germline transmission/aging.

A major goal of this study was to investigate how high levels of SERCA pump overexpression in the heart alter intracellular Ca\(^{2+}\) homeostasis. Specifically, we investigate the following: (1) whether SR Ca\(^{2+}\) stores, Ca\(^{2+}\) release, and Ca\(^{2+}\) uptake functions are altered; (2) whether SERCA1a is structurally and functionally an integral component of cardiac SR; (3) whether long-term expression of SERCA1a can be detrimental to myocyte structure and function; and (4) whether overexpression of SERCA pump leads to alter-
ations in the protein levels or functional performance of other SR and sarcolemmal Ca\(^{2+}\) handling proteins.

**Materials and Methods**

**Isolation of Ventricular Myocytes**

Ventricular myocytes were isolated from wild-type (WT) and SERCA1a hearts from adult mice (10 to 16 weeks old) using a Langendorff perfusion system with 0.8 mg/mL collagenase type 2 for 12 to 20 minutes until the heart became soft.\(^1\)\(^2\)

**Fluorescent Antibody Staining/Confocal Microscopy**

Adult cardiac myocytes were fixed in 4% paraformaldehyde/PBS, permeabilized with 0.2% Triton X-100, exposed overnight to appropriate antibody, and viewed by confocal microscopy.\(^2\)\(^2\) Slides were quickly scanned to determine an appropriate intensity setting, and this setting was used for all samples.

**Intracellular Ca\(^{2+}\) Measurements**

Intracellular free Ca\(^{2+}\) transients were measured as ratio of 340 to 380 nm excitation fluorescence of fura-2 acetoxymethyl ester (AM) (emission wavelength, 510 nm) using a photo scan dual spectrofluorometer in individual myocytes loaded with 7.5 μmol/L fura-2 AM at 37°C for 10 to 15 minutes in the dark.\(^3\)\(^4\) Cells were field stimulated at 0.5 Hz (Grass SD9 stimulator) until twitch characteristics were repeatable. Caffeine was applied for 10 seconds.

**Line-Scan Imaging and Ca\(^{2+}\) Spark Analyses**

Line-scan imaging was performed using fluo-3 Ca\(^{2+}\) fluorescent indicator and confocal microscopy (Molecular Dynamics). A single cell was scanned repetitively at 500 Hz for 5 seconds along a horizontal line. Ca\(^{2+}\) sparks were recorded from a central region of the cell using the x-y scan mode with pixels set to 512×512.

**L-Type Ca\(^{2+}\) Channel Current Measurements**

Whole-cell Ca\(^{2+}\) channel currents were recorded as reported.\(^2\)\(^5\) – \(^2\)\(^6\) External solution contained the following (in mmol/L): CaCl\(_2\) 2 or BaCl\(_2\) 2, MgCl\(_2\) 1, TEA-Cl 135, 4-aminopyridine 5, glucose 10, and HEPES (pH 7.3) 10. Pipette solution contained (in mmol/L) cesium aspartate 100, CsCl 20, MgCl\(_2\) 1, MgATP 2, GTP 0.5, EGTA 5, and HEPES 5 (pH 7.3). Membrane capacitance was measured using voltage ramps of 0.8 V/second from a holding potential of ~50 mV. Rapid solution changes were made using a modified Y-tube.\(^2\)\(^3\)

**\[^{3}\]H\)**Ryanodine Receptor (RyR) Binding Assay

Total ryanodine binding to cardiac homogenates was measured after incubation with \[^{3}\]H\)ryanodine (56.9 Ci/mmol, DuPont New Research Products) for 90 minutes at 37°C.\(^2\)\(^7\) Binding data were analyzed by a radioligand analysis program (G.A. McPherson, Elsevier-BIOSOFT).

**Ribonuclease Protection Assays**

The riboprobes for mouse cardiac RyR and rat SERCA1a were generated from respective cDNA clones. Ribonuclease protection assay was performed using the RPAIII kit (Ambion, Inc), and protected fragments were separated by electrophoresis in a 5% denaturing polyacrylamide gel.\(^2\)\(^8\)

**Quantitative Immunoblotting**

Quantitative immunoblotting of cardiac homogenates was used to determine the protein levels of SERCA1a, RyR, sodium-calcium exchanger, triadin, L-type channel, and actin.\(^1\)\(^2\)\(^9\) Homogenates were electrophoretically separated, blotted to membrane, and probed with appropriate antibodies. Quantification of the signals was performed by densitometry (UMAX Astra 1200) and analyzed (NIH Image, version 6.1).\(^1\)\(^2\)\(^3\)

**Simultaneous Intracellular Ca\(^{2+}\) and Twitch Force Measurements**

Geometrically regular trabeculae (dimensions in mm, 1.03±0.20 length, 0.26±0.09 width, and 0.13±0.04 thickness) were mounted to force transducers and superfused with buffer.\(^2\)\(^9\)–\(^3\)\(^2\) After equilibration, trabeculae were stimulated at 1.0 Hz using a Grass SD9 stimulator. Fura-2 potassium salt was microinjected\(^3\)\(^3\) and [Ca\(^{2+}\)]\(_i\) was determined by measuring the epifluorescence of fura-2 signal. [Ca\(^{2+}\)]\(_i\) was calculated using the Grynkiewicz equation.\(^3\)\(^4\)

**Statistical Analyses**

WT and SERCA1a parameters were compared using the Student t test and/or ANOVA. Results are expressed as mean±SEM.

**Results**

**SERCA1a Structurally Substitutes for SERCA2a in the Heart**

To link enhanced Ca\(^{2+}\) transport with SERCA1a overexpression, it was necessary to show at the subcellular level that SERCA1a is an integral component of the cardiac SR. Immunostaining of adult mouse myocytes with tropomodulin, an actin binding protein, gave a uniform striated pattern in rod-shaped cells (Figure 1, top and middle, green); this striated pattern was used as a selection criterion. Cells were costained with SERCA1a antibody (red) to determine the pattern of SERCA1a distribution. WT cells showed no specific staining with SERCA1a antibody (Figure 1, upper left), whereas SERCA1a TG myocytes showed a distinctive horizontal and vertical pattern (Figure 1, middle and bottom), which was indistinguishable from that seen with SERCA2a antibody staining (Figure 1, bottom, green). Thus, these data demonstrate that SERCA1a and SERCA2a exhibit subcellular colocalization within the limits of resolution of confocal microscopy (200 nm).

To determine possible long-term detrimental effects of SERCA1a overexpression, blinded comparative histological studies were performed from 20-month-old animals. Both WT hearts (Figure 2A, bottom panels) and TG hearts (Figure 2A, top panels) showed mild myocyte hypertrophy, fibrosis, and nuclear rowing. No significant morphological differences were evident at the gross anatomic level (Figure 2A, left panels, ×10; right panels, ×40). In addition, both control and TG mice had the same mortality curves. Consistent with histological analysis, there was no change in size of left ventricular myocytes as estimated by the cell capacitance (127.2±3.4 pF [n=60, WT] versus 118.9±3.3 pF [n=78, TG]). Thus, SERCA1a overexpression does not result in pathology or hypertrophy. To confirm that these aged hearts still expressed SERCA1a at high levels, Western blot analysis was performed. Figure 2B clearly shows robust expression of SERCA1a in both male (M) and female (F) mice at 20 months.

**Ca\(^{2+}\) Transient Amplitude and Contractility Are Altered in SERCA1a Hearts**

A major target of Ca\(^{2+}\) released from the SR is troponin-C, which on binding Ca\(^{2+}\) undergoes a conformational change and initiates actin-myosin interaction. By mass action, increased [Ca\(^{2+}\)]\(_i\) leads to increased Ca\(^{2+}\)–troponin-C complex activation and results in greater force production. Thus, to determine how Ca\(^{2+}\) cycling and force were altered in...
SERCA1a overexpressing heart muscle, contractile force and intracellular Ca\(^{2+}\) were measured simultaneously in fura-2–loaded isometrically contracting trabeculae. Figure 3A shows representative tracings of Ca\(^{2+}\) transients (top) and twitch force (bottom) in muscles from WT (left) and SERCA1a TG (right) hearts. Trabeculae from TG versus WT hearts exhibited an increase in Ca\(^{2+}\) transient amplitude (1.25 \pm 0.21 versus 0.9 \pm 0.11 \text{ mmol/L}) whereas the time course of Ca\(^{2+}\) removal was significantly shorter (74.9 \pm 3.5 versus 142.3 \pm 7.1 ms). Force generation was also increased in TG trabeculae (28.1 \pm 1.4 versus 14.8 \pm 3.0 \text{ mN/mm}^2 \text{ [n=4, TG and WT], } P<0.01), whereas relaxation times were unchanged. Diastolic [Ca\(^{2+}\)], was not different between the two groups. In summary, trabeculae data support isolated myocyte data and show that SERCA1a TG hearts have greater rates of Ca\(^{2+}\) cycling than do WT hearts.

**SERCA1a Overexpression Results in a Significant Increase in SR Ca\(^{2+}\) Load**

Both trabeculae (Figure 3) and isolated myocytes\(^{13}\) from SERCA1a hearts showed increased amplitude of calcium signal on field stimulation. Thus, we sought to test whether there was also an increased SR Ca\(^{2+}\) load. Caffeine binds to the RyR, keeping it open, thereby emptying the SR of Ca\(^{2+}\), giving a measure of total SR Ca\(^{2+}\) load (Figure 3B). We used 10-second pulses of caffeine to empty Ca\(^{2+}\) from the SR. Peak Ca\(^{2+}\) signal from SERCA1a myocytes was 2-fold greater than that seen in WT cells as measured by fura-2; thus, SERCA1a overexpression results in a significant increase in SR Ca\(^{2+}\) stores (Table 1).

The rate of Ca\(^{2+}\) removal after caffeine exposure is a measure of the ability of non-SR Ca\(^{2+}\) extrusion mechanisms to operate. We hypothesized that an increase in SR Ca\(^{2+}\) uptake function might diminish the role played by other Ca\(^{2+}\) removal mechanisms, such as the Na\(^{+}\)-Ca\(^{2+}\) exchanger (NCX).\(^{35,36}\) The exponential rate of decay of the Ca\(^{2+}\) signal, which is a measure of NCX activity, showed no significant difference in the first or second time constants (Figure 3B, Table 1). In addition, Western blot analysis (Figure 4A) revealed that there was no significant difference in NCX protein levels (2.59 \pm 0.08 versus 2.81 \pm 0.18, NS). This finding, coupled with the lack of change in the rates of Ca\(^{2+}\) removal after caffeine exposure, implies that NCX is not altered by SERCA1a overexpression.

**RyR Levels Are Decreased in SERCA1a TG Hearts**

We next sought to determine whether increased Ca\(^{2+}\) release was also partly due to alterations in RyR expression. Quan-
binding (Bmax) of the receptor is decreased by 32.5% in SERCA1a, whereas the Kd value remained unchanged (Table 2). RyR binding is Ca2+-dependent; thus, to investigate whether the decrease of the Bmax was due to a change in receptor sensitivity to Ca2+, we determined RyR binding in the presence of increasing free Ca2+ concentrations. The Ca2+ sensitivity of ryanodine binding (Kd) was unchanged. Thus, the difference in Bmax is due to decreased RyR level in SERCA1a hearts (Figure 4D; Table 2).

**SERCA1a Myocytes Showed Greatly Increased Frequency of Ca2+ Spark Activity and Enhanced Rate of Ca2+ Removal**

RyR activity is a critical determinant of SR Ca2+ release. In SERCA1a TG myocytes, RyR protein levels were decreased, yet global SR Ca2+ release was not decreased but rather increased (Figure 3). To determine whether the decrease in RyR protein level was offset by an increase in RyR channel opening, Ca2+ spark analysis was performed. Ca2+ sparks were recorded from healthy, quiescent WT and TG cells (Figure 5). SERCA1a myocytes showed greatly increased frequency of spark activity under basal conditions (≈2- to 4-fold increase over WT); spark amplitude was also approximately double in SERCA1a myocytes versus WT (1.83±0.48 versus 0.92±0.37 units; F/Fo). Often TG myocytes showed an “intense burst” of Ca2+ sparks after field stimulation, which lasted for 10 to 15 seconds. Cells then returned to a quiescent state and could be again field-stimulated.

In addition, we recorded line-scan images of Ca2+ transients from WT and transgenic myocytes loaded with fluo-3 and field-stimulated cells (Figure 5 top). Line-scan images were thinner in SERCA1a myocytes compared with WT; thus, the time course of Ca2+ removal in SERCA1a TG myocytes was significantly faster than in WT myocytes (109.8±13.7 ms [n=22, TG] versus 204.5±33.8 ms [n=36, WT]; P<0.001). This is consistent with trabecula data in Figure 3 and shows that overexpression of SERCA1a isoform leads to increased Ca2+ removal.

### L-Type Ca2+ Channel Current Amplitude Is Significantly Decreased in SERCA1a Myocytes, but Inactivation Time Is Unchanged

The cellular Ca2+ transients and contraction elicited by electric excitation are strongly influenced by the amount of Ca2+ influx through the L-type Ca2+ channel. Thus, we next determined whether L-type Ca2+ channel properties

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**TABLE 2. RyR Binding Studies**

<table>
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<th>Wild Type</th>
<th>SERCA1a</th>
<th>P Value</th>
<th>WT</th>
<th>TG</th>
<th>P Value</th>
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<tr>
<td>Bmax, nmol/L</td>
<td>112.6±6.83</td>
<td>75.9±5.60*</td>
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<tr>
<td>Kd, µmol/L</td>
<td>2.70±0.38</td>
<td>2.76±0.31</td>
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<td>5</td>
<td>NS</td>
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<tr>
<td>Hill coefficient</td>
<td>2.78±0.49</td>
<td>3.39±0.45</td>
<td>4</td>
<td>5</td>
<td>NS</td>
<td></td>
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<tr>
<td>Kd, µmol/L</td>
<td>0.37±0.03</td>
<td>0.40±0.01</td>
<td>4</td>
<td>5</td>
<td>NS</td>
<td></td>
</tr>
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</table>

Bmax and Kd are from Scatchard plot analysis of RyR binding study. Hill coefficient and Kd are from RyR binding vs pCa relationship. *Significant.

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**TABLE 1. Peak Ca2+ Amplitude and Rate of Decay After Caffeine Stimulation**

<table>
<thead>
<tr>
<th>Amplitude, F/Fo</th>
<th>Rate of Ca2+ Decline, 1/sec</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0.81±0.29</td>
<td>...</td>
</tr>
<tr>
<td>SERCA1a</td>
<td>1.64±0.42</td>
<td>&lt;0.01</td>
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</tbody>
</table>

Amplitude is peak Ca2+ transient of fura-2 ratio signal. Rate of Ca2+ decline was fitted using a double-exponential decay equation (y(t) = A0 + Ae(t/τ1) + Ae(t/τ2)). Tau1 is shown under Rate of Ca2+ Decline (n=17 [WT], n=19 [TG]).
were altered. Interestingly, peak Ca\(^{2+}\) current amplitude, normalized relative to cell capacitance (pA/pF), was significantly decreased in SERCA\(^{1a}\) myocytes compared with WT (3.8 ± 0.2 pA/pF \[n = 65\] versus 8.5 ± 0.4 pA/pF \[n = 48\]; \(P < 0.001\); Figure 6A). There was no change in the voltage range for current activation (Figure 6B). In both groups, the current began to activate around \(-30 mV\) and reached its maximum value near \(-110 mV\). At this potential, Ca\(^{2+}\) current inactivated rapidly during maintained depolarization in both groups. Consistent with electrophysiological analysis, Western blotting analysis showed that channel protein expression was decreased \(-30\) to \(-35\)% in TG hearts (70.5 ± 3.2% normalized to WT; Figure 6D). This finding was corroborated by the amplitude of Ba\(^{2+}\) current through the L-type Ca\(^{2+}\) channels. Because Ba\(^{2+}\) can permeate the L-type Ca\(^{2+}\) channels, but Ba\(^{2+}\) itself cannot inactivate the channel or trigger the release of Ca\(^{2+}\) from the SR, it can be used as an effective charge carrier to record maximal channel amplitude. In the presence of Ba\(^{2+}\), maximal current in SERCA\(^{1a}\) myocytes was significantly smaller than in WT (6.3 ± 0.6 versus 10.9 ± 0.9, \(P < 0.001\), data not shown).

It has previously been shown that L-type Ca\(^{2+}\) current inactivation in mouse myocytes involves voltage-dependent and Ca\(^{2+}\)-dependent mechanisms and that the local increase in Ca\(^{2+}\) released from the SR promotes Ca\(^{2+}\)-dependent inactivation.\(^{24,25}\) Thus, we next analyzed the time to half-decay of the current (\(T_{1/2}\)). Although the L-type channel current amplitude was significantly reduced in SERCA\(^{1a}\) myocytes, there was no significant difference in inactivation time between the two groups (22.8 ± 1.2 ms \[n = 60, TG\] versus 20.9 ± 1.2 ms \[n = 45 WT\]).

Decreased current amplitude is often attended by prolonged inactivation time, as less Ca\(^{2+}\) entering via the L-type channel means there is less Ca\(^{2+}\) available for Ca\(^{2+}\)-induced channel inactivation. Thus, to further explore why channel inactivation is not prolonged in SERCA\(^{1a}\) myocytes, L-type currents were recorded in the presence of 10 \(\mu\)mol/L ryanodine. Inactivation time is prolonged in the presence of ryanodine, because ryanodine holds RyR in an open state,
effectively depleting the SR of Ca\(^{2+}\). Thus, the only Ca\(^{2+}\) available for channel inactivation in the presence of ryanodine is that which enters via the L-type channel itself. In the presence of ryanodine, inactivation time was longer in SERCA1a myocytes than in WT (39.6 ± 2.3 versus 34.6 ± 1.7 ms; Figure 6C). This implies that SR Ca\(^{2+}\) load and release play a role in shortening the L-type channel inactivation time in SERCA1a hearts.

Discussion

SERCA1a Traffics to the Cardiac SR and Structurally Substitutes for the Endogenous SERCA2a Isoform

The purpose of this study was to investigate the molecular basis for enhanced Ca\(^{2+}\) uptake and increased contractility in SERCA1a TG hearts. In this study we convincingly demonstrate that SERCA overexpression results not only in an increased rate of SR Ca\(^{2+}\) transport but increased SR Ca\(^{2+}\) load and release. We show for the first time that SERCA1a traffics to the cardiac SR and structurally substitutes for the endogenous SERCA2a isoform. This is consistent with our previous observation that SERCA1a overexpression results in a ≈50% reduction in endogenous SERCA2a pump levels, which tends to argue that SERCA1a and SERCA2a compete for the same “sites” within the SR. This finding is corroborated by the confocal immunostaining of isolated myocytes; SERCA1a and SERCA2a proteins show distribution patterns that are indistinguishable from each other. Recent studies using adenovirus-mediated gene transfer into embryonic cardiac myocytes showed that SERCA1a was targeted to intracellular membranes; cytosolic Ca\(^{2+}\) transients were greatly increased and rates of shortening and relengthening were faster. These data suggest that SERCA1a, a protein not endogenously found in cardiac tissue, can be ectopically expressed yet properly trafficked into the cardiac SR and can functionally substitute for SERCA2a.

Increase in SERCA Pump Level Contributes to Increased SR Ca\(^{2+}\) Load

Using caffeine to empty the SR free of Ca\(^{2+}\), we demonstrate that SR Ca\(^{2+}\) load is increased ≈2.0-fold in SERCA1a TG myocytes. This further confirms that ectopically expressed SERCA pump is functional in the cardiac environment. Our study suggests that an important effect of SERCA pump overexpression is to increase the SR Ca\(^{2+}\) load, which in turn is responsible for the increased intracellular Ca\(^{2+}\) transients. Previous studies have also shown a positive correlation between SERCA level and SR Ca\(^{2+}\) load.

Increase in SR Ca\(^{2+}\) Load Contributes to a Higher Frequency of RyR Channel Opening in SERCA1a Myocytes

An important finding of the SERCA1a hearts is that the increased rate of SR Ca\(^{2+}\) transport and release, 49 an increase in spark frequency, 46 a decrease in RyR protein levels, and unchanged RyR mRNA levels. Thus, it may be that RyR downregulation compensates for the increased SR Ca\(^{2+}\) load and thereby finely regulates Ca\(^{2+}\) release during excitation-contraction coupling.
Altered L-Type Channel Expression and Properties Help to Regulate Excitation-Contraction Coupling in SERCA1a Hearts

Ca²⁺ influx through the L-type Ca²⁺ channel is a critical trigger for SR Ca²⁺ release. In SERCA1a hearts, peak Ca²⁺ current amplitude (pA/pF) was significantly decreased (≈50%; Figure 6A). Western blotting analysis showed that L-channel α-subunit expression levels were decreased (≈30% to 35%). This finding was corroborated by measuring Ba²⁺ currents through the L-type Ca²⁺ channel. In the phospholamban knockout model, L-type current amplitudes are unchanged, whereas Ca²⁺ transients and increased SR Ca²⁺ load are similar to those seen in the SERCA1a TG model. It is unclear whether these differences are due to different genetic background (FVBN versus SVJ/BL6) and/or model.

Although the L-type Ca²⁺ current amplitude in SERCA1a TG myocytes was decreased ≈50% in comparison with WT myocytes, surprisingly, there was no change in time course of Ca²⁺ current inactivation. Because Ca²⁺ entry via the channel itself plays a role in channel inactivation, it is often the case that decreased channel amplitude is accompanied by prolonged channel inactivation. However, another important source of Ca²⁺ for channel inactivation is that released from the SR. 24,50,51 To address this question, Ca²⁺ currents were recorded in the presence of ryanodine to essentially remove the SR Ca²⁺-release component. L-type Ca²⁺-channel inactivation is prolonged in the presence of ryanodine because the only Ca²⁺ available for channel inactivation is Ca²⁺ that enters through the channel itself. In the presence of ryanodine, T_{1/2} was prolonged in SERCA1a myocytes. Thus, these data allow us to conclude that SR Ca²⁺ load and release plays an important role in channel inactivation in SERCA1a myocytes and that privileged communication may exist in this source of Ca²⁺ for channel inactivation that is released from the SR. 24,50,51

In conclusion, we show that SERCA1a can substitute both structurally and functionally for SERCA2a in the heart and that SERCA1a overexpression can be used to enhance SR Ca²⁺ transport and cardiac contractility. Thus, SERCA1a represents an attractive candidate for gene therapy in patients with impaired cardiac contractility.

Acknowledgments

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References


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