Sorcin Inhibits Calcium Release and Modulates Excitation-Contraction Coupling in the Heart*

Received for publication, June 5, 2003
Published, JBC Papers in Press, June 24, 2003, DOI 10.1074/jbc.M305931200

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Activation of Ca\(^2\+)
release channels/ryanodine receptors (RyR) by the inward Ca\(^2\+)
current (I\(_{\text{Ca}}\)) gives rise to Ca\(^2\+)
-induced Ca\(^2\+)
release (CICR), the amplifying Ca\(^2\+)
signaling mechanism that triggers contraction of the heart. CICR, in theory, is a high-gain, self-regenerating process, but an unidentified mechanism stabilizes it in vivo. We reported previously (Lokuta, A. J., Meyers, M. B., Sander, P. R., Fishman, G. I., and Valdivia, H. H. (1997) J. Biol. Chem. 272, 25333–25338) that sorcin, a 22-kDa Ca\(^2\+)
-binding protein, binds to cardiac RyRs with high affinity and completely inhibits channel activity. Here we show that sorcin significantly inhibits both the spontaneous activity of RyRs in quiescent cells (visualized as Ca\(^2\+)
sparks) and the I\(_{\text{Ca}}\)-triggered activity of RyRs that gives rise to [Ca\(^{2+}\)]\(_i\) transients. Because sorcin decreased the amplitude of the [Ca\(^{2+}\)]\(_i\) transient without affecting the amplitude or kinetics of I\(_{\text{Ca}}\), the overall effect of sorcin was to reduce the "gain" of excitation-contraction coupling. Immunocytochemical staining shows that sorcin localizes to the dyadic space of ventricular cardiac myocytes. Ca\(^2\+)
induces conformational changes and promotes translocation of sorcin between soluble and membranous compartments, but the [Ca\(^{2+}\)]\(_i\) required for the latter process (E\(_{\text{D50}}\) ~ 200 \(\mu\)M) appears to be reached only within the dyadic space. Rapid injection of 5 \(\mu\)M sorcin onto the cytosolic face of RyRs reconstituted in lipid bilayers resulted in complete inhibition of channel activity in \(\approx\) 20 ms. Thus, sorcin is a potent inhibitor of both spontaneous and I\(_{\text{Ca}}\)-triggered RyR activity and is kinetically capable of playing a role in terminating the positive feedback loop of CICR.

Sorcin, a fairly novel 22-kDa Ca\(^{2+}\)
-binding protein, is known to be present in cardiac myocytes (1), but its function remains to be elucidated. Sorcin was first discovered in multidrug-resistant cells (2, 3), where it acquired its name (soluble resistance-related calcium-binding protein), but it was later found to play a secondary role in multidrug resistance. Meyers et al. (4) found that forced expression of sorcin in fibroblasts was accompanied by unexpected expression of Ca\(^2\+)
release channels/ryanodine receptors (RyR) and that antibodies against sorcin co-precipitated RyR2. These findings suggested a physical association between the two proteins, but the function of sorcin to terminate RyR activity in the intact cell. This has been subsequently demonstrated, suggesting a potential role for sorcin in RyR2 modulation, but questions regarding the role of sorcin in RyR2-initiated processes such as excitation-contraction (e-c) coupling and Ca\(^2\+)
-induced Ca\(^2\+)
release (CICR) remained outstanding.

The structural properties of sorcin make it a good candidate for having a potential role in these basic cellular processes. Sorcin belongs to the penta-EF-hand family of proteins (5) and binds Ca\(^{2+}\)
with high affinity (\(K_a\) Ca ~ 1 \(\mu\)M). At low [Ca\(^{2+}\)], sorcin is readily soluble but exposes hydrophobic residues and tends to associate to membranes when [Ca\(^{2+}\)] is high. It has been suggested that Ca\(^{2+}\)
binding induces conformational changes in sorcin (7), prompting it to translocate from cytosol to membrane-bound target proteins (5, 6, 8). This Ca\(^{2+}\)
dependence of sorcin could be advantageous in the setting of e-c coupling and CICR, where dynamic Ca\(^{2+}\)
signaling occurs. E-C coupling and CICR in cardiac muscle are fairly well understood, but it is still unclear how these two processes achieve stability in intact cells. CICR is conceptually an explosive and self-perpetuating process, but an unidentified mechanism finely grades and interrupts Ca\(^{2+}\)
release in vivo despite Ca\(^{2+}\)
being high. Although several theories have been offered to explain the phenomenon of RyR closure in the presence of Ca\(^{2+}\), such as Ca\(^{2+}\)
-induced inactivation (9), stochastic attrition (10), luminal Ca\(^{2+}\)
depletion (11), or RyR adaptation (12), no mechanism has been completely demonstrated. A recent, seemingly paradoxical, finding concerns Ca\(^{2+}\)
release from RyRs at rest, visualized as Ca\(^{2+}\)
sparks. Ca\(^{2+}\)
spark frequency in cardiac myocytes increases after cell permeabilization (14), suggesting that a cytosolic substance, which would be removed upon permeabilization, is acting to restrain RyR activity in the intact cell. This substance(s), if present, may also be responsible in whole or in part for interrupting Ca\(^{2+}\)
release from activated RyRs during CICR.

* This work was supported by National Institutes of Health Grants R01 HL55438 and P01 HL47053 (to H. H. V.) and the Fondation de France (to A. M. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: RyR, ryanodine receptors; e-c, excitation-contraction; CICR, Ca\(^{2+}\)
-induced Ca\(^{2+}\)
release; DHPR, dihydropyridine receptor; PBS, phosphate-buffered saline; I\(_{\text{Ca}}\), inward Ca\(^{2+}\)
current; MOPS, 4-morpholinepropanesulfonic acid; SR, sarcoplasmatic reticulum.
In this study, we explored the functional role of sorcin at several integrative levels of RyR2 function. By examining the Ca\(^{2+}\)-dependent properties and kinetics of interaction with isolated RyRs, we determined that sorcin is capable of dynamic interaction with the RyR at a rate that would allow for sorcin modulation of the channel on a beat-to-beat basis. Using permeabilized and intact cardiac myocytes, we found that sorcin attenuates both Ca\(^{2+}\)- sparks and Ca\(^{2+}\) transients and establishing the physiological significance of the effects of sorcin in vivo. Finally, we also explored the potential interaction of sorcin with dihydropyridine receptors (DHPRs), another key player in e-c coupling, and found that most of the cellular effects of sorcin may be explained by its predominant interaction with RyRs. Part of this work has been published in abstract form (15).

EXPERIMENTAL PROCEDURES

Cloning and Expression of Sorcin—The human sorcin gene was obtained from ATCC (New York, NY) and amplified by PCR to introduce XhoI sites. The PCR product was subcloned into pGEX-5x-3 (Amersham Biosciences) using the protocol suggested by the manufacturer. Sorcin was expressed in Escherichia coli BL21 (Promega, Madison, WI) and then purified in a glutathione-Sepharose column. Sorcin was eluted into PBS and aliquoted before storage at −80 °C. To avoid denaturation of sorcin, we avoided repetitive freeze-thaw cycles.

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Ventricular Cell Isolation and Calcium Spark Measurements—Mouse ventricular myocytes were isolated by collagenase digestion of perfused mice hearts using the method of Mitra and Morad (16) with modifications addressed by Wolska and Solaro (17). Mice were anesthetized by peritoneal pentobarbital injection (50 mg/kg) before removal and fixed on the coverslips with 4% paraformaldehyde at room temperature. After washing, membranes were incubated with secondary goat anti-rabbit antibodies (IgG) conjugated to horseradish peroxidase (Calbiochem; 1:70,000 dilution in PBST). Protein-antibody reactions were detected by Super Signal chemiluminescent (Pierce) using Kodak X-OMAT film. The relative amount of proteins on the blots was determined by densitometric analysis using the computer program LaserPix (BioRad). Exogenous sorcin was used as positive control.

Fluorescence Measurements—The intrinsic fluorescence of sorcin was measured using a Hitachi Fluorometer F4500; fluorescence quenching was achieved by adding increasing amounts of CaCl\(_2\). The excitation wavelength was 280 nm, and the fluorescence emission spectra were measured starting from a peak at 330 nm. After washing, membranes were incubated with secondary goat anti-rabbit antibodies (IgG) conjugated to horseradish peroxidase (Calbiochem; 1:70,000 dilution in PBST). Protein-antibody reactions were detected by Super Signal chemiluminescent (Pierce) using Kodak X-OMAT film. The relative amount of proteins on the blots was determined by densitometric analysis using the computer program LaserPix (BioRad). Exogenous sorcin was used as positive control.

Whole-cell Patch Clamping—Mouse cardiomyocytes were whole-cell patch-clamped in an external solution (in mM. 140 NaCl, 5.5 MgCl\(_2\), 5 CaCl\(_2\), 5.5 glucose, 1.8 CaCl\(_2\), pH 7.4). Patch pipette contained 300 mmol/liter CsCHSO\(_3\) (cesium methanesulfonate), 10 phosphocreatine, 5 units ml\(^{-1}\) creatine phosphokinase, and 8% dextran, pH 7.2. For measurements of Ca\(^{2+}\) sparks, myocytes were perfused with the internal solution described above with the addition of 0.03 pentapotassium Fluo-4 salt and 60 mM free Ca\(^{2+}\). All solutions were applied onto the cells with a microperfusion system that uses a three-barreled pipette (Warner Instruments). Sorcin concentration was 1 μM. Confocal images were acquired with a BioRad MR-1 confocal microscope coupled to a Nikon Diaphot microscope and a ×40 n.a. oil immersion objective. Images were recorded with the scan line oriented along the long axis of the cell at a speed of 2 μm/s line, using the Lasersharp 2000 software. Fluo-4 was excited at 488 nm with an Ar ion laser, and emitted fluorescence was collected at >515 nm. Ca\(^{2+}\) sparks were measured with a computer program running IDL software. All experiments were performed at room temperature (21–23 °C).

Single-channel Recording of RyR2 and Fast Sorcin Steps—RyRs in SR vesicles were incorporated into planar lipid bilayers as described above (13, 18). The trans (650 μl) and the cis (500 μl) chambers (corresponding to the luminal and cytoplasmic side of the channel, respectively) contained 300 mMmolar/liter CsCHSO\(_3\) (cesium methanesulfonate), 10 μM CaCl\(_2\), and 20 mM MOPS (pH 7.2). A phospholipid bilayer of phosphatidylethanolamine:phosphatidylserine (1:1 dissolved in n-decane to 25 mg/ml) was “painted” with a glass rod across an aperture of ~200-μm diameter in a dehrin cup. The trans chamber was the voltage control side and was connected to the head assembly of a 200A Axopatch. The cis side was held at virtual ground. Signals were analyzed after filtering with an 8-pole Bessel filter at a sampling frequency of 1.5–2 KHz. Data acquisition and analysis were done with Axon Instruments software and hardware (pClamp v6.0, Digidata 1200 AD/DA interface).

The kinetics of sorcin interaction with RyRs were estimated with a rapid exchange reaction method (19). Rapid exchange of solutions at the cytosolic side of the channel was achieved with a 250-ml syringe under the control of a microprocessor (Model MicroTM, World Precision Instruments). The 0.5-mm internal diameter stainless-steel needle was introduced into the cis chamber and positioned with a micromanipulator −50 μm directly in front of the bilayer aperture. A piezo-electric actuator actuated the microprocessor to shuttle the plunger at 200 μm/sec to drive a step increments to infuse solutions. Activation of the piezo-electric circuit charged the bilayer membrane transiently, allowing recording of capacitative currents that marked the duration of the injection. Once the injection was complete, the concentration of the injected modulators...
Adult mouse cardiomyocytes were permeabilized with saponin, and amplitude (Fig. 1C) in situ activity to assess sorcin modulation of RyRs and half-width of Ca²⁺ into several groups. Each group of cells was pelleted and suspended in a solution containing a given [Ca²⁺] in the absence (“intact”) or the presence (“permeabilized”) of 0.01% saponin for 1 min. All groups were then run in SDS gels and immunoblotted with the sorcin antibody (Fig. 3). Sorcin did not vary with [Ca²⁺] in the pellets of intact cells, as expected, because both cytosolic and membrane-bound sorcin were retained inside the intact cell (Fig. 3A). Conversely, sorcin retention in membranes of permeabilized cells was a function of [Ca²⁺] (Fig. 3A). The Ca²⁺ dependence of translocation in permeabilized cells is sigmoidal and may be fitted with a Hill equation that yields a half-maximal effective [Ca²⁺] (EC₅₀) = 202 μM and cooperativity coefficient (n) = 1.8 (Fig. 3B). The latter suggests that each sorcin molecule binds ~2 Ca²⁺ ions for translocation, which is consistent with previous studies that indicate that only two of the five EF-hands of sorcin bind Ca²⁺ (6). The EC₅₀, on the other hand, appears high and physiologically unattainable, but mathematical models estimate [Ca²⁺] at the dyadic junction to be several hundreds of micromolar during Ca²⁺ release (21, 22). It is possible, then, that sorcin translocation may be restricted to dyadic junctions and parajunctional areas, because the measured global [Ca²⁺] appears insufficient to induce translocation in intradyadic spaces. Thus, the privileged location of sorcin around T-tubule/SR junctions (Fig. 2), its capacity to modulate RyRs (Figs. 1 and 6), and its ability to translocate at high [Ca²⁺] all strengthen the notion that sorcin modulates e-c coupling.

Ca²⁺-dependent translocation of sorcin is proposed to be preceded by conformational rearrangements of the protein that expose hydrophobic residues and decrease its solubility in water (5). We exploited the intrinsic fluorescence of sorcin, conferred by two tryptophans in its amino acid sequence, to track its conformational changes in response to Ca²⁺. Fig. 4A shows that sorcin fluorescence, peaking at 330 nm, is highest when [Ca²⁺] is low (nanomolar) and is gradually quenched by increasing [Ca²⁺]. A plot of peak fluorescence versus pCa yields a sigmoidal relationship with EC₅₀ = 5.3 μM and n = 0.68 (Fig. 4B). These values are markedly different from those obtained in Ca²⁺-dependent translocation of sorcin (Fig. 3) and suggest that the latter may be controlled by more than one dynamic step (see “Discussion”).

Sorcin Modulation of Ca²⁺ Transients in Whole Cells—Excitation-contraction coupling in cardiac myocytes brings about abrupt and extensive changes in [Ca²⁺], that, in principle, may induce structural changes in sorcin and stimulate its translo-
**Fig. 2.** Immunostaining of mouse cardiomyocytes with an anti-sorcin antibody. Isolated mouse cardiomyocytes were fixed, permeabilized, and incubated with anti-C-terminal sorcin antibody (1:2000) alone (A) or along with 4.2 μM sorcin protein (control) (B). Both images were obtained with a confocal microscope. Scale bar in panel A applies to panel B.

**Fig. 3.** Ca^{2+}-dependent translocation of sorcin in mouse cardiomyocytes. A. Western blot of sorcin in adult cardiomyocytes, detected with anti-C-terminal sorcin antibody (1:5000). Exogenous sorcin (lanes 1–3) was used as positive control. Cells were exposed to indicated [Ca^{2+}] in the presence of 0.01% saponin (lanes 4–6) or in the absence of saponin (lanes 7–9). B, data points representing the mean intensity of bands in (A) were fitted with a Hill equation that yielded an ED_{50} = ~200 μM and n = 1.8. Data are representative of n = 7 experiments.

Activation from cytosol to membrane-bound target proteins. We patch-clamped adult mouse cardiomyocytes and measured Ca^{2+} transients, the inward Ca^{2+} current (I_{Ca}), and cell shortening in the absence and the presence of 3 μM sorcin (Fig. 5). A voltage step from ~45 mV to a test potential (~40 to +60 mV) was used to induce entry of external Ca^{2+} through L-type Ca^{2+} channels (I_{Ca}), and to elicit CICR. The amplitude of the [Ca^{2+}] transient in cells dialyzed with sorcin was invariably lower than those in control cells (Fig. 5, A and C). This depression of the Ca^{2+} transient was apparent at all tested potentials (Fig. 5C), but time to peak was unaffected (Fig. 5F). Sorcin also shortened the duration of the [Ca^{2+}] transient (Fig. 5A). The decay rate (τ_{decay}) was significantly faster in sorcin-dialyzed cells (Fig. 5F). These sorcin-induced alterations of the [Ca^{2+}] transient were accompanied by a corresponding decrease in cell shortening (Fig. 5D).

Cardiac DHPRs have also been identified as molecular targets for sorcin (23). We found that the amplitude and kinetics of I_{Ca} were unaltered by sorcin (Fig. 5A). A current-voltage relation for I_{Ca} in control and sorcin-dialyzed cells reveals no significant differences (Fig. 5B). Thus, it appears that the decrease in the [Ca^{2+}] transient amplitude is not simply a product of decreased Ca^{2+} entry through DHPRs but more likely a direct effect of sorcin on RyRs. The ratio of [Ca^{2+}] transient amplitude versus the integral of I_{Ca} (F/F_{0})/\Sigma I_{Ca} is a voltage-dependent process that is high at negative voltages and approaches zero at the Ca^{2+} reversal potential (Fig. 5E). This ratio, then, reflects the capacity of I_{Ca} to elicit Ca^{2+} release and may be used as an empirical index of e-c coupling gain (24). Because sorcin decreased the amplitude of the [Ca^{2+}] transient without a concomitant decrease in I_{Ca}, the overall effect of sorcin was to reduce e-c coupling gain (Fig. 5E).

**Kinetics of Sorcin-RyR2 Interaction**—The simplest explanation for sorcin reduction of e-c coupling gain in whole cells is that sorcin interacts with RyRs and directly inhibits Ca^{2+} release by decreasing channel activity. Our previous findings that sorcin inhibits isolated RyRs (1) bodes well with this notion. However, Ca^{2+} release from RyRs, calculated from the amplitude and kinetics of the [Ca^{2+}] transient, is a very fast process (τ_{on} = 2–5 ms, τ_{off} = 15–30 ms (25)). The brief lifetime of Ca^{2+} release thus imposes constraints on the capacity of sorcin to inhibit RyRs on a beat-to-beat basis. We used a fast solution-exchange system (see “Experimental Procedures”) to test the kinetics of sorcin interaction with RyRs. Sorcin (5 μM) was quickly injected onto the cytosolic face of Ca^{2+}-activated RyRs from mouse heart reconstituted into lipid bilayers (Fig. 6). Fig. 6A (top trace) shows that immediately upon solution exchange, sorcin completely abolished RyR activity. In 45 out of 45 injections (n = 10 channels) sorcin injection produced sudden and complete inhibition of RyR current. As sorcin diffused away from the reconstituted channel, activity gradually recovered to full control levels (Fig. 6A, top trace). The average time to recovery of RyR activity to control P_{o} was ~30 s (n = 41) but varied widely with the preceding number of injections. The graph of cumulative P_{o} (NP_{o}) versus time (Fig. 6B) for the trace in Fig. 6A displays the time course of RyR blockade by sorcin and its reversibility. The onset of RyR blockade by sorcin, on the other hand, was extremely fast and technically difficult to estimate. First, activation of the micropump produces an electrical artifact (downward deflections of the baseline current), which sorcin begins to make contact with the reconstituted channel (Fig. 6C). This mechanical disturbance is useful because it indicates the precise time in which sorcin begins to make contact with the reconstituted channel, but being of equal polarity to channel openings, it totally obscures channel recording. Thus, the onset of sorcin inhibition of RyRs (lag time between the point at which sorcin reaches the channel and the resultant inhibition) cannot be determined from the artifact.
precisely determined if channel inhibition occurs within that time window (~20 ms). Because complete cessation of RyR activity was seen immediately after the second artifact in the vast majority of trials, we can safely conclude that sorcin is able to interact with and inhibit single, Ca$^{2+}$-activated RyRs with a time constant ≤ 20 ms. This time frame is compatible with the notion that sorcin may inhibit Ca$^{2+}$ release on a beat-to-beat basis by inducing closure of the channel and possibly helping to terminate CICR.

**DISCUSSION**

In a previous study (1), we reported that sorcin inhibits cardiac RyRs completely and with high affinity ($K_d = -1 \mu M$). More recently, Meyers et al. (26) reported that a transgenic mouse with overexpression of sorcin has reduced [Ca$^{2+}$] transients and depressed cardiac contractility. We show here that sorcin is able not only to completely inhibit RyR2, but it does so rapidly enough to be a potential modulator of Ca$^{2+}$ release during e-c coupling. We also show that sorcin localizes to z-lines in cardiac myocytes, a place ideally suited for interaction with key players of e-c coupling, and that sorcin translocates between cytosol and z-line-related structures in a Ca$^{2+}$-dependent manner. Because sorcin dramatically modifies the frequency and characteristics of Ca$^{2+}$ sparks as well as the amplitude and time course of [Ca$^{2+}$] transients, we propose...
that sorcin may interact with and inhibit RyRs at rest and when activated by $I_{Ca}$. Although sorcin did not seem to significantly alter $I_{Ca}$ under the conditions of this study, Meyers et al. (26) found that sorcin overexpression modifies $I_{Ca}$ kinetics. Thus, it is possible that $I_{Ca}$ is indeed modified by sorcin if higher concentrations of sorcin than those used here exist in the dyadic space. Altogether, the results suggest that sorcin modulates e-c coupling by at least inhibiting RyRs dynamically. This inhibition is fast enough that it is congruent to postulate a role for sorcin in quenching CICR.

Sorcin is a relatively novel protein that, like calmodulin, binds Ca$^{2+}$ with high affinity and has several potential targets. Using immunological assays, sorcin has been shown to interact with annexin VII (synexin) in neurons (27), with RyRs in striated muscle (1), and with DHPRs in cardiac tissue (23). It is possible that the list of sorcin-interacting molecules will continue to grow as sorcin becomes better characterized. It is therefore difficult to ascertain the role of sorcin in whole cells, where sorcin is capable of interacting with multiple partners that complement or antagonize a defined cellular process. This study shows that sorcin influences Ca$^{2+}$ homeostasis in both the resting (Ca$^{2+}$) sparks and contracting (Ca$^{2+}$, transients) states of the cardiac myocyte. Although sorcin inhibition of [Ca$^{2+}$] transients may be affected via several target molecules, the modulation of Ca$^{2+}$ sparks indicates that sorcin directly inhibits RyRs. The latter effect suggests that sorcin in cells is effective even at resting [Ca$^{2+}$] and that the whole pool of sorcin, or part of it, is poised to decrease the spontaneous openings of RyRs that give rise to Ca$^{2+}$ sparks in quiescent cells. Because Ca$^{2+}$ spark frequency in ventricular cells increases upon permeabilization (14), it is tempting to speculate that sorcin may be the critical factor that controls RyR opening rate in intact cells and that is lost upon permeabilization.

As mentioned above, sorcin also has a significant modulatory effect on RyR activity by $I_{Ca}$. During e-c coupling, sorcin imposes two types of inhibitory effects. Voltage-induced [Ca$^{2+}$] transients in sorcin-dialyzed cardiomyocytes have a reduced amplitude compared with control that is apparent at the very start of the transient. Because of the extremely fast rate at which Ca$^{2+}$ activates RyR ($\tau_{on} = 2$ ms; Refs. 12 and 13), it is unlikely that sorcin translocates to RyR upon Ca$^{2+}$ entry through DHPRs and imposes inhibitory effects on RyR activity that are evident so early in the transient. Sorcin’s lack of effect on time to peak of the [Ca$^{2+}$], transient (Fig. 5F) supports this notion. Therefore, the sorcin-induced [Ca$^{2+}$], transient depression is most likely because of the proposed basal inhibition on the RyR mentioned previously. In support of this notion, we previously showed that sorcin may bind to and inhibit RyR activity at resting [Ca$^{2+}$] (1). The second effect, that of sorcin shortening the duration of the Ca$^{2+}$ transient, may be ascribed to the larger sorcin population, which remains cytosolic at resting Ca$^{2+}$ levels (Fig. 3) but is dynamically translocated to membrane proteins upon Ca$^{2+}$ release. Barring effects of sorcin on Ca$^{2+}$ transport molecules such as SERCA2a or the sarcolemmal Na/Ca exchanger, this sorcin population would interrupt Ca$^{2+}$ release faster than in cells lacking sorcin by directly inhibiting RyRs. Sorcin thus provides a potential mechanism that induces closure of RyRs in the presence of activating levels of Ca$^{2+}$.

This study reports for the first time that endogenous sorcin moves between soluble, cytosolic compartments and membrane-embedded targets of ventricular cells as a function of [Ca$^{2+}$]. Surprisingly, we found that the [Ca$^{2+}$] required for membrane translocation is relatively high (EC$_{50} = 200 \mu M$, Fig. 3), or at least higher than previously measured in in vitro assays (5, 8). However, the high [Ca$^{2+}$] required for this phenomenon is congruent with the physiological role for sorcin proposed here. Although [Ca$^{2+}$] in the dyadic space has not yet been measured, most mathematical models place this value at several hundreds of micromolar during Ca$^{2+}$ release (21, 22). It is conceivable then, that sorcin translocation occurs only in “hot Ca$^{2+}$ spots,” such as those close to Ca$^{2+}$ entry or release sites, i.e. the dyadic and parajunctional compartments, which would explain the preferential location of sorcin in these areas (Fig. 2). Nonetheless, it is worth mentioning that other processes not considered here may decrease the [Ca$^{2+}$] required for sorcin for a role in dyadic homeostasis.
translocation. For example, preliminary data suggest that PKA phosphorylation of sorcin shifts the Ca\(^{2+}\) dependence of translocation toward lower [Ca\(^{2+}\)] (not shown). Other structural changes such as dimerization or tetramerization of sorcin that may occur in physiological conditions (28) have yet to be tested. These little-studied conformational changes of sorcin in vivo may also account for the disparity between the \(\text{ED}_{50}\) for Ca\(^{2+}\)-dependent conformational change, measured by changes in protein autofluorescence (Fig. 4) and sorcin translocation. The latter is proposed to be prompted by conformational changes in protein structure brought about by Ca\(^{2+}\) binding to high affinity sites (EF-hands).

The notion that endogenous modulators or accessory proteins of RyRs may quench the inherently positive feedback of CICR is not unprecedented. A notable example is calmodulin, a Ca\(^{2+}\)-binding protein that binds to cardiac RyRs over a wide range of [Ca\(^{2+}\)] but inhibits their activity only when [Ca\(^{2+}\)] is high (29). Calmodulin and sorcin thus share common structural and functional features that place them in a privileged position to inhibit RyRs despite the presence of activating Ca\(^{2+}\). However, a defining feature that makes sorcin unique is its fast rate of RyR inhibition. In intact rat cardiomyocytes, SR Ca\(^{2+}\) release, calculated from the measured global Ca\(^{2+}\) transient (25), displays a time constant of decay equal to 25–30 ms. This fast process defines the necessary time frame in which RyR inhibition by accessory proteins must occur. In the fast solution exchange experiments presented here (Fig. 6), we estimated that sorcin inhibits RyRs in less than 20 ms. The rate of calmodulin inhibition of cardiac RyRs has not yet been measured, but estimates with skeletal RyRs indicate that calmodulin requires several tens of seconds to exert inhibition (29). Furthermore, calmodulin is only a partial antagonist of RyR activity, whereas sorcin inhibits channels completely. We thus conclude that sorcin is a viable candidate to restrain CICR.

In summary, sorcin is a novel Ca\(^{2+}\)-binding protein that appears to play an important role in e-c coupling of cardiac cells. Among accessory proteins of RyRs, sorcin is unique in its ability to inhibit RyR activity rapidly and completely. In intact and permeabilized cells, sorcin modifies Ca\(^{2+}\) sparks characteristics and [Ca\(^{2+}\)], transient waveforms by reducing Ca\(^{2+}\) release and e-c coupling gain. Sorcin’s remarkable inhibition of RyR2 and the speed with which it inhibits, along with its Ca\(^{2+}\)-dependent dynamics, make it a practical candidate for playing a role in quenching the positive feedback of CICR. Although sorcin may not act alone in this important mechanism, elucidating sorcin as a player is a defining step in uncovering the elusive mechanism by which CICR is terminated.

Acknowledgment—We thank Dr. Enrico Stefani (University of California, Los Angeles) for deconvoluting the confocal images used in Fig. 2 and Nancy A. Benkusky for help in obtaining recombinant sorcin.

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