Accelerated onset of heart failure in mice during pressure overload with chronically decreased SERCA2 calcium pump activity

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Heart plays a pivotal role in the beat-to-beat function of the heart. The SERCA pump promotes muscle relaxation by lowering the cytosolic Ca2+ concentration and through active transport restores the intracellular Ca2+ stores, thus providing Ca2+ needed for the next contraction. Decreases in SERCA pump expression and activity have been observed in a variety of pathological conditions. In particular, defects in the sarcoplasmic reticulum (SR) Ca2+-ATPase (SERCA)2 levels in combination with an increased hemodynamic load result in an accelerated pathway to heart failure. Age-matched wild-type and Serca2+/− mice were subjected to 10 wk of pressure overload via transverse aortic coarctation surgery. Cardiac hypertrophy and heart failure were assessed by echocardiography, gravimetry/histology, hemodynamics, and Western blotting analyses. Our results showed that ~64% of coarcted Serca2+/− mice were in heart failure compared with 0% of coarcted wild-type mice (P < 0.05). Overall, morbidity and mortality were greatly increased in Serca2+/− mice under pressure overload. Echocardiography assessment revealed a significant increase in left ventricular (LV) mass, and LV hypertrophy in coarcted Serca2+/− mice converted from a concentric to an eccentric pattern, similar to that seen in human heart failure. Coarcted Serca2+/− mice had decreased contractile/systolic and relaxation/diastolic performance and/or function compared with coarcted wild-type mice (P < 0.05), despite a similar duration and degree of pressure overload. SERCA2a protein levels were significantly reduced (≥50%) in coarcted Serca2+/− mice compared with noncoarcted and coarcted wild-type mice. Our findings suggest that reduction in SERCA2 levels in combination with an increased hemodynamic load results in an accelerated pathway to heart failure.

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namic load. In the present study, we tested the hypothesis that chronic reduction in SERCA2 levels in combination with an increased hemodynamic load will result in an accelerated pathway to heart failure. Our results show that heart failure occurs more rapidly with reduced SERCA2 levels in conjunction with pressure overload.

MATERIALS AND METHODS

Mice were housed in a specific pathogen-free facility and handled in accordance with standard use protocols and animal welfare regulations. The generation of Serca2+/− mice was described previously (29). All protocols were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Wild-type and Serca2+/− mice (10–12 wk of age) were randomly assigned to the present study, and 11 wild-type and 11 Serca2+/− mice subjected to aortic coarctation (AC) completed the study, which included echocardiography, blood pressure and left ventricular (LV) measurements, gravimetry/histology, and SERCA2a and calsequestrin (Csq) protein determination. After AC, four Serca2+/− mice died because of heart failure. No mortality was observed in wild-type mice committed to the 10-wk study.

AC surgery. Age-matched wild-type and Serca2+/− mice of either sex weighing 22–26 g were anesthetized intraperitoneally with 2.5% Avertin (0.037 ml/g). A tracheotomy was performed, and the mouse was artificially ventilated with room air (120 breaths/min with a tidal volume of 0.3 ml) with a Harvard Apparatus mouse ventilator (model 687; South Natick, MA). AC surgery was performed as described previously (35), and the surgeon was blinded to the identity of the mice. In brief, a blunt dissection was performed at the second intercostal space to expose the aortic arch. A 7-0 silk suture was placed around the aorta between the left common carotid artery and brachiocephalic trunk. A 27-gauge needle, bent to an L shape, was placed on top of the exposed transverse aorta. Tying the suture around this needle and subsequent removal of the needle produced banding of the aorta. The lungs were reexpanded with positive pressure, and the chest and skin incisions were closed with 5-0 silk and 5-0 Ti-cron, respectively. The trachea incision was closed with 7-0 silk. The mouse was monitored daily for 10 wk.

Echocardiography. Echocardiography was performed preoperatively and once a week postoperatively for 10 wk on wild-type and Serca2+/− mice as previously described (35). Intra- and interobserver variability was similar to that previously noted in our laboratory (36), and the sonographers were blinded to the identity of the mice. Two-dimensional and M-mode measurements of LV end-diastolic and end-systolic chamber size, septal wall thickness and LV posterior wall thickness (PWT) in diastole and systole, and R-R interval were made from original tracings, as recommended by the American Society of Echocardiography (33). LV mass was estimated with the cube formula, and cardiac function (fractional shortening, FS) was calculated (35). Changes in diastolic function were assessed by calculating the peak E-to-A ratio and isovolumic relaxation time (IVRT). Peak E (rapid ventricular filling) velocity and peak A (filling during atrial contraction) velocity through the mitral valve were measured from the apical four-chamber view at the tips of the mitral leaflets. Peak E/A is an indicator of LV filling. IVRT was obtained from continuous-wave Doppler of the LV outflow and inflex tracts. IVRT is the time interval from aortic valve closure to mitral valve opening and is a measure of LV relaxation as related to left atrial pressure.

In vivo hemodynamic measurements. Blood pressure measurements and cardiac function were obtained with a Millar catheter as described previously (34, 35). After echocardiographic assessment, age- and sex-matched noncoarcted (NC) and 10-wk AC wild-type and Serca2+/− mice were surgically instrumented as described previously (21). Average values for heart rate, mean arterial blood pressure, systolic blood pressure, and left intraventricular pressure (LVP) were measured directly from the pressure waveforms. Maximum and minimum change in pressure over time (dP/dt) were derived from a

Fig. 1. Tissue weight-to-body-weight (BW) ratios for age- and sex-matched noncoarcted (NC) and 10-wk aortic coarctation (AC) wild-type and Serca2+/− mice. Mice were subjected to sham surgery or AC for 10 wk to induce a chronic pressure overload. Tissues were harvested to identify mice that underwent cardiac hypertrophy and/or had signs of pulmonary edema, an indication of heart failure. Values are expressed as means ± SE. n = 4 for NC wild-type mice, 4 for NC Serca2+/− mice, 11 for AC wild-type mice, and 7 for AC Serca2+/− mice. Four AC Serca2+/− mice died during the course of the study and were not included in the tissue weight analysis. LV, left ventricle.

Fig. 2. Percentage of Serca2+/− mice in failure (based on observed pulmonary edema from lung weight measurements) during chronic pressure overload. No wild-type mice went into failure during the course of the study.

Fig. 3. Serial echocardiographic results for wild-type and Serca2+/− mice before and after AC. Calculated, estimated LV mass for prebanded and AC wild-type and Serca2+/− mice is shown. LV mass was calculated from the echocardiographic measurements of septal wall thickness, posterior wall thickness, and LV end-diastolic chamber size. Values are expressed as means ± SE. n = 11 for wild-type and 7 for Serca2+/− mice.
a subpopulation of suggesting a compensated stage of hypertrophy. However, after 10 wk of AC, Serca2/H11001 isovolumic relaxation time (IVRT) for prebanded and AC wild-type and 7 for AC wild-type mice. Values are expressed as means ± SE; LVED, left ventricular end-diastolic chamber size (mm); LVES, left ventricular end-systolic chamber size (mm); HR, heart rate (beats/min); SV, stroke volume (ml); CO, cardiac output (ml/min); AC, aortic coarctation. *P < 0.05 vs. prepanded cohort; †P < 0.05 vs. wild-type 10-wk AC.

Table 1.  Echocardiographic measures before and during pressure overload

<table>
<thead>
<tr>
<th>Pressure Gradient</th>
<th>LVED</th>
<th>LVES</th>
<th>SWT</th>
<th>PWT</th>
<th>HR</th>
<th>SV</th>
<th>CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-AC</td>
<td>3.78 ± 0.11</td>
<td>2.21 ± 0.14</td>
<td>0.61 ± 0.03</td>
<td>0.59 ± 0.03</td>
<td>418 ± 22</td>
<td>0.032 ± 0.003</td>
<td>13.3 ± 1.3</td>
</tr>
<tr>
<td>Wild type</td>
<td>4.03 ± 0.24</td>
<td>2.38 ± 0.12</td>
<td>0.61 ± 0.03</td>
<td>0.54 ± 0.03</td>
<td>358 ± 27</td>
<td>0.055 ± 0.017</td>
<td>20.0 ± 6.2</td>
</tr>
<tr>
<td>Nonfailing Serca2+/−</td>
<td>3.89 ± 0.19</td>
<td>2.25 ± 0.08</td>
<td>0.69 ± 0.04</td>
<td>0.49 ± 0.07</td>
<td>410 ± 42</td>
<td>0.041 ± 0.002</td>
<td>16.9 ± 2.6</td>
</tr>
<tr>
<td>5 wk AC Wild type</td>
<td>4.04 ± 0.12</td>
<td>2.62 ± 0.12</td>
<td>0.70 ± 0.04*</td>
<td>0.64 ± 0.04*</td>
<td>473 ± 22</td>
<td>0.035 ± 0.007</td>
<td>16.9 ± 3.7</td>
</tr>
<tr>
<td>Nonfailing Serca2+/−</td>
<td>4.16 ± 0.20</td>
<td>2.82 ± 0.24*</td>
<td>0.75 ± 0.06*</td>
<td>0.64 ± 0.01*</td>
<td>414 ± 22</td>
<td>0.046 ± 0.009</td>
<td>18.3 ± 3.2</td>
</tr>
<tr>
<td>Failing Serca2+/−</td>
<td>4.23 ± 0.13</td>
<td>2.72 ± 0.06*</td>
<td>0.74 ± 0.03</td>
<td>0.70 ± 0.02*</td>
<td>445 ± 16</td>
<td>0.026 ± 0.005</td>
<td>11.9 ± 2.6</td>
</tr>
<tr>
<td>10 wk AC Wild type</td>
<td>3.88 ± 0.19</td>
<td>2.54 ± 0.12</td>
<td>0.68 ± 0.02</td>
<td>0.69 ± 0.02*</td>
<td>448 ± 22</td>
<td>0.032 ± 0.003</td>
<td>14.6 ± 1.5</td>
</tr>
<tr>
<td>Nonfailing Serca2+/−</td>
<td>4.20 ± 0.16</td>
<td>2.72 ± 0.13*</td>
<td>0.76 ± 0.06*</td>
<td>0.67 ± 0.05*</td>
<td>386 ± 24</td>
<td>0.042 ± 0.010</td>
<td>16.2 ± 4.0</td>
</tr>
<tr>
<td>Failing Serca2+/−</td>
<td>4.69 ± 0.09*‡</td>
<td>3.00 ± 0.29*</td>
<td>0.81 ± 0.08*</td>
<td>0.78 ± 0.04*</td>
<td>379 ± 59</td>
<td>0.026 ± 0.006*</td>
<td>10.2 ± 1.7†</td>
</tr>
</tbody>
</table>

Values are means ± SE. LVED, left ventricular end-diastolic chamber size (mm); LVES, left ventricular end-systolic chamber size (mm); SWT, septal wall thickness at end diastole (mm); PWT, posterior wall thickness at end diastole (mm); HR, heart rate (beats/min); SV, stroke volume (ml); CO, cardiac output (ml/min); AC, aortic coarctation. *P < 0.05 vs. prepanded cohort; †P < 0.05 vs. wild-type 10-wk AC.

differential tracing of LVP. At the end of the experimental protocol, mice were killed, organs including the heart, lungs, kidney, and colon were harvested, and the tissue was weighed and normalized to mouse body weight and tail length. The tissue was either placed in liquid nitrogen and stored at −80°C for Western blot analysis or placed in 4% paraformaldehyde for histological determination.

Western blot analysis. To determine the protein levels, hearts from AC and NC animals were homogenized in 10 mM imidazole (pH 7.0), 0.3 M sucrose, 0.3 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 10 mM sodium fluoride. Protein concentration was determined by the Bradford method with bovine serum albumin for the standard curve. The homogenates were electrophoretically separated on a 10% SDS polyacrylamide gel, blotted onto a 0.22-μm nitrocellulose membrane, and probed with polyclonal antibody raised against SERCA2a (16). In addition, antibodies specific for phospholamban (PLB) and Csq were used to probe the Western blots (polyclonal PLB and anti-Csq antibodies were from Affinity BioReagents, Golden, CO). Csq was used to correct loading variations between samples because it did not vary significantly between control and banded samples. The signal obtained for each sample was quantified by densitometry with a UMAX Astra 1200s scanner and NIH Image software (version 1.62) (16).

Statistical analysis of data. All values are expressed as means ± SE. Tissue weight-to-body weight ratios, in vivo hemodynamic measurements, and SERCA2a and Csq protein levels were compared by one-way ANOVA with Fisher’s least-significant difference post hoc test. Differences between echocardiographic data were compared with two-way ANOVA for time and treatment with repeated measures with Fisher’s least-significant difference test. Statistical differences were considered significant at P < 0.05.

RESULTS  

Gravimetric analysis reveals a heart failure phenotype in Serca2+/− mice after 10 wk of pressure overload. Wild-type and Serca2+/− mice were subjected to 10 wk of pressure overload via transverse AC surgery. Echocardiography was performed at 1-week intervals to follow the development and progression of cardiac hypertrophy and heart failure. Gravimetric, histological, hemodynamic, and molecular measurements were also obtained for age- and sex-matched NC and 10-wk AC wild-type and Serca2+/− mice.

Gravimetric analysis showed that both 10-wk AC wild-type and Serca2+/− mice had a significant increase in whole heart weight- and LV weight-to-body weight ratio compared with NC cohorts (P < 0.05; Fig. 1). Interestingly, there was a...
further significant increase in whole heart weight- and LV weight-to-body weight ratio in AC Serca2+/- mice compared with AC wild-type mice (P < 0.05; Fig. 1). Heart weights were not significantly different between NC wild-type and Serca2+/- mice, whereas AC animals had a significant increase in heart weight (NC wild type = 128 ± 11 mg; NC Serca2+/- = 127 ± 13 mg; AC wild type = 152 ± 10 mg; AC nonfailing Serca2+/- = 160 ± 14 mg; AC failing Serca2+/- = 159 ± 9 mg). Body weights were not different between NC and AC wild-type and Serca2+/- mice, whereas AC animals had a significant increase in heart weight (NC wild type = 31 ± 3 g; NC Serca2+/- = 30 ± 4 g; AC wild type = 30 ± 2 g; AC nonfailing Serca2+/- = 27 ± 2 g; AC failing Serca2+/- = 24 ± 4 g), indicating that the increase in whole heart weight- and LV weight-to-body weight ratio in AC Serca2+/- mice was not the result of diminished murine body weight. Wet lung weight-to-body weight ratio, an experimental measure of pulmonary edema, revealed a heart failure phenotype in AC Serca2+/- mice (P < 0.05; Fig. 1). The heart failure phenotype was determined retrospectively and was based on the criterion of wet lung weight-to-body weight ratio (NC wild type range 5.4–7.1; NC Serca2+/- range 5.6–6.9; 10-wk AC wild type range 4.0–6.3; 10-wk AC Serca2+/- range 5.1–17.7). In fact, the incidence of heart failure was genotype dependent, as 64% of AC Serca2+/- mice had pulmonary edema (i.e., significant increase in wet lung weight-to-body weight ratio) compared with 0% of AC wild-type mice (P < 0.05; Fig. 2). Dry lung weight-to-body weight ratio was not significantly different in NC and AC wild-type and Serca2+/- mice, confirming that the excess wet lung weight was due to edema (data not shown). No mortality occurred in 10-wk AC wild-type mice; however, four AC Serca2+/- mice died between 4 and 8 weeks of pressure overload (i.e., 1 mortality at 4 wk of AC, 2 mortalities at 6 wk of AC, and 1 mortality at 8 wk of AC). Histological assessment of 10-week NC and AC wild-type and Serca2+/- hearts indicated that AC wild-type hearts had mild myocyte hypertrophy with mild perivascular fibrosis whereas AC Serca2+/- hearts had diffuse, moderate myocyte hypertrophy and moderate multifocal fibrosis (data not shown).

Echocardiographic assessment demonstrates LV hypertrophy and systolic and diastolic dysfunction in Serca2+/- mice after pressure overload. Echocardiography showed that AC wild-type and nonfailing Serca2+/- mice had similar increases in LV mass; however, the increase in LV mass was
There was a 51% reduction in FS in 10-wk AC dysfunction compared with AC wild-type mice (Serca2 mice. Table 1), indicating a concentric form of hypertrophy in these and nonfailing mice. A significant greater for failing Serca2+/− mice compared with AC wild-type mice at 5 and 10 wk of AC (P < 0.05; Fig. 3). There were no differences in LV mass between wild-type and Serca2+/− mice before AC. No significant LV chamber dilation occurred in AC wild-type and nonfailing Serca2+/− mice (Table 1). However, there was a significant increase in LV chamber dilation and PWT in AC failing Serca2+/− mice (P < 0.05; Table 1), demonstrating an eccentric, dilated form of hypertrophy. A significant increase in PWT in AC wild-type and nonfailing Serca2+/− mice was also observed (P < 0.05; Table 1), indicating a concentric form of hypertrophy in these mice.

Cardiac function assessed by echocardiography demonstrated that AC Serca2+/− mice had significant systolic (measured as FS) and diastolic (measured as E/A) dysfunction compared with AC wild-type mice (P < 0.05; Figs. 4 and 5). There was a 51% reduction in FS in 10-wk AC failing Serca2+/− mice compared with AC wild-type and nonfailing Serca2+/− mice (Fig. 4A). Serca2+/− mice had a slower IVRT before AC compared with wild-type mice (P < 0.05; Fig. 4B), which supports our earlier findings (29) of a slower rate of relaxation in Serca2+/− mice. After AC, IVRT was further elevated in Serca2+/− mice, indicating severe diastolic dysfunction (Fig. 4B). Another measure of diastolic function is E/A, which is a measure of ventricular filling, both passive (E) and via atrial contraction (A), and is influenced by the speed of LV relaxation and LV compliance. Before AC, Serca2+/− mice had an elevated E/A compared with wild-type mice (P < 0.05; Fig. 5B). After 10 wk of AC, failing Serca2+/− mice had a significant increase in E/A compared with 10-wk AC wild-type mice and prebanded Serca2+/− mice (P < 0.05; Fig. 5B). Although not significant, there was also an elevation of E/A of nonfailing Serca2+/− mice after AC compared with wild-type mice and prebanded Serca2+/− mice. Different E/A patterns of diastolic filling were observed in coarcted Serca2+/− mice (Fig. 5A). The images depicted in Fig. 5A are representative of E/A patterns observed in coarcted Serca2+/− at different time points of AC. The E/A reversal pattern was observed at early time points (between 2 and 4 wk) in AC Serca2+/− mice, but this pattern was not observed in AC wild-type mice. At later stages (8–10 wk) of pressure overload, the E/A pattern demonstrated restrictive LV relaxation in Serca2+/− mice but not in AC wild-type mice.

There was no difference in the heart rate or pressure gradient produced in wild-type and Serca2+/− mice to account for the cardiac hypertrophy and/or remodeling and functional differences observed (see Tables 1 and 2). The echocardiographic measurements of pressure gradient after AC are comparable to the gradients obtained from catheterizations of the LV and femoral artery as shown previously (35).

LV catheterization study reveals systolic and diastolic dysfunction in Serca2+/− mice after pressure overload. As previously observed (29), LV catheterization demonstrated that age-and sex-matched NC Serca2+/− mice had a significant decrease in +dP/dt and −dP/dt compared with NC wild-type mice (P < 0.05; Fig. 6). Consistent with the functional assessment obtained by echocardiography, a subpopulation of 10-wk AC Serca2+/− mice had significant systolic and diastolic cardiac dysfunction, as measured by the rates of contraction and relaxation (+dP/dt and −dP/dt, respectively), compared with AC wild-type mice (P < 0.05; Fig. 6), despite a similar duration and degree of pressure overload (Table 2). In addition, peak LV systolic pressure, as measured by the Millar catheter

![Figure 6](http://ajpheart.physiology.org/)

**Table 2. Hemodynamic measurements in noncoarcted and 10-week AC wild-type and Serca2+/− mice**

<table>
<thead>
<tr>
<th></th>
<th>HR (beats/min)</th>
<th>Systolic BP (mmHg)</th>
<th>LVPsys (mmHg)</th>
<th>Pressure Gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>400±25</td>
<td>94±9</td>
<td>114±9</td>
<td>20±3</td>
</tr>
<tr>
<td>Serca2+/−</td>
<td>350±28</td>
<td>80±7</td>
<td>98±8</td>
<td>18±4</td>
</tr>
<tr>
<td>10-wk AC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>448±18</td>
<td>85±5</td>
<td>160±4*</td>
<td>75±6*</td>
</tr>
<tr>
<td>Nonfailing Serca2+/−</td>
<td>395±25</td>
<td>107±11</td>
<td>160±22*</td>
<td>63±14*</td>
</tr>
<tr>
<td>Failing Serca2+/−</td>
<td>359±35</td>
<td>89±19</td>
<td>148±30*</td>
<td>59±10*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Systolic BP, systemic systolic blood pressure (mmHg); LVPsys, left intraventricular systolic pressure (mmHg); NC, no AC; Pressure gradient, difference between LVP and systolic BP (mmHg). *P < 0.05 vs. NC cohort.
placed proximal to the aortic coartation, was not different between Serca2+/- mice and wild-type mice after 10 wk of AC (Table 2), confirming matched hemodynamic loads. Both AC wild-type and nonfailing Serca2+/- mice had enhanced rates of contraction and relaxation, suggesting that these mice were in a compensatory stage during pressure overload. LV end-diastolic pressure was significantly elevated in AC failing Serca2+/- mice compared with AC wild-type mice (6.2 ± 1.7 mmHg in AC failing Serca2+/- vs. 2.1 ± 0.9 mmHg in AC wild type; \( P < 0.05 \)).

Pressure overload results in a further decrease in SERCA2 protein levels in Serca2+/- mice. Western blot analysis revealed that 10-wk AC wild-type mice had a 14% reduction in SERCA2a protein levels compared with their NC cohorts (\( P < 0.05 \); Fig. 7B). AC Serca2+/+ mice had a further 36% reduction in SERCA2a protein levels (for a total reduction of 56% compared with NC wild-type mice) compared with their NC cohorts, which started with an initial 20% reduction in SERCA2a protein levels (\( P < 0.05 \); Fig. 7B). Compared with AC wild-type mice, SERCA levels were significantly decreased in Serca2+/- mice after 10 wk of pressure overload. On the other hand, Csq protein levels were not significantly different between NC and AC cohorts. Our analysis of PLB expression showed that PLB was not significantly different between wild-type and Serca2+/+ mice (data not shown). These data suggest that the severity of heart failure may relate to the greater decrease in SERCA levels observed in the AC Serca2+/- mice.

### DISCUSSION

A number of animal models of cardiac hypertrophy and human heart failure have decreased levels of SERCA2 mRNA and/or protein (2, 5, 6, 9, 20, 24). However, the question remained as to whether there was a causal relationship between decreased levels of SERCA2 and heart failure. We reported previously (16, 29) that disruption of a single copy of the SERCA2 gene (Serca2+/− mice) results in decreased SERCA2 protein and activity and causes an impairment of basal cardiac performance and altered Ca\(^{2+}\) homeostasis, with no overt signs of cardiac disease.

Our present study has demonstrated that heart failure occurs more rapidly and more frequently in mice with chronically reduced SERCA2 levels in conjunction with a pathological stress (i.e., increased hemodynamic load). Systolic and diastolic performance is significantly decreased in 10-wk AC Serca2+/− mice. Also, these mice (Serca2+/−) had a significant degree of cardiac hypertrophy and dilatation compared with wild-type mice after pressure overload, which suggests that Ca\(^{2+}\) handling via the SERCA2 pump may not only regulate cardiac function but also influence cardiac remodeling during disease events. The functional and remodeling parameters were significantly different between AC wild-type and Serca2+/− mice despite a similar duration and degree of pressure overload. Western blot analysis revealed that Serca2+/− mice subjected to pressure overload had a further reduction in SERCA2, which may account for the systolic and

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**Fig. 7.** Western blot analysis depicting % sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA2a) levels in NC and 10-wk AC wild-type and Serca2+/− mice. A: representative image of Western blot for SERCA2a and calsequestrin (Csq) levels in NC and 10-wk AC wild-type and Serca2+/− mice. B: quantitation of the SERCA2a protein levels in NC and 10-wk AC wild-type and Serca2+/− mice. Values are expressed as means ± SE; \( n = 3 \) for NC wild-type mice, 3 for NC Serca2+/− mice, 3 for AC wild-type mice, and 3 for AC Serca2+/− mice. The 4 AC Serca2+/− mice that died during the course of the study were not included in this analysis.
diastolic dysfunction observed in AC Serca2a+/− mice (see Fig. 7). These data suggest that depressed levels of SERCA2 protein and its activity below a certain threshold (e.g., >35% reduction in SERCA2 protein and activity) contribute to the onset and progression of heart failure. Our findings correlate well with recent studies showing that a defect in SR Ca2+ uptake function can contribute to cardiac dysfunction and pathology. Dash et al. (4) showed that fourfold overexpression of PLB (inhibitor of SERCA) in transgenic mice resulted in depressed Ca2+ transport (as evidenced by prolonged Ca2+ transients) and mechanical function. Young transgenic animals showed no signs of heart failure and maintained a full response to isoproterenol in vivo. However, on aging (15–18 mo), transgenic mice exhibited a progression to cardiac hypertrophy, LV chamber dilatation, and heart failure and sudden death (4). In addition, treatment of mice with ryanodine, an inhibitor of the SR Ca2+ release channel, resulted in cardiac hypertrophy and contractile dysfunction, suggesting that defects in SR Ca2+ handling can cause cardiac pathology (25). Although we have suggested that the level of SERCA2 protein and/or activity may impact the heart’s ability to handle chronically increased pressure load, ultimately leading to heart failure, modifying other signaling mechanisms involved in cardiac remodeling and/or contractility could exhibit profound effects on the maintenance of cardiac function in response to hemodynamic overload. For example, mitogen-activated protein kinase and ERK kinase kinase 1 knockout mice were shown to be susceptible to pressure overload and to developing cardiac dysfunction compared with control wild-type mice, although it is not known whether SERCA2 levels were decreased in this model as they were not studied (32). However, a study by Kiriakiz and colleagues (17) demonstrated that SERCA2 protein levels were markedly decreased (50%) in coarcted failing wild-type and PLB knockout mice, whereas coarcted nonfailing wild-type mice had a 24% reduction in SERCA2 protein, and these levels of SERCA2 protein correlated with FS. Our data are consistent with those of Kiriakiz et al. (17) in that we detected a 56% reduction in SERCA2 protein levels in AC failing Serca2a+/− mice and AC nonfailing wild-type mice had only a 14% reduction in SERCA2 (see Fig. 7). Targeted expression of the SERCA pump in transgenic mouse hearts has been shown to improve cardiac function, increase systolic Ca2+ transients, and accelerate relaxation rate (13, 22, 19). In addition, studies have examined the benefit of increased SERCA pump levels during pressure overload-induced cardiac hypertrophy in genetically altered animal models. Ito et al. (15) found decreased mortality in 7-wk banded transgenic SERCA2a mice vs. banded controls showed that transgenic animals maintained increased SERCA levels and enhanced contractile function even after banding. However, the magnitude of hypertrophy was similar in both controls and transgenic mice (15). These studies conclude that enhanced SERCA levels maintain increased SR Ca2+ load and contractile function, which therefore modify the outcome of pressure overload-induced remodeling.

In general, genetic manipulations that improve Ca2+ transport either by SERCA gene therapy or PLB ablation have been shown to be beneficial and to prevent cardiac dysfunction and ventricular remodeling in both transgenic and surgically induced heart failure models (7, 8, 10, 14, 26, 27). Recently, it was shown that ablation of PLB could prevent heart failure in a mouse model of dilated cardiomyopathy caused by deficiency in a cytoskeletal protein the muscle-specific Lim protein (26). Further PLB ablation prevented systolic dysfunction and exercise intolerance, but not hypotrophy (10), in a hypertrophic cardiomyopathy mouse model resulting from Arg403Gln mutation in myosin heavy chain. Hoshijima et al. (14) showed that sustained expression of pseudophosphorylated PLB into Bio 14.6 cardiomyopathic hamsters improves Ca2+ transport and contractile function. Adenoviral gene transfer studies by Hajjar and colleagues (7, 8, 27) showed that SERCA2a gene transfer can improve survival, ventricular function, and energy metabolism in an experimentally induced heart failure model. These studies clearly point out that restoration of normal Ca2+ transport can be beneficial; it can prevent ventricular dysfunction as well as cardiac remodeling triggered by a defect in Ca2+ transport.

In conclusion, our findings suggest that a decrease in SERCA pump level can lead to cardiac dysfunction and heart failure when the heart is subjected to an increase in physiological demands. This study presents evidence that hearts with decreased SERCA levels are at greater risk and develop heart failure, thus suggesting that a decrease in SERCA levels may contribute to worsening function, thus leading to failure.

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