Constitutive Cardiac Overexpression of Sarcoplasmic/Endoplasmic Reticulum Ca\(^{2+}\)-ATPase Delays Myocardial Failure After Myocardial Infarction in Rats at a Cost of Increased Acute Arrhythmias

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Background—Heart failure often complicates myocardial infarction (MI), and sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA2a) is underexpressed in the failing myocardium. We examined the effect of preexisting cardiac SERCA2a protein overexpression on rat survival and left ventricular (LV) remodeling after MI.

Methods and Results—Baseline myocardial SERCA2a expression was 37% higher in transgenic (TG) rats than in their wild-type (WT) controls, consistent with enhanced myocardial function. The mortality rate of TG rats during the 24 hours after surgical MI was higher than that of WT rats (71% versus 35%, \(P<0.001\)), associated with a higher frequency of ventricular arrhythmias, and was normalized by lidocaine treatment. The increased acute-phase mortality in TG rats was not accompanied by increased 6-month mortality. Function of the noninfarcted myocardium, as assessed by tissue Doppler imaging, was higher in TG rats than in WT rats for up to 1 month after MI, a beneficial effect no longer observed at 3 months. LV remodeling and global function were similar in TG and WT rats. No difference in papillary muscle function was found at 6 months.

Conclusions—Constitutive cardiac SERCA2a overexpression has a transient beneficial effect on remote myocardium function in rat MI, with no improvement in LV global function or prevention of LV remodeling and failure. This benefit is associated with a higher risk of acute mortality, which is prevented by lidocaine treatment. (Circulation. 2004;109:1898-1903.)

Key Words: contractility ■ arrhythmia, ventricular ■ heart failure ■ myocardial infarction ■ echocardiography

Heart failure (HF) is a common outcome of myocardial infarction (MI) and is associated with a poor prognosis. Because of the loss of contractile function in the infarcted area, MI results in increased mechanical load on the intact myocardium, which undergoes molecular, cellular, morphological, and functional remodeling. Decreased sarcoplasmic/endoplasmic reticulum (SR) Ca\(^{2+}\) uptake and decreased expression of the SR Ca\(^{2+}\)-ATPase, SERCA2a, are key features of cardiac myocyte dysfunction in both experimental\(^1\) and human\(^2\) HF.

Available drugs do not adequately improve the condition of many patients with HF. Treatments targeting the SERCA gene might be able to rescue the compromised myocardial function. Indeed, in cardiomyocytes isolated from the left ventricle (LV) of patients with end-stage HF, SERCA2a overexpression can restore normal Ca\(^{2+}\) cycling.\(^3\) In rats, LV function improved 2 days after in vivo SERCA2a gene transfer,\(^4\) and survival increased in a model of aortic banding–induced HF.\(^5\) SERCA2a overexpression in transgenic (TG) mouse hearts preserved SERCA2a levels 7 weeks after banding of the ascending aorta, preserved contractility of isolated myocytes, and prevented the onset of LV dysfunction in vivo.\(^6\) A 30% to 70% SERCA2a overexpression in TG rat hearts was associated with shortened intracellular Ca\(^{2+}\) transients (Ca\(^{2+}\)-tr) and enhanced LV functional parameters.\(^7\) Moreover, banding of the abdominal aorta revealed improved LV function in TG rats compared with wild-type (WT) controls, correlating with the myocardial level of SERCA2a.\(^7\)
No data are available on the short- and long-term effects of MI in SERCA-overexpressing animals. Hypertrophic cardiomyocytes with reduced contractility and relaxation have been found in the failing rat heart after MI, accompanied by a reduced peak Ca$^{2+}$-tr and prolonged Ca$^{2+}$-tr decay. SERCA mRNA and protein levels decreased with increasing severity of HF after left coronary artery ligation. Arrhythmia is a common complication of acute MI, and most fatal arrhythmias result from abnormal Ca$^{2+}$ cycling because of severe acute myocardial ischemia. The influence of the SERCA2a expression level in these arrhythmias is unknown.

The aim of the present study was to examine the effects of constitutive, ie, preexisting, SERCA2a overexpression on survival, arrhythmia, and late cardiac remodeling and function after MI in rats.

**Methods**

**SERCA2a TG Rats and Experimental Design**

TG rats with cardiac-specific SERCA2a overexpression were generated by Dr W. Franz. The rat SERCA2a cDNA was linked to the rat 2.1-kb myosin light chain-2v (MLC-2v) promoter and a fragment of the human cytomegalovirus immediate-early enhancer. Male homozygous TG offspring and their age-matched Sprague-Dawley WT controls were used. Animal care and procedures were in accordance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, revised 1985).

Three studies were performed. In study 1, 12-week-old TG and WT rats underwent echocardiography, followed by invasive hemodynamic investigations as described previously. Isolated LV papillary muscles were studied in a subgroup of rats. In study 2, 12-week-old rats underwent left coronary artery ligation to induce MI as described previously. ECG was recorded for the first 24 hours after ligation. Lidocaine (20 mg/kg, AstraZeneca) was administered intraperitoneally to a subgroup of TG rats 10 minutes before surgery and intramuscularly 2, 4, 6, and 8 hours after surgery to reduce acute-phase mortality. Study 3 included rats surviving more than 3 months after MI, with an echocardiographic follow-up at 1 week and 1 and 3 months. Rat survival rates, LV papillary muscle mechanics, and SERCA2a protein levels in the noninfarcted myocardium were determined 6 months after MI.

**Continuous ECG**

A 24-hour continuous single-lead ECG trace was obtained with an implantable transmitter and electrodes (TA10EFAF20, Data Sciences International), implanted at the time of coronary artery ligation. The ECG signal (400 to 2000 Hz) was recorded in animals housed in individual cages (RLA1020, DSI) and stored for off-line analysis (PowerLab16SP, ADInstruments). Ventricular arrhythmias were identified and quantified visually. Ventricular tachycardia (VT) was defined as a run of 4 or more consecutive premature ventricular beats (including torsades de pointes) and ventricular fibrillation (VF) as signals with changing morphology and rate from beat to beat (Figure 1). Results are reported as the total number of episodes of VT and VF per hour per rat.

**Transthoracic Echocardiography**

Echocardiography was performed with a Toshiba Powervision 6000, SSA 370A device equipped with an 8- to 14-MHz linear transducer under isoflurane anesthesia (1.0% to 1.5% in oxygen) and spontaneous ventilation. Data were transferred to a computer for offline analysis (Ultrasound Image Workstation-300A, Toshiba). The LV was imaged in both parasternal long-axis and short-axis views at the papillary muscle level. A scar score was established to estimate infarct size: 1 point was given for each akinetic or dyskinetic LV segment, and 1 point was added for the presence of an aneurysm on each short- and long-axis view. LV ejection fraction (EF) was calculated using Simpson’s modified biplane equation. LV diastolic parameters were derived from pulsed-wave spectral Doppler mitral flow and from pulsed-wave spectral mitral tissue Doppler imaging (TDI) from the apical view, with the sample volume placed at the lateral corner of the mitral annulus. Peak systolic ($S_{PV}$) and diastolic ($E_{PV}$) velocities of the noninfarcted myocardium were measured by pulsed-wave TDI at the LV inferior wall (see Figure 5A). Myocardial velocity gradients (MVGs) were derived from time-motion-color TDI, as previously described. Briefly, MVP was computed offline as the difference between the epicardium and the endocardium velocities divided by wall thickness, sampled every 2 ms and averaged over 5 to 10 systoles or diastoles (see Figure 5D).

**Papillary Muscle Mechanics and SERCA2a Protein Assay**

Hearts were removed from rats anesthetized with pentobarbital. The whole heart and the LV were dissected and weighed, and...
papillary muscles were isolated. The LV was laid out on a board and covered with a transparent sheet, allowing us to draw the contour of the scar and to calculate its area. The scar was then removed before LV freezing for SERCA2a protein assay. Papillary muscle mechanics were studied in Krebs-Henseleit bicarbonate buffer solution as described previously.16 Briefly, the maximum shortening (maxVc) and lengthening (maxVr) velocities were determined from isometric twitch; the maximum isometric active force normalized per cross-sectional area and maximum rate of force rise (dF/dt) and decay (−dF/dt) from isometric twitch. R1 (maxVc/maxVr) and R2 (maxVr/ΔF, cm/s) were calculated to characterize muscle relaxation at low and high load, respectively.16 SERCA2a protein levels were determined immunochemically as described previously.17

### Results

#### Study 1: SERCA2a TG rats

The main characteristics of the rats are summarized in Table 1. TG rats were smaller than WT rats, but no differences were found with respect to heart weight or ratio of LV weight to body weight or LV diameter (LVEDD/BW). On echocardiography, LV relaxation was faster in TG than in WT rats, but LV systolic and diastolic function was similar. Positive and negative dP/dt were higher in TG than in WT rats. Papillary muscle Vmax, −dF/dt, maxVr, and maxVc were higher in TG than in WT rats. However, the larger increase in maxVr resulted in a decrease in R1, which is characteristic of increased SR Ca2⁺-uptake function. Consistent with these results, the myocardial SERCA2a protein level was increased by 37% in TG rats (92.7±7.5 versus 67.4±5.1 pmol/mg total protein; P<0.01).

#### Study 2: Acute Phase of MI

Because the mortality rate of TG rats (29 of 41, 71%) was markedly higher than that of WT rats (18 of 51, 35%, P<0.001) during the first 24 hours after MI, arrhythmias were investigated by continuous ECG in 7 TG and 8 WT rats. During the first 24 hours after MI, ventricular arrhythmias were more frequent in TG than in WT rats (4.5±2.0 versus 0.3±0.2 episodes per hour per rat, P<0.05), suggesting that the increased mortality rate of TG rats was related to fatal arrhythmias. Lidocaine treatment reduced the mortality rate in a subgroup of TG rats (12 of 25, 48%) to the WT level.

#### Study 3: Long-Term Survival and LV and Myocardial Remodeling After MI

At 6 months, the mortality rates of TG and WT rats surviving the first 24 hours after MI were similar (50% and 42% in TG and WT rats, respectively; P=NS). WT rats surviving the acute phase started to die from the end of the third month after MI; TG rats were similar in this respect (Figure 2). During the

### Table 1. SERCA2a Transgenic Rats

<table>
<thead>
<tr>
<th>Anatomic parameters</th>
<th>WT (n=10)</th>
<th>TG (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>422±9</td>
<td>392±9*</td>
</tr>
<tr>
<td>Heart weight/BW, mg/g</td>
<td>3.67±0.16</td>
<td>3.50±0.12</td>
</tr>
<tr>
<td>LV weight/BW, mg/g</td>
<td>2.40±0.09</td>
<td>2.38±0.09</td>
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<tr>
<td>Echocardiography</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>345±8</td>
<td>345±5</td>
</tr>
<tr>
<td>LVEDD/BW, mm/mg</td>
<td>25.6±0.5</td>
<td>25.7±0.4</td>
</tr>
<tr>
<td>EF, %</td>
<td>74±2</td>
<td>76±2</td>
</tr>
<tr>
<td>Sa, cm/s</td>
<td>3.6±0.1</td>
<td>3.8±0.2</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>25.5±1.7</td>
<td>18.7±0.9*</td>
</tr>
<tr>
<td>Ea, cm/s</td>
<td>6.3±0.3</td>
<td>6.0±0.3</td>
</tr>
<tr>
<td>E/Ea</td>
<td>18±1</td>
<td>17±1</td>
</tr>
<tr>
<td>Hemodynamic data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, bpm</td>
<td>362±10</td>
<td>379±6</td>
</tr>
<tr>
<td>+dP/dt, mm Hg/s</td>
<td>5531±337</td>
<td>7263±467*</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>108±3</td>
<td>114±1</td>
</tr>
<tr>
<td>−dP/dt, mm Hg/s</td>
<td>412±359</td>
<td>5178±198*</td>
</tr>
<tr>
<td>LVEDD, mm Hg</td>
<td>8±1</td>
<td>8±1</td>
</tr>
<tr>
<td>Papillary muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vmax, Lmax/s</td>
<td>3.04±0.09</td>
<td>3.46±0.09*</td>
</tr>
<tr>
<td>MaxVc, Lmax/s</td>
<td>1.88±0.08</td>
<td>2.14±0.08*</td>
</tr>
<tr>
<td>MaxVr, Lmax/s</td>
<td>−2.39±0.18</td>
<td>−3.15±0.13*</td>
</tr>
<tr>
<td>R1</td>
<td>0.81±0.04</td>
<td>0.68±0.02*</td>
</tr>
<tr>
<td>AF, mN/mm²</td>
<td>51±7</td>
<td>70±11</td>
</tr>
<tr>
<td>+dF/dt, mN/s</td>
<td>639±84</td>
<td>921±157</td>
</tr>
<tr>
<td>−dF/dt, mN/s</td>
<td>−259±32</td>
<td>−407±52*</td>
</tr>
<tr>
<td>R2</td>
<td>2.49±0.19</td>
<td>2.26±0.17</td>
</tr>
</tbody>
</table>

BW indicates body wt; LVEDD, LV end-diastolic diameter; EF, LV ejection fraction; Sa, peak mitral annulus systolic velocity; IVRT, isovolumic relaxation time; E, peak velocity of early mitral flow; Ea, peak mitral annulus diastolic velocity; LVSP, LV peak systolic pressure; +dP/dt, −dP/dt, the maximum rate of LV pressure rise and decay; LVEDP, LV end-diastolic pressure; Vmax, maximum unloaded shortening velocity; maxVc, maximal shortening velocity; maxVr, maximal lengthening velocity; R1, maxVc/maxVr; AF, active force per muscle cross-sectional area; +dF/dt and −dF/dt, maximum rate of papillary muscle force rise and decay during the isometric twitch; and R2, +dF/dt⁻¹−dF/dt⁻¹.

*P<0.05.
observation period, rats grew and their heart rates decreased with time in both groups. However, TG rats had a smaller BW and higher heart rate than WT rats at each time point (data not shown). Echographic parameters were thus normalized to BW or heart rate, as appropriate.

Thirteen WT and 14 TG rats completed the echocardiographic study. The MI size, estimated by use of the echocardiographic scar score, was similar in the 2 groups at all time points (Figure 3A). This was consistent with autopsy findings at 6 months (Figure 3B). LV end-diastolic volume to body weight ratio increased with time in both groups (1.46±0.21 at 3 months versus 1.14±0.10 at 1 week in WT; 1.21±0.11 at 3 months versus 1.03±0.07 at 1 week in TG, P<0.05 for both comparisons). The increase was larger in WT than in TG rats (25% versus 18%), but the difference did not reach statistical significance. In both groups, EF decreased markedly 1 week after MI, and there was no difference between TG and WT rats at any time point after MI (Table 1 and Figure 4A). One month after MI, isovolumic relaxation time was smaller in TG than in WT rats (P<0.05) (Figure 4B).

Parameters of systolic and diastolic function of the noninfarcted LV inferior wall in TG and WT rats after MI are shown in Figure 5. Two different echocardiographic methods, ie, pulsed-wave TDI and TM-color TDI, consistently showed that myocardial systolic and diastolic function of the noninfarcted LV inferior wall was enhanced in TG compared with WT rats up to 1 month after MI.

Six months after MI, the papillary muscles of WT and TG rats showed no significant differences in contraction, relaxation, or active force (Table 2). SR Ca2+-uptake function, as assessed by R1, was similar in the 2 groups. Similarly, the SERCA2a protein level in noninfarcted LV myocardium did not differ between TG and WT rats (74.3±3.7 [n=9] versus 66.6±3.0 [n=10] pmol/mg total protein, P=NS).

**Discussion**

The in vivo assessment of baseline LV function in SERCA2a TG rats showed increased LV relaxation (isovolumic relaxation time, −dP/dt) and normal (echo) or improved (+dP/dt) LV contractility. LV papillary muscles showed faster contraction and relaxation rates and increased R1, indicative of an increased SR Ca2+-uptake function, in good agreement with the 37% increase in SERCA2a protein expression. These data are in keeping with previous findings in SERCA2a TG mice and in the same rat line. In our study, LV global systolic and diastolic function, as assessed by echocardiography, were not different between TG and WT rats, probably because these

**TABLE 2. LV Papillary Muscle Study 6 Months After MI**

<table>
<thead>
<tr>
<th>Papillary Muscles</th>
<th>WT (n=13)</th>
<th>TG (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmax, Lmax/s</td>
<td>3.16±0.11</td>
<td>3.40±0.13</td>
</tr>
<tr>
<td>MaxVc, Lmax/s</td>
<td>1.83±0.08</td>
<td>1.97±0.06</td>
</tr>
<tr>
<td>MaxVr, Lmax/s</td>
<td>−2.86±0.14</td>
<td>−2.73±0.20</td>
</tr>
<tr>
<td>R1</td>
<td>0.65±0.02</td>
<td>0.75±0.05</td>
</tr>
<tr>
<td>AF, mN/mm²</td>
<td>51±5</td>
<td>56±9</td>
</tr>
<tr>
<td>+dF/dt, mN/s</td>
<td>576±42</td>
<td>728±113</td>
</tr>
<tr>
<td>−dF/dt, mN/s</td>
<td>−239±19</td>
<td>−301±51</td>
</tr>
<tr>
<td>R2</td>
<td>2.50±0.16</td>
<td>2.47±0.20</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1. No significant difference between groups.
parameters do not directly reflect muscle function, because they are dependent on LV loading conditions.

After a large MI, the LV dilates, a process that contributes to the increase in both preload and afterload of the noninfarcted myocardium, in which SERCA2a expression is downregulated proportionately to the severity of HF. Interestingly, an early decrease in SERCA expression was found in the noninfarcted myocardium adjacent to the scar but not in the remote myocardium, in which the decrease in SERCA is delayed by about 2 months. The impairment of LV function after MI is directly related to the mass of infarcted myocardium. In the present study, infarct size was not different between TG and WT rats; accordingly, LV global systolic function was similarly decreased in the 2 groups at 1 week after MI. The SERCA2a overexpression in TG rats would have been expected to delay LV remodeling through improved myocardial function. However, similar LV dilatation occurred in TG and in WT rats, and no increase in LV EF was seen in the TG rats, despite the increase in contractile function of the noninfarcted myocardium. This increase may thus have been too small to delay LV remodeling and failure. Alternatively, it can be speculated that increasing the function of the noninfarcted myocardium at the acute phase of MI is not sufficient to prevent or delay LV remodeling. Additional experiments are needed to clarify this point.

Our finding that echographic TDI parameters can show subtle changes in LV myocardial function compared with pump function parameters, as previously shown in humans, is of importance for future experimental studies. The LV EF is influenced by preload and afterload, whereas the MVG is more directly related to intrinsic myocardial properties. Furthermore, the fact that spectral pulsed-wave TDI gave results similar to those of MVG measurements is of interest, because the former is technically simple, whereas MVG assessment requires dedicated offline computer analysis.

The SERCA2a protein level in noninfarcted myocardium of TG rats 6 months after MI was not different from that in WT rats. This could explain why the improvement in myocardial function is transient after MI. The decrease of SERCA2a protein level after MI in TG rats, with no change in WT rats, is unexpected. It could result from decreased expression of the transgene if chronic hemodynamic overload acts negatively on the MLC-2v promoter. However, this seems unlikely, because this promoter has been used successfully to induce HF in mice. Moreover, MLC-2v is not downregulated in failing human ventricles. It can also be speculated that the increased mechanical stress on the noninfarcted myocardium after MI has led to a downregulation of SERCA2a gene expression in TG rats as a means to optimize the economy of cellular Ca2+ cycling, as shown in several models of chronic hemodynamic overload.
ble that the death of the TG rats throughout the study has selected rats with the lowest SERCA2a levels.

We found higher early post-MI mortality rates and increased rates of ventricular arrhythmias in TG rats compared with WT rats. 3D mapping studies have shown that most fatal arrhythmias occurring at the acute phase of MI are initiated by a nonreentrant mechanism,9 such as early or delayed afterdepolarizations, that triggers for trigger activities. Increased SR Ca\(^{2+}\) content might increase Ca\(^{2+}\) leakage from the SR to the cytosol,22 a mechanism enhanced by \(\beta\)-adrenergic stimulation.\(^{10}\) High SR Ca\(^{2+}\) load is expected in TG rats with SERCA2a overexpression, as in TG mice.\(^{2}\) Accordingly, with severe ischemia and its subsequent sympathetic system activation, SERCA2a TG rats are likely to be at a higher risk of ventricular arrhythmias. In addition, a reentry mechanism could also be involved, because of the juxtaposition of ischemic and nonischemic tissues. Interestingly, the increased acute-phase mortality in SERCA2a TG rats was not accompanied by an increase in subsequent mortality up to 6 months after MI. This suggests that any arrhythmias occurring after the acute phase of MI do not increase mortality and that SERCA2a gene therapy in chronic HF might not have the deleterious proarrhythmic effects seen when SERCA2a overexpression preexists before MI. Although care should be taken before extrapolating data from a model of constitutive SERCA overexpression to the gene therapy setting, the similar levels of total SERCA2a expression and LV function at later stages in the 2 rat lines favor the use of induced-by-disease promoters for future gene therapy of HF.\(^{28}\)

In the present study, we demonstrated that cardiac SERCA2a overexpression had a beneficial effect on myocardial function as long as SERCA2a is overexpressed, with no significant effect on LV global systolic and diastolic function. We also demonstrated that cardiac SERCA2a overexpression increased the risk of sudden death during acute MI. This should be taken into account for future gene therapy in patients at risk for myocardial ischemia.

Acknowledgments

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References

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