Excessive Sarcoplasmic/Endoplasmic Reticulum Ca\(^{2+}\)-ATPase Expression Causes Increased Sarcoplasmic Reticulum Ca\(^{2+}\) Uptake but Decreases Myocyte Shortening

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**Background**—Increasing sarcoplasmic/endoplasmic reticulum (SR) Ca\(^{2+}\)-ATPase (SERCA) uptake activity is a promising therapeutic approach for heart failure. We investigated the effects of different levels of SERCA1a expression on contractility and Ca\(^{2+}\) cycling. We tested whether increased SERCA1a expression levels enhance myocyte contractility in a gene-dose–dependent manner.

**Methods and Results**—Rabbit isolated cardiomyocytes were transfected at different multiplicities of infection (MOIs) with adenoviruses encoding SERCA1a (or \(\beta\)-galactosidase as control). Myocyte relaxation half-time was decreased by 10\% \((P=0.052)\) at SERCA1a MOI 10 and by 28\% at MOI 50 \((P<0.05)\). Myocyte fractional shortening was increased by 12\% at MOI 10 \((P<0.05)\) but surprisingly decreased at MOI 50 \((-22\%, P<0.05)\) versus control. SR Ca\(^{2+}\) uptake (in permeabilized myocytes) demonstrated a gene-dose–dependent decrease in \(K_n\) by 29\% and 46\% and an increase in \(V_{\text{max}}\) by 37\% and 72\% at MOI 10 and MOI 50, respectively (all \(P<0.05\) versus control). Ca\(^{2+}\) transient amplitude was increased in Ad-SERCA1a–infected myocytes at MOI 10 (by 121\%, \(P<0.05\)) but at MOI 50, the Ca\(^{2+}\) transient amplitude was not significantly changed. Caffeine-induced Ca\(^{2+}\) transients indicated significantly increased SR Ca\(^{2+}\) content in Ad-SERCA1a–infected cells, by 72\% at MOI 10 and by 87\% at MOI 50. Mathematical simulations demonstrate that the functional increase in SR Ca\(^{2+}\)-ATPase uptake activity at MOI 50 (and increased cytosolic Ca\(^{2+}\) buffering) is sufficient to curtail the Ca\(^{2+}\) transient amplitude and explain the reduced contraction.

**Conclusions**—Moderate SERCA1a gene transfer and expression improve contractility and Ca\(^{2+}\) cycling. However, higher SERCA1a expression levels can impair myocyte shortening because of higher SERCA activity and Ca\(^{2+}\) buffering.

*Key Words:* heart failure ■ gene therapy ■ calcium ■ sarcoplasmic reticulum

Changes in myocardial excitation-contraction coupling are important in the pathophysiology of heart failure. Increased expression of Na\(^{+}\)-Ca\(^{2+}\) exchanger (NCX), altered ryanodine receptor (RyR) function, and decreased expression of SR Ca\(^{2+}\)-ATPase (SERCA) contribute to reduced SR Ca\(^{2+}\) accumulation. Furthermore, an increased phospholamban (PLB)/SERCA2a ratio reduces SERCA activity.\(^1\)

Decreased SR Ca\(^{2+}\) uptake activity slows relaxation and depresses contractility (because of reduced SR Ca\(^{2+}\) loading). In mice, disruption of 1 copy of the SERCA2 gene reduced the maximum velocity of SR Ca\(^{2+}\) uptake in homogenates, SR Ca\(^{2+}\) content and contractility in isolated cardiomyocytes, and in vivo cardiac performance.\(^2,3\)

Thus, restoring SR Ca\(^{2+}\) uptake may be valuable therapeutically in heart failure. Accordingly, transgenic mice overexpressing SERCA2a exhibited improved cardiac function and Ca\(^{2+}\) handling.\(^4,5\) In neonatal rat cardiomyocytes with normal and depressed SERCA2a expression, adenovirus-mediated overexpression of SERCA2a resulted in enhanced SR Ca\(^{2+}\) uptake and accelerated decay of Ca\(^{2+}\) transients.\(^6,7\) Furthermore, catheter-based transfection with an adenovirus encoding SERCA2a restored cardiac function in rats in transition to heart failure\(^8\) and improved survival.\(^9\) In human cardiomyocytes isolated from end-stage failing hearts, adenovirus-mediated augmented expression of SERCA2a resulted in enhanced contractility and Ca\(^{2+}\) handling.\(^10\)
Here, we tested the hypothesis that increasing levels of SERCA expression progressively enhance contractility and relaxation rate. We used adenovirus-mediated gene transfer of SERCA1a into isolated rabbit ventricular cardiomyocytes. SERCA1a is a splice transcript of the SERCA1 gene (expressed in adult fast-twitch skeletal muscle) that has faster Ca\(^{2+}\) transport kinetics\(^1\) and might achieve higher levels of SERCA activity than equivalent upregulation of SERCA2a.\(^2\) Rabbit ventricular myocytes were used because excitation-contraction coupling in rabbit myocardium is similar to that in humans.\(^3\),\(^4\)

**Methods**

**Primary Culture of Rabbit Ventricular Myocytes and Adenovirus Transfection**

Ventricular cardiomyocytes were isolated from adult female Chinchilla Bastard rabbits (2.0 to 2.5 kg) as previously described.\(^5\) Myocytes were counted, and adenoviral transfection was performed at the indicated multiplicity of infection (MOI) while the cells were plated on laminin-coated 35-mm dishes at a density of 5 \times 10^6 rod-shaped myocytes/dish. This study was designed and performed in accordance with institutional guidelines for the care and use of animals.

**Recombinant Adenovirus**

Adenovirus containing the SERCA1a gene (Ad-SERCA1a) was generated as described previously.\(^6\) Briefly, cDNA of the chicken SERCA1a gene was expressed as a fusion protein with a c-terminal c-myc tag under control of the constitutively active cytomegalovirus promoter.

**Verification of Transgene Expression and Expression Pattern**

Reverse transcriptase–polymerase chain reaction (RT-PCR) was performed after 48 hours of culture. Specific primers for chicken SERCA1a and rabbit calsequestrin were used for amplification. For Western immunoblot, a polyclonal anti–c-myc antibody (PA1-981, ABR) was used at a dilution of 1:5000 to detect the expressed protein.

**Single Myocyte Shortening and Intracellular Ca\(^{2+}\) Measurements**

The stimulation frequency was 1 Hz, and myocytes were continuously perfused with Krebs buffer (2 mmol/L CaCl\(_2\), at 36°C to 37°C). In separate experiments, intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) was measured with Fura-2 (-AM loaded) was used as previously outlined.\(^7\) After 60 to 90 seconds of continuous 1-Hz stimulation, [Ca\(^{2+}\)]\(_i\) was measured with Fura-2 (10 \mu mol/L, Molecular Probes) and ruthenium red (2.7 \mu mol/L) to block SR Ca\(^{2+}\) efflux.

**Measurements of SR Ca\(^{2+}\) Uptake Characteristics**

Myocytes (\(=5\times10^6/mL\), 48 hours after transfection) were permeabilized using 0.1 mg/mL \(\beta\)-escin. Myocyte suspensions (\(=4\times10^6\) cells/mL) were stirred, and Fura-2 (10 \mu mol/L, Molecular Probes) was used to monitor [Ca\(^{2+}\)]\(_i\) (20°C to 22°C). Oxalate (10 mmol/L) was included to maintain low and constant intra-SR [Ca\(^{2+}\)]\(_i\) and ruthenium red (2.7 \mu mol/L) to block SR Ca\(^{2+}\) efflux.

**Mathematical Modeling and Analysis**

Simulations were based on mean measured Ca\(^{2+}\) transient parameters (see Table 2). Equations were derived to describe the intrinsic [Ca\(^{2+}\)]\(_i\), dependence of Ca\(^{2+}\) transport by the SR Ca\(^{2+}\) pump (J\(_{\text{SR, Pump}}\)) and NCX (J\(_{\text{NCX}}\), reported in \mu mol/L cytosol per second (or simply \mu mol/L/s).\(^8\) J\(_{\text{SR, Pump}}\) transients were simulated using these fluxes plus fluxes for SR Ca\(^{2+}\) release (J\(_{\text{SR, rid}}\)) and Ca\(^{2+}\) current (I\(_{\text{Cai}}\)) as required to produce the measured Ca\(^{2+}\) transient characteristics.

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MOI 50) is not a result of the different MOIs (not shown) but is likely to be a result of variation in isolated myocytes batches, in which MOI 10 studies (±SERCA1a) were performed in 1 set of cells and the MOI 50 studies were performed with separate batches of cells on different days. To exclude the influence of isolation and culture, mean values for each experimental day (for LacZ and SERCA) were compared using a paired t test (Table 1). Ca\(^{2+}\) transient measurements were made separately, and both MOI values were studied at the same time.

**Stimulated and Caffeine-Induced Ca\(^{2+}\) Transients**

As shown in Figures 3 and 4 and Table 2, the Ca\(^{2+}\) transient amplitude in cells infected with Ad-SERCA1a at MOI 10 was increased significantly, by 121%, versus control (Ad-LacZ), whereas the 18% increase in Ca\(^{2+}\) transient amplitude at MOI 50 was not significant (versus control). At MOI 10, diastolic [Ca\(^{2+}\)], was not significantly different, but peak [Ca\(^{2+}\)], was increased by 75%. At MOI 50, diastolic [Ca\(^{2+}\)], was decreased significantly, by 32%, but peak [Ca\(^{2+}\)], was not significantly different from that with Ad-LacZ-50. At both MOIs, the rate constant of the decline of [Ca\(^{2+}\)], was increased in the Ad-SERCA1a group compared with the control cells (Table 2). As illustrated in Figures 3 and 4, SR Ca\(^{2+}\) content (assessed by rapid caffeine application) after 1-Hz stimulation was 72% higher in Ad-SERCA1a (MOI 10) than in control Ad-LacZ-10 myocytes. At MOI 50, caffeine-induced Ca\(^{2+}\) transient was 87% higher compared with the Ad-LacZ control group (Figure 4, Table 2). Thus, there is a SERCA1 gene-dose–dependent increase in the rate of diastolic [Ca\(^{2+}\)], decline, consistent with increased SR Ca\(^{2+}\)-ATPase function, but Ca\(^{2+}\) transient amplitude did not increase at the higher gene dose, and contraction decreased.

**SR Ca\(^{2+}\) Uptake Rates**

As shown in Figure 5, oxalate-supported Ca\(^{2+}\) uptake was measured in permeabilized cardiomyocytes after transfection with Ad-LacZ and Ad-SERCA1a. The decay of the [Ca\(^{2+}\)] in the cuvette after addition of an aliquot of CaCl\(_2\) (50 nmol) was faster after Ad-SERCA1a transfection compared with the Ad-LacZ control, indicating increased Ca\(^{2+}\) uptake rate (Figure 5C). A quantitative description of SERCA activity was obtained by calculating the changes of total [Ca\(^{2+}\)] on the basis of the known Ca\(^{2+}\) buffering capacity of the extracellular solution (Figure 5A). Differentiation of the total [Ca\(^{2+}\)] signal reveals the rate of Ca\(^{2+}\) uptake that can be plotted against the associated free [Ca\(^{2+}\)] to obtain a sigmoidal relationship (Figure 5B). This relationship was fitted with a

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**TABLE 1. Mechanical Parameters of Isotonically Contracting Myocytes**

<table>
<thead>
<tr>
<th></th>
<th>Ad-LacZ MOI-10</th>
<th>Ad-SERCA1a MOI-10</th>
<th>Ad-LacZ MOI-50</th>
<th>Ad-SERCA1a MOI-50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolations/cells, n</td>
<td>11/91</td>
<td>11/89</td>
<td>8/73</td>
<td>8/71</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>2.24±0.1</td>
<td>2.51±0.1*</td>
<td>2.83±0.4</td>
<td>2.22±0.4*</td>
</tr>
<tr>
<td>TTP, ms</td>
<td>170±8</td>
<td>153±9*</td>
<td>156±14</td>
<td>120±10*</td>
</tr>
<tr>
<td>RT(_{50}), ms</td>
<td>107.5±5.5</td>
<td>96.1±5.3†</td>
<td>98.1±8.9</td>
<td>70.2±7.4*</td>
</tr>
</tbody>
</table>

Fractional shortening is expressed as percentage of diastolic cell length; TTP, time to minimal cell length; and RT\(_{50}\), time from minimal cell length to 50% relaxation.

\(P<0.05\) vs Ad-LacZ; \(\dagger P=0.052\) vs Ad-LacZ.

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**Figure 2.** SERCA1a dose–dependent changes in myocyte contraction. Contraction parameters were normalized against Ad-LacZ–transfected controls (at same MOI). A representative recording from a control cell is shown. Fractional shortening is expressed relative to diastolic cell length; TTP, time to peak shortening; RT\(_{50}\), time from peak shortening to half relaxation. \(*P<0.05\) vs Ad-LacZ at corresponding MOI; †\(P=0.052\) vs Ad-LacZ at corresponding MOI.

**Figure 3.** Ca\(^{2+}\) transients. A, [Ca\(^{2+}\)] recorded 48 hours after transfection with (i) Ad-LacZ (MOI 10), (ii) Ad-SERCA1a (MOI 10), and (iii) Ad-SERCA1a (MOI 50). Last 3 steady-state twitches at 1 Hz and caffeine-induced Ca\(^{2+}\) transients are shown. B, Average and normalized twitch Ca\(^{2+}\) transients from these groups.
logistic curve to estimate the free [Ca\textsuperscript{2+}] that generated half the maximal Ca\textsuperscript{2+} uptake rate (K\textsubscript{m}) and the value of the maximal rate of Ca\textsuperscript{2+} uptake (V\textsubscript{max}). Table 2 shows mean values of V\textsubscript{max} and K\textsubscript{m} for Ad-LacZ and Ad-SERCA1a transfection at the 2 MOIs used. Cells infected with Ad-LacZ (MOI 10 versus MOI 50) exhibited comparable Ca\textsuperscript{2+} affinity (K\textsubscript{m}) and V\textsubscript{max}. Transfection with Ad-SERCA1a decreased the K\textsubscript{m} of SERCA-mediated SR Ca\textsuperscript{2+} uptake by 29% and 46% (at MOI 10 and MOI 50, respectively, versus controls). The V\textsubscript{max} increased by 37% and 71% at MOI 10 and MOI 50, respectively (versus controls). This is consistent with a gene-dose–dependent increase in SR Ca\textsuperscript{2+}-ATPase function.

**TABLE 2.** Parameters of SR-Ca\textsuperscript{2+} Uptake Activity and Intracellular Ca\textsuperscript{2+} Measurements

<table>
<thead>
<tr>
<th></th>
<th>Ad-LacZ MOI-10</th>
<th>Ad-SERCA1a MOI-10</th>
<th>Ad-LacZ MOI-50</th>
<th>Ad-SERCA1a MOI-50</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SR Ca\textsuperscript{2+} uptake in cell aggregates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolations/measurements, n</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>K\textsubscript{m}, nmol/L</td>
<td>819±225</td>
<td>581±12*</td>
<td>829±22</td>
<td>448±20*</td>
</tr>
<tr>
<td>V\textsubscript{max}, nmol/10\textsuperscript{6} cells/s</td>
<td>2.02±0.13</td>
<td>2.77±0.16*</td>
<td>1.90±0.17</td>
<td>3.26±0.42*</td>
</tr>
<tr>
<td><strong>Ca\textsuperscript{2+} measurements from intact cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolations/cells, n</td>
<td>4/12</td>
<td>4/12</td>
<td>5/12</td>
<td>5/13</td>
</tr>
<tr>
<td>Diastolic [Ca\textsuperscript{2+}], nmol/L</td>
<td>154±36</td>
<td>124±11</td>
<td>122±15</td>
<td>83±6*</td>
</tr>
<tr>
<td>Twitch peak [Ca\textsuperscript{2+}], nmol/L</td>
<td>465±45</td>
<td>813±79*</td>
<td>481±36</td>
<td>506±29</td>
</tr>
<tr>
<td>Twitch rate constant, s\textsuperscript{-1}</td>
<td>2.76±0.086</td>
<td>3.70±0.06*</td>
<td>2.89±0.08</td>
<td>5.34±0.10*</td>
</tr>
<tr>
<td>Twitch Δ[Ca\textsuperscript{2+}], nmol/L</td>
<td>312±19</td>
<td>688±81*</td>
<td>359±41</td>
<td>423±30</td>
</tr>
<tr>
<td>Caffeine: peak [Ca\textsuperscript{2+}], nmol/L</td>
<td>895±74</td>
<td>1425±63*</td>
<td>989±43</td>
<td>1639±70*</td>
</tr>
<tr>
<td>Caffeine: rate constant, s\textsuperscript{-1}</td>
<td>0.68±0.08</td>
<td>0.73±0.06</td>
<td>0.76±0.11</td>
<td>0.70±0.07</td>
</tr>
<tr>
<td>Caffeine: Δ[Ca\textsuperscript{2+}], nmol/L</td>
<td>737±53</td>
<td>1268±61*</td>
<td>824±42</td>
<td>1542±55*</td>
</tr>
</tbody>
</table>

Twitch rate constant indicates rate constant of decline of twitch [Ca\textsuperscript{2+}]; caffeine rate constant, rate constant of decline of caffeine-induced Ca\textsuperscript{2+} transients.

*P<0.05 vs Ad-LacZ at corresponding MOI.

**Intact Myocyte Ca\textsuperscript{2+} Flux Analysis**

Simulations were used to clarify the lack of Ca\textsuperscript{2+} transient enhancement at high SERCA1a MOI. Figure 6A shows the [Ca\textsuperscript{2+}]\textsubscript{i} dependence of Ca\textsuperscript{2+} transport by the SERCA and NCX inferred from stimulated and caffeine-induced Ca\textsuperscript{2+} transients in intact myocytes. All 4 J\textsubscript{NCX} curves were almost
Broken curve demonstrates expected intracellular Ca\(^{2+}\) dependence of rate of Ca\(^{2+}\) SERCA1a versus Ad-LacZ at MOI 10 and from 125 to 275 (although absolute values differ). These results are consistent with the increases measured in permeabilized cells Ad-SERCA1a MOI 50 (164 versus 183 nmol/L). These results indicate that high SERCA activity causes a paradoxical decrease in contractile activation because of greater Ca\(^{2+}\) removal from the cytosol.

Several studies have shown that increased SR Ca\(^{2+}\)-ATPase expression improves Ca\(^{2+}\) cycling and myocardial function. Here, the fast-twitch skeletal muscle SERCA1a was used because it has faster Ca\(^{2+}\) transport kinetics versus SERCA2a.\(^{11,16}\) Furthermore, SERCA1a transfection in cultured neonatal cardiomyocytes generated higher SERCA protein levels than parallel SERCA2a studies.\(^{12}\) SERCA1a targets to intracellular membranes after adenovirus-mediated gene transfer into embryonic cardiac myocytes in a pattern identical to that of SERCA2a.\(^{20}\) Confocal studies on transgenic mice expressing SERCA1a in the heart showed traffic of SERCA1a to cardiac SR.\(^{21}\) A similar expression pattern was found in the present study.

In both our \(\beta\)-escin–permeabilized and intact cells, SR Ca\(^{2+}\) uptake demonstrated a gene-dose–dependent increase in \(V_{\text{max}}\) and Ca\(^{2+}\) affinity (reduced \(K_{m}\)) in SERCA1a-expressing cells (Figures 5C and 6A and Table 2). Although SERCA1a and SERCA2a have identical Ca\(^{2+}\) concentration dependence\(^{11}\) and are both regulated by PLB,\(^{22,23}\) the shift in \(K_{m}\) in SERCA1 cells may indicate an increased SERCA/PLB ratio, particularly (ie, 60% and 85% increases in \(V_{\text{max}}\)). The apparent Ca\(^{2+}\) affinity was only slightly enhanced in the Ad-SERCA1a MOI 10 (\(K_{m}=197\) versus 210 nmol/L), but the shift was greater in Ad-SERCA1a MOI 50 (164 versus 183 nmol/L). These results are consistent with the increases measured in permeabilized cells (although absolute values differ).
consistent with previous studies with SERCA1a transfection in chicken or rat cardiomyocytes.\textsuperscript{12,20,21}

Interestingly, higher virus titer (MOI 50) and SERCA1a protein expression decreased twitch contraction amplitude and failed to enhance Ca\(^{2+}\) transient amplitude, despite enhanced SERCA activity, SR Ca\(^{2+}\) load, twitch [Ca\(^{2+}\)], decline, and relaxation rates. This indicates that above an optimum SERCA1a expression level (for Ca\(^{2+}\) transient amplitude), greater SERCA1a expression may limit Ca\(^{2+}\) transient amplitude. The higher rate of [Ca\(^{2+}\)]\(_{i}\) decline after SERCA1a overexpression is paralleled by the increase in caffeine-releasable Ca\(^{2+}\). This strongly suggests that the additional efflux of Ca\(^{2+}\) from the cytosol is directed into the SR and not the sarcolemma. The unaltered NCX function (based on rate constants of [Ca\(^{2+}\)], in the presence of caffeine) indicates that there were no compensatory changes in NCX transport during these studies. Thus, the net effect of high levels of SERCA1a overexpression is to enhance relaxation and, by shortening of the duration of activation of the contractile proteins, to reduce the amplitude of myocyte shortening.

On the basis of the rate constants of [Ca\(^{2+}\)], decline,\textsuperscript{19} the contribution of NCX to [Ca\(^{2+}\)], decline was 25% to 26% in Ad-LacZ-infected myocytes, as in previous data in rabbit.\textsuperscript{13} However, with enhanced SERCA1a expression (MOI 10 and MOI 50), NCX contribution decreased to 20% and 13%, respectively. This is consistent with the stronger competition of SERCA versus NCX. The Ca\(^{2+}\) flux analysis and simulations help to explain the unaltered Ca\(^{2+}\) transient amplitude and decreased isotonic shortening despite increased SR Ca\(^{2+}\) load and pump function at high SERCA overexpression. Greater SR Ca\(^{2+}\) pump expression can limit the Ca\(^{2+}\) transient by curtailing the peak. The impact of this effect is shown in Figure 6, B and C. Analogous data in rabbit myocytes showed that acute SERCA block with thapsigargin (without unloading the SR) increased Ca\(^{2+}\) transient amplitude by \(\approx\)20%.\textsuperscript{13} A second means by which increased SERCA expression can limit [Ca\(^{2+}\)], is that it adds to cytosolic Ca\(^{2+}\) buffering. After troponin C (\(\approx 70\) \(\mu\)mol/L), the SR Ca\(^{2+}\) pump is the most prominent cytosolic Ca\(^{2+}\) buffer (\(\approx 50\) \(\mu\)mol/L), with equally high affinity.\textsuperscript{19} Thus, a 50% increase in SR Ca\(^{2+}\) pump expression would compete for Ca\(^{2+}\) binding to troponin C and limit the amplitude of the Ca\(^{2+}\) transient (see Figure 6C). The lower diastolic [Ca\(^{2+}\)] in SERCA-overexpressing cells will also contribute to the absence of improved inotropy, because a larger SR Ca\(^{2+}\) release would be required to achieve peak systolic [Ca\(^{2+}\)] levels that were comparable to control. Third, more SR Ca\(^{2+}\) pumps can increase the rate of SR Ca\(^{2+}\) uptake but may not increase maximal SR Ca\(^{2+}\) load for a given [Ca\(^{2+}\)].\textsuperscript{24-26} This is because the SR Ca\(^{2+}\)-ATPase can only build a [Ca\(^{2+}\)]\(_{i}\) gradient (or potential energy) related to \(\Delta G_{ATP}\) (eg, \(\Delta G_{SR,Pump}=2\) RT ln ([Ca\(^{2+}\)]\(_{SR}\)/[Ca\(^{2+}\)]\(_{i}\)) or [Ca\(^{2+}\)]\(_{i}\) ([Ca\(^{2+}\)]\(_{SR}\)/[Ca\(^{2+}\)]\(_{i}\), \(\approx 7000\)).\textsuperscript{25} Thus, higher SR Ca\(^{2+}\) pump expression may allow a closer approach toward this limit (and get there faster), but there is a limit. It is notable that the diastolic [Ca\(^{2+}\)], was significantly lower and SR Ca\(^{2+}\) load significantly higher in SERCA-50 versus Lac-Z-50, and this may represent an approach to this limiting [Ca\(^{2+}\)]\(_{i}\)/[Ca\(^{2+}\)]\(_{SR}\) gradient. Thus, at high SERCA levels, one may approach a maximal SR Ca\(^{2+}\) load that more SERCA cannot further improve.

Very high levels of SERCA1a gene transfer into neonatal rat and embryonic chicken cardiomyocytes could also produce cytotoxic effects, possibly related to SR dysfunction.\textsuperscript{27} This did not occur here, where we saw enhanced SR Ca\(^{2+}\) transport and load and reduced diastolic [Ca\(^{2+}\)], at the highest SERCA1a expression levels.

In conclusion, the dose of SERCA1a overexpression is critical for improved myocardial function. Although increased SR Ca\(^{2+}\) pump expression can enhance SR Ca\(^{2+}\) content, Ca\(^{2+}\) transient amplitude, and diastolic function, there may be an optimum value above which limiting factors prevent further increase in Ca\(^{2+}\) transients and can even reduce myocyte shortening. Although we used chicken SERCA1a and rabbit myocytes, we believe that the present findings are transferable to other species and SERCA isoforms. Increasing SR Ca\(^{2+}\) pump function in failing hearts (with low functional SERCA2a expression) may be beneficial, but the same SERCA1a expression in myocardium with normal SERCA2a levels could be detrimental. We conclude that the use of SERCA1a for gene therapy in heart failure requires careful control of transfection efficiency and induced expression levels.

Acknowledgments

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References


Excessive Sarcoplasmic/Endoplasmic Reticulum Ca\(^{2+}\)-ATPase Expression Causes Increased Sarcoplasmic Reticulum Ca\(^{2+}\) Uptake but Decreases Myocyte Shortening

Nils Teucher, Juergen Prestle, Tim Seidler, Susan Currie, Elspeth B. Elliott, Deborah F. Reynolds, Peter Schott, Stefan Wagner, Harald Kogler, Giuseppe Inesi, Donald M. Bers, Gerd Hasenfuss and Godfrey L. Smith

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