Sorcin modulates mitochondrial Ca\textsuperscript{2+} handling and reduces apoptosis in neonatal rat cardiac myocytes

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Sorcin modulates mitochondrial Ca\textsuperscript{2+} handling and reduces apoptosis in neonatal rat cardiac myocytes. Am J Physiol Cell Physiol 304: C248–C256, 2013. First published November 14, 2012; doi:10.1152/ajpcell.00039.2012.—Sorcin localizes in cellular membranes and has been demonstrated to modulate cytosolic Ca\textsuperscript{2+} handling in cardiac myocytes. Sorcin also localizes in mitochondria; however, the effect of sorcin on mitochondrial Ca\textsuperscript{2+} handling is unknown. Using mitochondrial pericam, we measured mitochondrial Ca\textsuperscript{2+} concentration and fluxes in intact neonatal cardiac myocytes overexpressing sorcin. Our results showed that sorcin increases basal and caffeine-stimulated mitochondrial Ca\textsuperscript{2+} concentration. This effect was associated with faster Ca\textsuperscript{2+} uptake and release. The effect of sorcin was specific for mitochondria, since similar results were obtained with digitonin-permeabilized cells, where cytosolic Ca\textsuperscript{2+} flux was disrupted. Furthermore, mitochondria of cardiac myocytes in which sorcin was overexpressed were more Ca\textsuperscript{2+}-tolerant. Experiments analyzing apoptotic signaling demonstrated that sorcin prevented 2-deoxyglucose-induced cytochrome c release. Furthermore, sorcin prevented hyperglycemia-induced cytochrome c release and caspase activation. In contrast, antisense sorcin induced caspase-3 activation. Thus, sorcin ant apoptotic properties may be due to modulation of mitochondrial Ca\textsuperscript{2+} handling in cardiac myocytes.

Sorcin, a 22-kDa Ca\textsuperscript{2+}-binding protein member of the penta EF-hand family (19), was initially identified in multidrug-resistant cells (20, 23, 36). Subsequently, sorcin has been detected in a wide variety of mammalian tissues, including heart and skeletal muscle (22). Sorcin induces a drug-resistant phenotype and shows ant apoptotic properties in human colorectal cancer cells, although the mechanism is incompletely understood (17, 18).

Sorcin translates from the cytosol to membranes upon binding of Ca\textsuperscript{2+}. Translocation takes place at micromolar Ca\textsuperscript{2+} concentrations and is reversed when the cation concentration is lowered by addition of EGTA (24, 37). Translocation from the cytosol to membranes allows sorcin to interact with specific target proteins. Sorcin is primarily localized to endoplasmic reticulum and mitochondria in neuronal cells (29). In cardiac cells, sorcin localizes to junctions between the transverse tubule system and junctional sarcoplasmic reticulum (SR) and to mitochondria only at points directly in contact with or in close proximity to the SR near transverse tubules (22). Furthermore, we found that expression of sorcin was increased in the mitochondrial fraction from adult cardiac myocytes overexpressing sorcin (35). However, the function of sorcin in mitochondria is unknown. Recently, evidence that sorcin has an important role in modulating cardiac contractility through its effects on ryanodine receptor (RyR2), L-type Ca\textsuperscript{2+} channels, or Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX) has accumulated (21, 33, 35). We demonstrated that cardiac overexpression of sorcin, achieved via adenoviral gene therapy techniques, reverses cardiac dysfunction in diabetic mice by improving cytosolic Ca\textsuperscript{2+} handling (35). Taken together, these findings suggest a role for sorcin as a Ca\textsuperscript{2+}-handling-modulating protein.

Although interaction of sorcin with plasma membrane and SR proteins has been studied, the role of sorcin in mitochondria is completely unexplored. Participation of mitochondria in regulation of cytosolic Ca\textsuperscript{2+} may be minimal. However, cytosolic Ca\textsuperscript{2+} seems to have a major role in modulating mitochondrial energy production by stimulating dehydrogenases and ATP synthase (12). Furthermore, excess Ca\textsuperscript{2+} accumulation in mitochondria is a common event in the process of cell death by necrosis and apoptosis (8, 31). Therefore, changes in mitochondrial Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{m}) are an important signal in cellular physiology. However, measurement of [Ca\textsuperscript{2+}]\textsubscript{m} in the intact cell has been difficult because of the practical problem of distinguishing signals originating from mitochondria from those originating from cytoplasmic locales. Despite these difficulties, beat-to-beat oscillations of [Ca\textsuperscript{2+}]\textsubscript{m} have been reported using Ca\textsuperscript{2+} indicators that are not specific for mitochondria. Recently, the ratiometric Ca\textsuperscript{2+}-sensitive yellow fluorescent protein pericam, selectively expressed in mitochondria, was used in a study showing beat-to-beat Ca\textsuperscript{2+} oscillations in neonatal cardiac myocytes (32). Using targeted mitochondrial pericam after sarco/endoplasmic reticulum Ca\textsuperscript{2+}-ATPase (SERCA2a) overexpression, we successfully determined mitochondrial Ca\textsuperscript{2+} (2).

The goal of this work was to determine whether sorcin modulates [Ca\textsuperscript{2+}]\textsubscript{m} homeostasis in intact neonatal rat cardiac myocytes and explore whether the effects of sorcin on [Ca\textsuperscript{2+}]\textsubscript{m} are part of the mechanisms of sorcin-induced ant apoptotic effects. Using adenoviral gene transfer of sorcin, we measured [Ca\textsuperscript{2+}]\textsubscript{m} with ratiometric pericam. Our results show, for the first time, that sorcin increases [Ca\textsuperscript{2+}]\textsubscript{m} by activating Ca\textsuperscript{2+} uptake and increasing mitochondrial Ca\textsuperscript{2+} tolerance, preventing Ca\textsuperscript{2+}-induced cytochrome c release and hyperglycemia-induced apoptosis.

MATERIALS AND METHODS

All animal protocols were approved by the University of California, San Diego, Institutional Animal Care and Use Committee and
conform to the Guide for the Care and Use of Laboratory Animals as outlined by the National Institutes of Health.

Cardiac myocyte culture and adenoviral infection. Rat neonatal cardiac myocytes were isolated as described previously (13). Briefly, ventricles from 1- to 2-day-old neonatal rats were minced, digested with collagenase and pancreatin, and subjected to discontinuous Percoll (Pharmacia LKB Biotechnology) gradient centrifugation. The myocyte-enriched fraction was washed twice and resuspended in culture medium. Cells were plated onto gelatin-coated culture dishes or glass chamber slides. Culture medium consisted of 4:1 DMEM-M199, 10% horse serum, 5% fetal bovine serum, and 1% penicillin-streptomycin-amphotericin B (Fungizone). Final glucose concentration was 20 mM. After 24 h of plating, myocytes were infected with a multiplicity of infection of 20 for all viruses. Studies were performed after 48 h of viral infection.

Cardiac myocytes exposed to high glucose. Cells were allowed to adhere to the plates for 24 h before change to basic experimental culture medium (4:1 DMEM-M199, 2% fetal bovine serum, and 1% penicillin-streptomycin-amphotericin B, supplemented with glucose) at physiological [normal (5.5 mM)] or elevated [high (25 mM)] glucose concentrations and maintained in these conditions for 72 h. Cells were subjected to viral infection 24 h after high-glucose exposure.

Construction of adenoviral vectors. Generation of sorcin adenovirus (Adv-sorcin) has been described previously (35). We cloned a mouse gene coding for sorcin (GenBank accession no. BF178692) into a replication-deficient adenoviral vector under control of the promoter-enhancer region of the human cytomegalovirus (Adv-sorcin). An empty adenovirus without transgene (Adv-control) was used in the control group. Antisense sorcin (AS-sorcin) was generated by cloning the sorcin cDNA in reverse orientation relative to the promoter, as described previously (14).

Mitochondrial pericam has been constructed and described by Nagai et al. (28). Pericam is a circularly permuted yellow fluorescent family protein that was fused to calmodulin (CaM) and M13, a 26-residue peptide derived from the CaM-binding region of the skeletal muscle myosin. The Ca$^{2+}$-bound CaM and M13 peptide form a stable and compact complex. We used the ratiometric pericam, which has a bimodal excitation spectrum peaking at 415 and 494 nm. Pericam was targeted specifically to the mitochondrial matrix (mitochondrial pericam) by fusion to a cytosolic oxidase sequence.

We cloned the gene coding for mitochondrial pericam into a replication-deficient adenoviral vector under control of the promoter-enhancer region of the human cytomegalovirus (Adv-CMVpericam). Cells were infected with Adv-CMVpericam using 20 plaque-forming units per cell.

Pericam fluorescence. Cells cultured on a chambered cover glass were perfused with HEPES-buffered medium containing 1.8 mM CaCl$_2$ at room temperature. Chambers were mounted in a Nikon Diaphot epifluorescence microscope equipped with a $\times$100 Fluor objective (oil immersion) interfaced to a dual-excitation lamp system (Solamere Technologies, Salt Lake City, UT) set at 410 nm for excitation 1 and 485 nm for excitation 2 via a set of filters. Fluorescence emission was filtered at 510 nm and directed to a photomultiplier tube. Additionally, an aperture mechanism allowed fluorescence to be collected from a selected portion of the field, which was always positioned over the peripheral, nonnuclear regions of individual cells. Data were collected from the emission channel at a rate of 20 Hz, and the ratio of intensity at 410-nm excitation to intensity at 485-nm excitation was calculated providing for relative comparisons of the [Ca$^{2+}$]$_{in}$ between experimental treatments. [Ca$^{2+}$]$_{in}$ was determined as follows: [Ca$^{2+}$]$_{in}$ = $K_a$ (R - $R_{min}$)/($R_{max}$ - R), where $R$ represents the fluorescence intensity ratio F$_{495}$/F$_{410}$, $K_a$ (~410 nm) and $K_b$ (~485 nm) are the fluorescence detection wavelengths for pericam]. Ratios corresponding to the titration end points are denoted by subscripts indicating the minimum (min) and maximum (max) Ca$^{2+}$ concentration. $K_a$ is the Ca$^{2+}$ dissociation constant of ratiometric pericam, which is 1.7 $\mu$M (28). $R_{max}$ was measured in situ by application of 5 mM Ca$^{2+}$ in the presence of ionophore (ionomycin). $R_{min}$ was obtained by application of EGTA (9).

Indo 1 fluorescence. The indo 1-facilitated Ca$^{2+}$ transient after adenoviral infection was measured as described previously (35).

Preparation of subcellular fractions. Mitochondria and SR microsomes were prepared using differential centrifugation, as described previously (35). Myocytes were homogenized with a Polytron in a buffer containing 30 mM Tris, 300 mM sucrose, and protease inhibitor cocktail (1:1,000 dilution; Sigma). The 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 and spun twice at the same speed. The supernatant was spun for 1 h at 160,000 g. The pellet was used as the SR microsome fraction. Fractions were kept frozen at −80°C until they were tested.

Western blot analysis. Myocytes were homogenized with a Polytron in lysis buffer. Protein content was measured by the Bradford method (Bio-Rad) and adjusted for equal loading. Protein extracts (20 $\mu$g) were separated by a 4–12% Bis-Tris-HCl-buffered polyacrylamide gel (Invitrogen, Carlsbad, CA) and subjected to Western blotting. SERCA2a and cytochrome c antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The primary antibody for Western blotting of sorcin was a rabbit anti-sorcin polyclonal antibody (a kind gift from Dr. Hector Valdivia, Dept. of Physiology, University of Wisconsin Medical School, Madison, WI). Antibodies against caspase-9, caspase-3, cleaved caspase-3, poly(ADP-ribose) polymerase (PARP), and cleaved PARP were obtained from Cell Signaling Technology (Danvers, MA). The secondary antibody was a horseradish peroxidase-conjugated anti-rabbit. The blots were also probed by a mouse monoclonal porin (voltage-dependent anion chan-
nel) antibody or actin antibody as internal controls to ensure equivalent protein loading and protein integrity. Signals on the films were digitized on a 350-dots/inch scanner.

**Analysis of mitochondrial Ca\(^{2+}\) transients.** Resting \([Ca^{2+}]_m\) was obtained from myocytes that did not present spontaneous contraction and were not paced. Diastolic and systolic \([Ca^{2+}]_m\) are the minimum and maximum pericam ratios, respectively, that were measured during contraction. \(Ca^{2+}\) uptake is the maximum slope of the upstroke. \(Ca^{2+}\) release is the maximum slope during \(Ca^{2+}\) decline. \(\Delta[Ca^{2+}]_m\) is the difference between diastolic and systolic \([Ca^{2+}]_m\).

**Statistical analysis.** Values are means ± SE from at least three different experiments. Comparisons between means were analyzed, as appropriate, by Student’s t-test or one-way ANOVA followed by Bonferroni’s post test. \(P < 0.05\) was considered statistically significant.

**RESULTS**

\([Ca^{2+}]_m\) in neonatal cardiac myocytes. Results from a typical experiment in which pericam was used to measure \([Ca^{2+}]_m\) are shown in Fig. 1A. \([Ca^{2+}]_m\) in resting cells was 255 ± 27 nM. \([Ca^{2+}]_m\) increased immediately after electrical stimulation of the cell at 0.3 Hz, and beat-to-beat oscillations were observed (Fig. 1A). After stimulation was stopped, \([Ca^{2+}]_m\) slowly returned to resting levels (Fig. 1A). Interestingly, increasing the rate of pacing further increased \([Ca^{2+}]_m\), which is in accordance with previous reports (data not shown) (4, 26).

Effect of sorcin overexpression on \([Ca^{2+}]_m\). Adv-sorcin transfer in neonatal cardiac myocytes resulted in a dramatic
increase in sorcin protein in mitochondrial, as well as SR, fractions (Fig. 1B). This increase in mitochondrial sorcin was not due to contamination from the SR fraction, since SERCA2a was minimal in the mitochondrial fraction (Fig. 1B). [Ca$^{2+}$]$_{im}$ was higher in cardiac myocytes infected with Adv-sorcin than in cells infected with Adv-control (Fig. 2, A and B). Sorcin increased diastolic (60%) and systolic (80%) [Ca$^{2+}$]$_{im}$ (Fig. 2B). The magnitude of the mitochondrial Ca$^{2+}$ transient was increased approximately twofold by sorcin (Fig. 2C).

Effect of sorcin on mitochondrial Ca$^{2+}$ fluxes. Sorcin over-expression stimulated mitochondrial Ca$^{2+}$ uptake by 150% (Fig. 2D). Sorcin also stimulated Ca$^{2+}$ release by 250% (Fig. 2D). Caffeine induces SR Ca$^{2+}$ release via RyR2 and is able to increase [Ca$^{2+}$]$_{im}$ by increasing cytosolic Ca$^{2+}$ in microdomains (34). Therefore, we investigated the effect of sorcin on caffeine-induced [Ca$^{2+}$]$_{im}$ increase. Figure 3A shows a typical recording of cytosolic Ca$^{2+}$ assessed with indo 1. Recordings of caffeine-induced Ca$^{2+}$ release from SR in a control cell and a sorcin-infected cell are shown. Ca$^{2+}$ release by the sorcin-infected cell was increased. Figure 3B shows the effect of 10 mM caffeine on [Ca$^{2+}$]$_{im}$ in a cell infected with Adv-control and a cell infected with Adv-sorcin. Caffeine induced a slow rise in pericam signal in the control cell; however, the Adv-sorcin-infected cell presented an accelerated mitochondrial Ca$^{2+}$ uptake with a higher maximum peak than the control cell (Fig. 3B).

Effect of sorcin on [Ca$^{2+}$]$_{im}$ of permeabilized cardiac myocytes. To determine whether sorcin has a direct effect on the mitochondria, we permeabilized myocytes with 25 μM digitonin for 30 min and subsequently challenged mitochondria

![Figure 3](http://ajpcell.physiology.org/)
with 50 μM Ca\(^{2+}\). This manipulation induces a rapid Ca\(^{2+}\) uptake by the mitochondria, increasing [Ca\(^{2+}\)]\(_{m}\), which eventually will promote opening of the transition pore and other mechanisms of Ca\(^{2+}\) release that lead to a decrease in [Ca\(^{2+}\)]\(_{m}\),

Fig. 4. Influence of sorcin on cytochrome c (Cyt c) content. Western blot shows protein levels of cytochrome c in mitochondrial and cytosolic fraction in control (C or Ctr) and sorcin-overexpressing (S or Sor) cells. Values (means ± SE, n = 4 for each group) are averages of densitometry values [arbitrary units (AU)] and were normalized with porin and actin for mitochondrial and cytosolic fractions, respectively. *P < 0.05 vs. Ctr.

Fig. 5. Sorcin prevents cytochrome c release induced by 2-deoxyglucose (DOG). Western blot shows protein levels of cytochrome c in mitochondrial and cytosolic fractions in control cells (Ctr), cells treated with DOG, and sorcin-overexpressing cells treated with DOG (Sor + DOG). Values (means ± SE, n = 4 for each group) are averages of densitometry values of each sample and were normalized with porin and actin. *P < 0.05 vs. Ctr.

Fig. 6. Sorcin decreases cytochrome c release induced by hyperglycemia. Western blot shows protein levels of cytochrome c in the cytosolic fraction in normal glucose (NG, 5.5 mM), high glucose (HG, 25 mM), and HG + Adv-sorcin (HG + Sor). NG- and HG-treated cells received an empty adenovirus as control. Values (means ± SE, n = 4 for each group) are averages of densitometry values of each sample and were normalized with actin. *P < 0.05 vs. NG. &P < 0.5 vs. HG.
as shown at the end of the curves. Figure 3C shows a typical recording of the experiment. Ca\(^{2+}\) uptake was more rapid in sorcin-overexpressing than control cells (Fig. 3C, inset). The maximum Ca\(^{2+}\) uptake was higher in the cells that overexpressed sorcin (Fig. 3C).

**Sorcin reduces cytochrome c release and caspase activation.** Sorcin overexpression in cardiac cells increased mitochondrial protein levels of cytochrome c and decreased cytosolic cytochrome c levels (Fig. 4). Cytochrome c can be detected in the cytosolic fraction of control cells, because the normal culture medium contains 20 mM glucose, which has been demonstrated to induce apoptosis (5, 16). To investigate if sorcin could reduce cytochrome c release during apoptosis-induced stimulus, we incubated cardiac myocytes with 3 mM 2-deoxy-glucose (DOG) for 24 h (27). Figure 5 shows that DOG reduced mitochondrial and increased cytosolic cytochrome c protein levels, which is in agreement with results reported by others (27). Sorcin effectively reduced cytochrome c release (Fig. 5). Hyperglycemia is known to induce cytochrome c and trigger apoptosis in cardiac myocytes (5, 16). Thus we sought to investigate the effects of sorcin on glucose-induced apoptosis. Figure 6 shows cytosolic cytochrome c levels assessed by Western blotting. High glucose (25 mM) dramatically increased (by 7-fold) cytochrome c. Sorcin expression significantly decreased cytochrome c levels, despite high glucose. Caspase-9, caspase-3, and PARP are activated by high glucose, as demonstrated in Fig. 7A by Western blotting. Densitometric analysis (Fig. 7B) showed that high glucose reduced protein levels of caspase-9, caspase-3, and PARP. In contrast, cleaved caspase-3 and cleaved PARP were increased by high glucose. Sorcin expression in myocytes exposed to high glucose reduced caspase activation, as demonstrated by increased caspase-9, caspase-3, and PARP protein levels. In addition, cleaved caspase-3 and cleaved PARP protein levels were reduced in cells expressing sorcin, despite high glucose.

**Reducing sorcin protein levels using AS-sorcin RNA induces caspase activation.** AS-sorcin reduced sorcin protein levels by 32% (Fig. 8). In addition, caspase-3 was decreased and cleaved caspase-3 was increased, indicating activation of the caspase pathway (Fig. 8).

**DISCUSSION**

Our findings demonstrate, for the first time, that the sorcin-antiapoptotic effect is associated with alterations in mitochondrial Ca\(^{2+}\) by 10.220.33.4 on April 1, 2017 http://ajpcell.physiology.org/ Downloaded from

![Graphs and images showing molecular interactions and results from experiments](image-url)
drial Ca\(^{2+}\) handling in neonatal cardiac myocytes. We found an increase in \([\text{Ca}^{2+}]_\text{m}\) after adenoviral transfer of sorcin that can be due to activation of mitochondrial Ca\(^{2+}\) uptake by sorcin. In addition, mitochondria from cells overexpressing sorcin showed an increased tolerance to Ca\(^{2+}\) and decreased DOG-induced cytochrome \(c\) release, as well as inhibition of hyperglycemia-induced apoptotic signaling.

Recent work by us and others has established a role for sorcin in modulating cytosolic Ca\(^{2+}\) handling by affecting RyR2, NCX, SERCA2a, and L-type Ca\(^{2+}\) channel (1, 7, 21, 33, 35). However, despite data showing that sorcin is expressed also in mitochondria, studies on the effect of sorcin on mitochondria were not available. Accordingly, we sought to investigate the effects of sorcin on mitochondrial Ca\(^{2+}\) handling and whether these effects could explain the antiapoptotic effect of sorcin.

Our findings show that mitochondria are an important sorcin target, since we found a dramatic expression of sorcin in the mitochondrial fraction after Adv-sorcin infection (Fig. 1B).

Using mitochondrial pericam, we were able to measure \([\text{Ca}^{2+}]_\text{m}\) in the intact cardiac myocyte. Furthermore, we found beat-to-beat oscillations in the neonatal cardiac myocyte, which have been observed by others (32). Mitochondrial transients are characterized by a slow Ca\(^{2+}\) uptake followed by slower Ca\(^{2+}\) release (Figs. 1A and 2A). This pattern is in accordance with that predicted by simulations by Crompton (6) and was also observed by Robert et al. (32).

Overexpression of sorcin results in increased \([\text{Ca}^{2+}]_\text{m}\). This effect of sorcin can be explained by activation of Ca\(^{2+}\) uptake, possibly by activating the mitochondrial Ca\(^{2+}\) uniporter (MCU). An alternative explanation is that the higher \([\text{Ca}^{2+}]_\text{m}\) after sorcin overexpression is reflecting an increase in cytosolic Ca\(^{2+}\) concentration, which may be increased by sorcin, as has been demonstrated in the adult rat cardiac myocyte (35). We observed activation of Ca\(^{2+}\) uptake in the intact cell and the digitonin-permeabilized myocyte. These results indicate that sorcin has a direct effect on the mitochondria. However, steady-state \([\text{Ca}^{2+}]_\text{m}\) in sorcin-overexpressing cells may be the result of effects of sorcin on different cellular targets. Sorcin modulates cytosolic Ca\(^{2+}\) handling by its effects on RyR2, L-type Ca\(^{2+}\) channel, or NCX (21, 33, 35). A RyR isoform in mitochondria that participates in rapid Ca\(^{2+}\) uptake has been reported (3). In addition, it has been suggested that mitochondria can transiently experience microdomains of high Ca\(^{2+}\) concentration during systole (34). Furthermore, other studies have shown that the affinity of the MCU for Ca\(^{2+}\) is relatively
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low (10, 11, 15, 25) and only mitochondria in close proximity to the RyR\textsubscript{2} of SR may be exposed to high enough Ca\textsuperscript{2+} concentrations for optimal MCU function (30). Normal Ca\textsuperscript{2+} loading of the SR and Ca\textsuperscript{2+} release by RyR\textsubscript{2} are required for adequate mitochondrial Ca\textsuperscript{2+} uptake by the MCU. Sorcin may play a role modulating the SR-mitochondria interaction. In this work we showed that mitochondrial Ca\textsuperscript{2+} levels mirror cytosolic Ca\textsuperscript{2+} levels after caffeine-induced SR Ca\textsuperscript{2+} release and that sorcin increased cytosolic and mitochondrial Ca\textsuperscript{2+}. Increased mitochondrial Ca\textsuperscript{2+} after sorcin expression can be explained, because there is more Ca\textsuperscript{2+} in Ca\textsuperscript{2+} microdomains. However, increased mitochondrial Ca\textsuperscript{2+} uptake after sorcin overexpression suggests a specific mitochondrial effect of sorcin. This effect alone could explain the increase in mitochondrial Ca\textsuperscript{2+}. In fact, [Ca\textsuperscript{2+}]\textsubscript{m} was higher and Ca\textsuperscript{2+} uptake was greater in permeabilized cells expressing sorcin than control cells, indicating that the effect of sorcin on mitochondrial Ca\textsuperscript{2+} uptake can lead to increased [Ca\textsuperscript{2+}]\textsubscript{m}.

Sorcin was first described in multidrug-resistant cells (36). Recently, an antiapoptotic role of sorcin has been suggested (17). Our results demonstrate that mitochondria from sorcin-overexpressing cells tolerate more Ca\textsuperscript{2+} before opening of the mitochondrial transition pore. In addition, DOG-induced cytochrome c release was prevented by sorcin. We also tested the effect of sorcin on glucose-induced apoptosis. Our results demonstrated that sorcin inhibited glucose-induced cytochrome c release and caspase activation. These results support an antiapoptotic role of sorcin in cardiac myocytes. Further support was obtained in experiments using AS-sorcin to decrease sorcin protein levels. Decrease of sorcin levels in cells exposed to hyperglycemia triggered caspase-3 activation.

In conclusion, sorcin modulates mitochondrial Ca\textsuperscript{2+} handling in neonatal cardiac myocytes. Since [Ca\textsuperscript{2+}]\textsubscript{m} likely influences the efficiency of mitochondrial metabolism, sorcin may help provide a link between contractile activity and cardiac energy metabolism. Furthermore, sorcin may have an antiapoptotic role in the cardiac pericard.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.S. and W.H.D. are responsible for conception and design of the research; J.S., P.M.M., B.T.S., A.S.-R., and H.W. performed the experiments; J.S., A.S.-R., H.W., E.S.F., and W.H.D. analyzed the data; J.S. and P.M.M. interpreted the results of the experiments; J.S. prepared the figures; J.S. drafted the manuscript; J.S., B.T.S., E.S.F., and W.H.D. edited and revised the manuscript; J.S., P.M.M., B.T.S., A.S.-R., H.W., E.S.F., and W.H.D. approved the final version of the manuscript.

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