In vivo adenoviral transfer of sorcin reverses cardiac contractile abnormalities of diabetic cardiomyopathy


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CONGESTIVE HEART FAILURE is an important medical problem resulting in significant morbidity and mortality. Heart failure occurs at an increased incidence in patients with diabetes mellitus. In addition to an increased propensity for coronary vascular disease, resulting in ischemic heart disease, diabetic cardiomyopathy occurs in combination with or independent of coronary vascular disease. In the diabetic heart, abnormal Ca^{2+} handling during the contractile cycle results in a decreased upstroke phase of the Ca^{2+} transient due to diminished release of Ca^{2+} from the sarcoplasmic reticulum (SR) by ryanodine receptor (RyR)2 (23). In addition, the diastolic decline of the Ca^{2+} transient is diminished due to reduced activity of the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA)2a pump (4). Recently developed approaches are aimed at improving the abnormal Ca^{2+} flux of the heart with viral vector-based delivery of proteins to cardiac myocytes, resulting in normalization of the Ca^{2+} transient and improved contractile function. The identification of novel Ca^{2+}-modulating proteins suitable for viral vector-based delivery is therefore of interest to gain additional insight into the components governing calcium homeostasis in the cardiac myocyte and to potentially provide novel strategies for therapeutic intervention to achieve improved contractile function.

One of the Ca^{2+}-modulating proteins is sorcin, a 21.6-kDa calcium-binding protein that is a member of the penta EF-hand family (11). Sorcin was initially identified in multidrug-resistance cells, in which it is overexpressed because of a shared locus encompassing both the multidrug-resistance P-glycoprotein (mdr1) and the sorcin gene (12). Sorcin is expressed in a wide variety of mammalian tissues, including heart and skeletal muscle (14). However, the function of sorcin remains speculative in relation to both multidrug resistance and other functions, derived from its effects in excitable cells like neurons (6) and skeletal muscle (15). Sorcin has a wide tissue distribution and a highly conserved amino acid sequence among species, suggesting that its biological role transcends its potential involvement in multidrug resistance.

Sorcin translocates from the cytosol to membranes on binding of Ca^{2+}. Translocation takes place at micromolar Ca^{2+} concentrations, and it is reversed when the cation concentration is lowered by addition of EGTA (16, 28). Translocation from the cytosol to membranes allows sorcin to interact with specific target proteins. In cardiac cells, sorcin localizes to junctions between the transverse tubule system and junctional sarcoplasmic reticulum (SR) and antisera against either sorcin or the cardiac RyR2 precipitate both proteins (14). Furthermore, recombinant sorcin decreases the open probability of single RyR2 reconstituted in lipid bilayers (3, 9) and can inhibit Ca^{2+} sparks of RyR2 in saponin-permeabilized mouse cardiac myocytes (3). Therefore, sorcin may play a role in modulating intracellular Ca^{2+} levels in the heart (26). More recently, an association of sorcin with the pore-forming subunit of voltage-dependent L-type Ca^{2+} channels was found (15); however, the functional implication of this association is unknown.

Together, these observations suggest that sorcin may participate in regulating calcium homeostasis in the cardiac cell. Moreover, alterations in the expression of sorcin might contribute to impaired sarcoplasmic Ca^{2+} handling in pathological states of the myocardium. Sorcin was found to be elevated in transgenic mice with cardiac-specific expression of activating transcription factor 3, a stress-inducible gene, which have conduction abnormalities and contractile dysfunction (17). Recently, a transgenic mouse in which sorcin was overexpressed 20-fold in the heart showed reduced cardiac contractility (13). Unfortunately, exposure to high levels of sorcin during development and adaptive alterations provoked by long-term exposure to high sorcin levels may lead to a more complicated...
cardiac phenotype. Thus an approach that permits study of the short-term effect of sorcin in the intact cell is necessary.

Accordingly, we hypothesized that sorcin may modulate cardiac contractility. We therefore determined the effects of in vivo cardiac adenoviral gene transfer of sorcin on the contractile properties of the mouse heart under both physiological and pathological conditions and the influence of overexpression of sorcin on Ca\(^{2+}\) transients of adult cardiac myocytes with adenoviral vector-based expression of sorcin. Our results show, for the first time, that sorcin enhances cardiac contractile performance in the normal mouse heart. In addition, sorcin can also improve the decreased contractile function of the diabetic heart by enhancing the Ca\(^{2+}\) upstroke phase of the Ca\(^{2+}\) transient, which is diminished in diabetic cardiac myocytes.

**MATERIALS AND METHODS**

Mice were handled according to the animal welfare regulations of the University of California, San Diego, which are in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, Revised 1996), and the experimental protocol was approved by the Animal Subjects Committee of this institution.

**Adenoviral gene delivery.** Mice were anesthetized with a ketamine (100 mg/kg)-xylazine (8 mg/kg) mixture, intubated, and ventilated with room air. The heart was exposed by a lateral sternotomy at the level of the second intercostal space. Adenovirus (Adv) was administered by direct injection into the left ventricular (LV) free wall of mice heart at a rate of 10 pfu/ml per site with an insulin syringe and a 29-gauge needle. This method allowed for infection of \(-50\%\) of the volume of the total heart, with the majority of it being confined to the free wall of the LV. This value was determined by substituting Adv-cytomegalovirus (CMV) nuclear localization sequence (nls)-\(\beta\)-galactosidase (\(\beta\)-Gal) for Adv-sorcin, slicing the heart into 1-mm-thick segment rings from apex to base, and staining with 5-bromo-4-chloro-3-indolyl-\(\beta\)-D-galactopyranoside (X-Gal) after fixation of the rings with 1% glutaraldehyde. The ring segments were subsequently photographed, and the area stained with X-Gal relative to the total area of the ring segment was measured. This procedure was performed on a total of six mice (not shown). After the thoracic cavity was closed, the mice were extubated and allowed to recover for 5 days.

**Diabetic mice.** Mice were made diabetic by the injection (ip) of 200 mg/kg streptozotocin 3 wk before surgery as described previously (24). Diabetic mice had blood glucose levels in excess of 600 mg/dl, whereas nondiabetic mice had levels of 216 mg/dl. Adenovirus administration was performed as described for normal mice. In vivo cardiac performance was determined by echocardiography in three groups: 1) diabetic mice receiving Adv-sorcin, 2) diabetic mice receiving the empty construct Adv-control, and 3) normal mice receiving the empty construct Adv-control.

**Generation of sorcin adenovirus.** To study the effects of sorcin expression in vivo, we cloned the gene coding for sorcin into a replication-deficient adenoviral vector under control of the promoter-enhancer region of the human CMV (Adv-sorcin). The general procedure was described previously (7). An empty adenovirus without transgene (Adv-control) was injected in the control group.

**Isolated, perfused hearts.** Five days after viral vector injection, hearts were isolated and transferred to a miniaturized Langendorff setup for contractile studies in isolated mouse hearts as previously described (25). In brief, hearts were removed from anesthetized mice and immersed in cold Krebs-Henseleit buffer solution. The aorta was cannulated, and Langendorff perfusion was initiated with Krebs-Henseleit buffer at a perfusion pressure of 55 mmHg. A small fluid-filled balloon was inserted into the LV and inflated to an end-diastolic pressure of 10 mmHg. Pressure development was recorded digitally by connecting the intraventricular balloon to a 2-Fr Millar catheter. The hearts were paced at 400 beats/min, and the resulting pressure waves were analyzed for pressure derivatives [rate of contraction (+\(dP/dt\), rate of relaxation (\(-dP/dt\))] and peak systolic pressure. At the end of the experiment, hearts were frozen in liquid N\(_2\) for Western blot analysis of protein expression.

**Echocardiography.** Transthoracic echocardiography was performed as previously described (22). For acquisition of in vivo cardiac functional data, we used an Agiope CX (ATL Interspec) echocardiography system. For image acquisition, mice were anesthetized with pentobarbital (2.5% (10 \(\mu\)g/g body wt.). The mice were placed in the left lateral decubitus position, and the transducer was placed on the left hemithorax. Care was taken not to apply excessive pressure on the chest to avoid bradycardia. The two-dimensional parasternal short-axis view was used as a guide, and a LV M-mode tracing was obtained close to the papillary muscle level with a sweep speed of 100 mm/s. Pulsed Doppler tracings of the estimated LV outflow tract velocity were obtained in a modified parasternal long-axis view at a sweep speed of 100 mm/s. M-mode and Doppler tracings were recorded on a videotape for offline analysis on an Agilent Sonos 5500 system. After calibration of this system, LV end-diastolic and end-systolic internal diameter (LVDD and LVEDD, respectively) were measured in three consecutive heart cycles with the American Society of Echocardiography leading-edge method (20). LV fractional shortening (FS) was calculated as FSI = (LVDD - LVEDD)/(ET \(\times\) LVEDD). Using the mean aortic ejection time (ET) from three consecutive heart cycles obtained from the Doppler tracings of the LV outflow tract, we calculated the velocity of circumferential fiber shortening (VCF) as VCF (circ/s) = (\(\pi\)LVDDD - \(\pi\)LVEDD)/(ET \(\times\) LVEDD). Being sensitive to acute changes in loading conditions, mean VCF provides an approach for in vivo assessment of myocardial contractibility under basal conditions without acute changes in arterial pressure (10).

**Western blot analysis.** Ventricular tissue (area injected of the LV free wall) or isolated rat cardiac myocytes were homogenized with a Polytron homogenizer. Protein content was measured by the Bradford method (Bio-Rad) and adjusted for equal loading. Protein extracts from heart tissue (20 \(\mu\)g unless otherwise indicated) were separated by a 4-12% bis-Tris-HCl-buffered polyacrylamide gel (Invitrogen, Carlsbad, CA) and subjected to Western blotting for sorcin, phospholamban (PLB), and SERCA2a antibodies (Stressgen, Victoria, BC, Canada and Affinity Bioreagents, Golden, CO). The primary antibody for Western blot of sorcin was a rabbit anti-sorcin polyclonal antibody provided as a kind gift by Dr. Hector Valdivia (Dept. of Physiology, University of Wisconsin Medical School, Madison, WI). The secondary antibody was a horseradish peroxidase-conjugated anti-rabbit antibody. The blot was also probed by a mouse monoclonal \(\alpha\)-actin antibody or a mouse porin (voltage-dependent anion channel) antibody as an internal control to ensure equivalent protein loading and protein integrity. Signals on the films were digitized on a 350 dots/in. scanner.

**Isolation and adenoviral infection of adult ventricular rat cardiomyocytes.** Ca\(^{2+}\)-tolerant adult cardiomyocytes were isolated from ventricular tissue of rats by a standard enzymatic digestion procedure (8) and cultured either on a four-well Lab-Tek chambered coverglass system (Nalge Nunc) or on glass coverslips treated with laminin. Cells were infected with either Adv-sorcin or Adv-control 2 h after isolation with a multiplicity of infection of 20 pfu/cell. Calcium transients were determined 48 h after infection. To test the expression efficiency, myocytes were infected with an adenoviral vector carrying a marker gene, \(\beta\)-galactosidase, at a multiplicity of 20 pfu/cell. After 48 h of infection, myocytes were fixed in 0.5% glutaraldehyde for 5 min at room temperature. The myocytes were then washed twice with PBS and stained for 2 h at 37\(^\circ\)C in PBS containing 1 mg/ml X-Gal, 5 mM K\(_2\)Fe(CN)\(_6\), 5 mM K\(_3\)Fe(CN)\(_6\), and 1 mM MgCl\(_2\). The infection efficiency was determined under these conditions was 94% (results of 2 experiments, not shown).

**Preparation of subcellular fractions.** Cytosol, mitochondria, and SR microsomes were prepared with differential centrifugation. Car-
Diaic tissue or isolated myocytes were homogenized with a Polytron homogenizer in a buffer containing 30 mM Tris, 300 mM sucrose, and protease inhibitor cocktail (1:1,000, Sigma). First centrifugation was performed at 1,500 g for 15 min at 4°C, and the pellet containing the nuclear fraction and cellular debris was discarded. The supernatant was spun at 8,000 g for 15 min. The pellet containing mitochondria was washed twice, spinning two times at the same speed. Supernatant was spun for 1 h at 160,000 g. The pellet was used as the SR microsome fraction, and the supernatant was used as the cytosolic fraction. Fractions were kept frozen at −80°C until testing.

Indo 1 fluorescence. The indo 1-activated Ca2+ transient measurement after adenoviral infection was performed as described previously (5). In brief, cells cultured on chambered coverglass were loaded with indo 1 (3 μM indo 1-AM) via 20-min incubations at room temperature in an atmosphere of 5% CO2-95% air. The dispersing agent, Pluronic F-127 (BASF Wyandotte, Wyandotte, MI), was also present during indo 1 loading at a final concentration of 0.02 mg/ml. Chambers were rinsed to remove excess indo 1-AM and mounted in a Nikon Diaphot epifluorescence microscope equipped with a ×100 fluo objective (oil immersion) interfaced to a Solamere Technologies (Salt Lake City, UT) dual-emission lamp, with the excitation wavelength set to 365 nm via a filter. Fluorescence emission was split and directed to two photomultiplier tubes with 20-nm band-pass filters centered at 405 and 485 nm, respectively. Additionally, an aperture mechanism allowed fluorescence to be collected from a selected portion of the field, which was always positioned over the cytoplasmic region of individual cells. Data were simultaneously collected from each emission channel at a rate of 20 Hz. Fluorescence measurements were performed in Tyrode buffer with 2 mM CaCl2 containing 25 mM HEPES at room temperature beginning 15–20 min after loading with indo 1. For each well, measurements were typically carried out for 10–20 s on an individual cell, a time period during which there was minimal photobleaching of indo 1. This was repeated with additional cells in other fields so that a total of up to 20 cells after infection with Adv expressing sorcin or empty Adv was surveyed. Indo 1 fluorescence data are reported as ratios of fluorescence simultaneously obtained from the 405- and 485-nm channels, providing for relative comparisons of the cytoplasmic Ca2+ concentration ([Ca2+]i) between experimental treatments.

SR Ca2+ load. Experiments were performed at room temperature as described by Shannon et al. (21). In brief, cells were superfused with normal Krebs solution and paced at 0.3 Hz at least 20 times; the solution was then rapidly switched to 0 Na+, 0 Ca2+ normal Tyrode. After at least 30 s, 10 mM caffeine was added to cause SR Ca2+ release. The difference between the basal and peak total systolic [Ca2+] in the presence of caffeine is therefore the SR Ca2+ content.

Statistical analysis. Data are expressed as means ± SE. A Student’s t-test (P < 0.05) was used to make comparisons between controls and sorcin-transfected groups. ANOVA followed by a Student-Newman-Keuls test was used to determine differences among the nondiabetic, diabetic, and diabetic sorcin-transfected groups.

RESULTS

In vivo adenoviral gene transfer of sorcin improves cardiac contractility. To determine whether increasing sorcin levels result in altered contractile function, we increased expression of this protein by an in vivo adenoviral vector-mediated gene transfer approach and analyzed cardiac contractility in the isolated, perfused heart after 5 days of Adv-sorcin administration. Figure 1 shows LV pressure, maximum +dP/dt (+dP/dtmax) and maximum −dP/dt (−dP/dtmax) of isolated, perfused hearts obtained from normal mice that received either Adv-
sorcin or Adv-control. Overexpression of sorcin in the heart resulted in a 40% increase in LV developed pressure (Fig. 1B,a), 54% in +dP/dt max (Fig. 1B,b), and 72% in −dP/dt max (Fig. 1B,c) compared with the control group (158.3 ± 18.2 vs. 113.3 ± 9.1 mmHg, 8,099 ± 1,428 vs. 5,250 ± 763 mmHg/s, and 5,231 ± 891 vs. 3,034 ± 464 mmHg/s, respectively). These data demonstrate that increased expression of cardiac sorcin via gene therapy techniques leads to increased contractility.

Expression of sorcin protein in hearts injected with Adv-sorcin. To verify increased expression of sorcin protein after intramyocardial injection in the Adv-Sorcin group. Western blot analysis was performed in cardiac homogenates. Figure 2 shows an increase of sorcin in ventricular tissue taken from hearts that received Adv-sorcin compared with those that received Adv-control. An increase of 274% in sorcin protein expression was observed in hearts that received Adv-sorcin (3,493 ± 227 vs. 13,058 ± 1,974 arbitrary units for control and sorcin, respectively; P < 0.001; n = 10).

Effect of sorcin overexpression in Ca²⁺-handling proteins in the normal heart. Western blot analysis of SERCA2a, PLB, and RyR2 did not change in the normal heart after overexpression of sorcin (Fig. 2).

Intracellular localization of sorcin after overexpression. To investigate the distribution of sorcin in the cardiac myocyte after overexpression, we infected isolated adult rat myocytes with Adv-sorcin for 48 h and performed cellular fractionating. Western blot analysis of the subcellular fractions showed that sorcin was present in cytosol, mitochondria, and microsomes of control cells. There was a dramatic increase in all of the cellular fractions infected with Adv-sorcin (Fig. 3A). We found a fivefold increase of sorcin protein in total homogenate (Fig. 3A).

Enhancement of Ca²⁺ transients and SR Ca²⁺ load by sorcin overexpression in adult cardiomyocytes. It has been demonstrated that sorcin interacts with proteins involved in Ca²⁺ handling, for example, the RyR of the SR (RyR2) (9) and L-type calcium channels (15). Such interactions could conceivably affect Ca²⁺ handling in sorcin-infected cardiomyocytes and be responsible for the increase in contractility. Therefore, we tested whether sorcin overexpression could alter Ca²⁺ handling. Infection of adult rat myocytes with Adv-sorcin increased expression of sorcin compared with myocytes infected with Adv-control as detected by Western blot (Fig. 3A). Analysis of intracellular Ca²⁺ cycling in adult cardiac myocyte 2 days after Adv-sorcin infection revealed significant increases in both diastolic and maximal systolic indo 1 fluorescence ratios (Fig. 4 and Table 1). In addition, the half-time of [Ca²⁺]
Table 1. Effects of sorcin overexpression on [Ca\(^{2+}\)], of adult rat cardiac myocytes

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>(R_{\text{dia}})</th>
<th>(R_{\text{sys}})</th>
<th>(T_{\text{max}, S})</th>
<th>(t_{1/2, S})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>0.380 ± 0.004</td>
<td>0.515 ± 0.016</td>
<td>0.172 ± 0.007</td>
<td>0.375 ± 0.018</td>
</tr>
<tr>
<td>Sorcin</td>
<td>20</td>
<td>0.399 ± 0.007*</td>
<td>0.640 ± 0.031*</td>
<td>0.194 ± 0.004†</td>
<td>0.320 ± 0.016*</td>
</tr>
</tbody>
</table>

Data are means ± SE for n cells. \(R_{\text{dia}}\) and \(R_{\text{sys}}\), diastolic and maximal systolic indo 1 ratios for electrically paced (0.3 Hz) myocytes infected with empty adenovirus without transgene (control) or adenovirus expressing sorcin (sorcin); \(T_{\text{max}, S}\), time to reach peak systolic Ca\(^{2+}\) (\(R_{\text{sys}}\)); \(t_{1/2, S}\), half-life for intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]) decline. *P < 0.05, †P < 0.001 vs. control.

decline (\(t_{1/2}\)) in sorcin-treated cells was reduced compared with control cells (0.375 ± 0.018 and 0.320 ± 0.016 s, respectively, Table 1). In contrast, the time to reach peak systolic Ca\(^{2+}\) (\(T_{\text{max}}\)) was found to be increased by ~14% in sorcin-infected cardiac myocytes (Table 1). In a different group of experiments, we investigated whether sorcin affects SR Ca\(^{2+}\) load. Figure 5 shows a typical recording of caffeine-induced Ca\(^{2+}\) release from SR in a control cell (Fig. 5A) and a sorcin-infected cell (Fig. 5B). There was an increase in Ca\(^{2+}\) released by the cell that was infected with sorcin. SR Ca\(^{2+}\) content was 49% higher in cells overexpressing sorcin (control 0.291 ± 0.028 vs. sorcin 0.435 ± 0.044 indo 1 ratio; n = 17 cells for control and 8 for sorcin; P < 0.05).

Effect of sorcin overexpression on expression of Ca\(^{2+}\)-handling proteins in cardiac myocyte. RyR2, SERCA2a, and PLB protein levels were analyzed in isolated rat cardiac myocytes infected with Adv-control or Adv-sorcin by Western blot (Fig. 3, B and C). We were not able to detect statistically significant changes in Ca\(^{2+}\)-handling proteins in the total homogenate of cells with sorcin overexpression (Fig. 3B). However, there was a marked increase in expression of RyR2 by 71% (control 27,328 ± 1,262 vs. sorcin, 46,951 ± 2,325), a threefold increase in SERCA2a (control 15,055 ± 932 vs. sorcin 46,268 ± 2,540), and an increase in PLB by 66% (control 36,181 ± 1,461 vs. sorcin 60,281 ± 3,345) in the SR-rich fraction of the cells overexpressing sorcin (Fig. 3C; n = 4; values are given in arbitrary units and normalized by porin).

Improvement of in vivo contractile function of diabetic hearts after administration of Adv-sorcin. Because sorcin overexpression improved cardiac performance in normal mice, probably by enhancing cytosolic Ca\(^{2+}\) transient and SR Ca\(^{2+}\) load in the myocyte, we investigated whether overexpression of sorcin via in vivo cardiac adenoviral gene delivery may improve the diminished cardiac contractile function observed in diabetic mice. We used transthoracic echocardiography for in vivo evaluation of cardiac function. Mice were studied 5 days after adenoviral infection. Heart failure in the diabetic mice was characterized by a marked decrease in both %FS of the LV and VCF (Fig. 6). Adv-sorcin gene therapy rescued cardiac function by returning these parameters to the normal range, as shown in Fig. 6A. In particular, LV %FS and VCF were significantly higher (Fig. 6B). These data demonstrate that sorcin overexpression can counteract the pathological effects of diabetes on cardiac function by augmenting contractility.

Effect of sorcin overexpression in Ca\(^{2+}\)-handling proteins in diabetic heart. Injection of Adv-sorcin into the heart of diabetic mice resulted in a threefold increase in sorcin expression (control 3,523 ± 839 vs. sorcin 13,376 ± 2,495 arbitrary units; n = 4; P < 0.05; Fig. 7). However, we did not detect significant changes in the expression of RyR2 (control 12,762 ± 1,648 vs. sorcin 10,799 ± 1,197; n = 4), SERCA2a (control 12,447 ± 699 vs. sorcin 13,747 ± 3,183; n = 4), or PLB (control 4,251 ± 253 vs. sorcin 4,634 ± 653; n = 4) in the heart after overexpression of sorcin (Fig. 7).

DISCUSSION

Our results show that increased expression of sorcin in normal and diabetic mouse hearts under in vivo conditions results in increased contractile function. Increased sorcin expression was achieved by injecting an adenoviral vector encoding sorcin into the free wall of the LV of the mouse heart. The mechanism responsible for the sorcin-mediated increase in contractile function is most likely linked to sorcin-induced changes in Ca\(^{2+}\) handling by cardiac myocytes.

It is well established that the increase in cytosolic Ca\(^{2+}\) during systole occurs by release of Ca\(^{2+}\) from the SR through a specialized release channel, the RyR, via the process of Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) (2). The entry of a small amount of ("trigger") Ca\(^{2+}\) through the sarcoplasmic L-type Ca\(^{2+}\) current (I\(_{\text{Ca,L}}\)) produces a localized increase of intracellular [Ca\(^{2+}\)]\((i)\) in the small space between the surface and SR membranes. This increases the open probability of the RyR2.

![Fig. 5. Effect of sorcin overexpression on SR Ca\(^{2+}\) load. Representative original tracing of cardiac myocytes infected with a control adenovirus (A) and an adenovirus containing sorcin gene (B). In both cases, the SR is loaded by pacing at 0.3 Hz in normal Tyrode and then the solution is rapidly switched to 0 Na\(^+\), 0 Ca\(^{2+}\) Tyrode. After 30 s, Ca\(^{2+}\) release from SR is induced by rapid exposure to 10 mM caffeine. Sorcin produced a higher caffeine-induced Ca\(^{2+}\) release.](image-url)
reconstituted in lipid bilayers after exposure to recombinant sorcin (3, 9). We also found an increase in diastolic and systolic [Ca\(^{2+}\)], and higher SR Ca\(^{2+}\) load. It is possible that long-term exposure to sorcin or maneuvers that decrease the open probability of RyR2 may lead to higher [Ca\(^{2+}\)]. To support this idea, it has been reported that overexpression of FK506-binding protein (FKBP12.6), which also decreases the open probability of the RyR2, stabilizing it in the closed conformational state, provokes an increase in amplitude of twitch shortening in single cardiomyocytes associated with a reduced RyR2-mediated Ca\(^{2+}\) efflux from SR and a higher SR Ca\(^{2+}\) load (18). Thus FKBP12.6 has been proposed to diminish the Ca\(^{2+}\) leak from the SR (27). Therefore, a putative role for sorcin would be to function as an inhibitor of SR Ca\(^{2+}\) release similar to FKBP12.6 (2, 26). At this time, the mechanism by which sorcin increases contraction and [Ca\(^{2+}\)], has not been fully explored. However, an effect on SERCA2a may be involved because we found that T\(_{1/2}\) was decreased and SR Ca\(^{2+}\) load was higher in cardiac myocytes transfected with sorcin (Table 1 and Fig. 5). This effect of sorcin on SERCA2a activity and/or expression would contribute to the increase in the rate of intracellular Ca\(^{2+}\) decline and therefore be responsible for the higher SR Ca\(^{2+}\) load observed, thus providing more Ca\(^{2+}\) for contraction.

Diabetic cardiomyopathy is characterized by reduced cardiac contractility due to direct changes in heart muscle function independent of vascular disease (1, 19). An important contributor to contractile dysfunction in the diabetic state is an abnormal Ca\(^{2+}\) handling with diminished Ca\(^{2+}\) entry into the cytoplasm during systole and delayed lowering of diastolic Ca\(^{2+}\) levels. A diminished number of RyRs, increased phosphorylation of RyR2, as well as diminished SERCA2a activity and expression, are important contributors to the abnormal Ca\(^{2+}\) handling in diabetes (23). Because sorcin improved cardiac contraction in the normal mouse heart by enhancing Ca\(^{2+}\) transients in the cardiomyocytes, we investigated whether adenovirus-based sorcin expression could ameliorate the diabetes-induced contractile failure. Five days after administration of adenovirus expressing sorcin to diabetic mice, resulting in the efflux of Ca\(^{2+}\) from the SR into the cytoplasm. Amplification of I\(_{Ca}\) by CICR elevates myoplasmic [Ca\(^{2+}\)] to initiate muscle contraction. Relaxation is initiated by a lowering of [Ca\(^{2+}\)], produced either by pumping Ca\(^{2+}\) back into the SR by the SR Ca\(^{2+}\)-ATPase or out of the cell, largely by the sarcolemmal Na\(^+\)/Ca\(^{2+}\) exchanger.

Our findings show that a moderate increase (3- to 5-fold) in sorcin expression results in an enhanced peak of the systolic or upstroke phase of the Ca\(^{2+}\) transient. Additionally, an increase in SR Ca\(^{2+}\) load was observed after sorcin overexpression. Commensurate with these changes, an increase in systolic contractile performance occurs as indicated by enhanced FS, circumferential fiber shortening, and dP/dt\(_{max}\). This positive effect of sorcin on contraction may be expected because it has been demonstrated that introduction of sorcin into nonmuscle cells augments caffeine-activated intracellular Ca\(^{2+}\) release, suggesting a role for sorcin in modulating RyR2 function or expression (14). In agreement with these findings, we observed an increase in systolic Ca\(^{2+}\) and RyR2 protein expression of adult rat cardiomyocytes after sorcin gene transfer (Table 1, Fig. 3C). We found a delay in T\(_{max}\) consistent with delays in RyR2 opening that have been reported to occur in RyR2.
contractile function was markedly improved as determined by echocardiography. In recent studies, we demonstrated (24) that overexpression of SERCA2a improves myocardial contractility in diabetic mice. Sorcin increased SERCA2a expression in the normal adult rat cardiomyocyte. Furthermore, $dP/dt_{max}$ and $t_{1/2}$ were improved by sorcin, suggesting an increase in SERCA2a activity. Therefore, the recovery of the impaired SR function and consequently, the improvement of contractile properties of the diabetic mice, may be partially due to an effect of sorcin on SERCA2a activity. In addition, sorcin upregulated the expression of RyR2 and SERCA2a in the isolated cardiomyocyte. This effect of sorcin would be beneficial and may explain the recovery of heart function of the diabetic mice because RyR2 and SERCA2a are downregulated during diabetes. In the present study, we analyzed heart function in mice after 3–4 wk of diabetes induction. At this time only a decrease of 25–30% in SERCA2a protein was observed (24). RyR2 and PLB were not changed. Therefore, heart dysfunction in our diabetic model may be attributed mainly to functional alterations of the Ca$^{2+}$-handling proteins. Thus the beneficial effect of sorcin in the diabetic mouse heart may be due to improvement of SERCA2a and/or RyR2 activity. Perhaps a modest increase in expression of these proteins may also participate.

Physiological role of sorcin in heart. Despite a body of data provided by recent reports and the present study, the physiological role of sorcin is still not well understood. The transgenic model overexpressing sorcin presented a decreased cardiac performance (13). This model is characterized by a long-term exposure (including development) of the heart to very high sorcin levels (20-fold increase in expression). Therefore, the transgenic phenotype would be the sum of the effects of sorcin and the adaptive changes or deleterious effects due to long-term exposure to high sorcin. A different study performed in saponin-permeabilized mouse cardiac myocytes showed the acute inhibitory effect of pharmacological concentrations (3–5 μM) of recombinant-purified sorcin on Ca$^{2+}$ sparks (3). The same group demonstrated an attenuation of Ca$^{2+}$ transients of cardiac cells after superfusion with recombinant sorcin (3 μM).

In the present work, we induced a short-term (2–5 day) moderate overexpression (2- to 5-fold) of sorcin in the heart or isolated cardiac myocytes. Our results showed overall a beneficial effect of sorcin in cardiac performance and Ca$^{2+}$ handling. These apparent contradictory results cannot be compared with previous studies because the experimental conditions are quite different. Factors like time of exposure to sorcin, concentrations of sorcin reached in the cell, and the method used to expose sorcin targets to the protein may be determinant in the obtained response. It is possible that sorcin at high concentration or long-term exposure may lead to toxic effects.

In conclusion, this study demonstrates for the first time that sorcin overexpression enhances cardiac contractile performance and establishes the concept of sorcin as an in vivo regulator of myocardial contractility. The mechanism of action of sorcin involves regulation of cytosolic Ca$^{2+}$ fluxes. Viral vector-based delivery of specific proteins improving Ca$^{2+}$ flux, such as sorcin, provides a novel approach to improve contractile function in different pathophysiological conditions including diabetic cardiomyopathy.

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