Sorcin is a widely expressed, 22-kDa Ca\textsuperscript{2+}-binding protein initially identified in multidrug-resistant cells. In the heart, sorcin localizes to the dyadic junctions of transverse tubules and sarcoplasmic reticulum and co-immunoprecipitates with the Ca\textsuperscript{2+} release channel/ryanodine receptor (RyR) (Meyers, M. B., Pickel, V. M., Sheu, S.-S., Sharma, V. K., Scotto, K. W., and Fishman, G. I. (1995) J. Biol. Chem. 270, 26411–26418). We have investigated a possible functional interaction between sorcin and cardiac RyR using purified recombinant sorcin in \textsuperscript{[3]H}ryanodine binding experiments and single channel recordings of RyR. The open probability of single RyR was decreased significantly by the addition of sorcin to the cytoplasmic side of the channel (IC\textsubscript{50} = 480 nM). In addition, sorcin completely inhibited \textsuperscript{[3]H}ryanodine binding with an IC\textsubscript{50} = 700 nM. Inhibition occurred over a wide range of [Ca\textsuperscript{2+}], and sorcin-modulated RyR remained Ca\textsuperscript{2+}-dependent. Furthermore, caffeine-activated RyRs were also inhibited by sorcin at low [Ca\textsuperscript{2+}] (pCa 7), suggesting that Ca\textsuperscript{2+} is not an obligatory factor for sorcin inhibition of RyR. Comparisons of these inhibitory effects with those of calmodulin and calpain, proteins structurally related to sorcin, suggested that the interaction of sorcin with cardiac RyR was distinct from and independent of either of these modulatory proteins. Phosphorylation of sorcin with the catalytic subunit of protein kinase A significantly decreased the affinity of sorcin to modulate RyR. These results suggest that sorcin may modulate RyR function in a normal cell environment and that the level of modulation is in turn influenced by signaling pathways that increase protein kinase A activity.

Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (CICR),\textsuperscript{3} the process by which a small influx of extracellular Ca\textsuperscript{2+} triggers massive release of Ca\textsuperscript{2+} from intracellular stores, has gained acceptance as the mechanism responsible for excitation-contraction coupling in the heart (1, 2). The protein responsible for Ca\textsuperscript{2+} release from the sarcoplasmic reticulum (SR) of cardiac muscle is the Ca\textsuperscript{2+} release channel, a ~2 million-Da protein that binds ryanodine with high affinity and specificity, hence the name ryanodine receptor (RyR) (3–5). A variety of endogenous substances regulate the activity of RyR, including Ca\textsuperscript{2+} (which is the primary signal for Ca\textsuperscript{2+} release), Mg\textsuperscript{2+}, ATP, H\textsuperscript{+}, calmodulin, and several protein kinases (6, 7). Exogenous substances such as ryanodine (8), caffeine (9), and scorpion peptides (10), although without a role in CICR, also regulate RyRs and have contributed to define their pharmacological profile.

Recently it has become evident that a functional Ca\textsuperscript{2+} release channel includes not only the tetrameric RyR, but also the immunophilin FK506-binding protein (11, 12). Removal of FK506-binding protein from RyRs induces the appearance of subconducting states and causes the channel to become “leaky.” In neurons the Ca\textsuperscript{2+}/calmodulin-dependent phosphatase, calcineurin, is associated with the RyR-FK506-binding protein receptor complex and regulates channel activity (13). Thus, accessory proteins are also important regulators of RyR activity.

Sorcin is a 22-kDa protein originally isolated from multidrug-resistant cells in which it was overexpressed as a result of amplification of the sorcin gene (14). Although a role for sorcin in multidrug resistance has not been elucidated, the protein may have a normal function as an accessory protein of RyR/Ca\textsuperscript{2+} release channels. Within the heart, sorcin localizes to the dyadic junctions of transverse tubules and to the SR (15). Immunoprecipitation of cardiac lysates with antisera to either sorcin or cardiac RyR recovers both proteins. Moreover, forced expression of sorcin in fibroblasts results in caffeine-sensitive intracellular Ca\textsuperscript{2+} release, suggesting a functional association of sorcin with the RyR (15). Although sorcin is expressed widely in most tissues, and its complementary DNA predicts an amino acid sequence with some homology to the Ca\textsuperscript{2+}-binding proteins calpain and calmodulin (16), its precise functional role in the heart and other tissues is unknown.

To explore the potential functional consequences of a RyR-sorcin interaction and its implications for CICR in the heart, we conducted \textsuperscript{[3]H]ryanodine binding experiments and single channel recordings of cardiac RyR and tested the effect of recombinant sorcin. We found that sorcin inhibits RyR activity in a dose-dependent manner via a mechanism different from that exerted by calmodulin or calpain. Furthermore, phosphorylation of sorcin by protein kinase A (PKA) greatly decreases its capacity to inhibit RyRs. These results suggest that sorcin may regulate Ca\textsuperscript{2+} release in the heart by modulating RyR function. Part of these results have been published in an abstract form (17).
EXPERIMENTAL PROCEDURES

Materials—Radiolabeled chemicals, \(^{3}H\)ryanodine (60–80 Ci/mmol), and \(^{32}\text{P}\)ATP (3,000 Ci/mmol), were from NEN Life Science Products. Bovine brain phosphatidylethanolamine and phosphatidylserine were from Avanti Polar Lipids (Birmingham, AL). Bovine brain calmodulin, AMP-PCP, caffeine, and the catalytic subunit of PKA were from Sigma. Monoclonal cardiac RyR antibody was from Affinity Bioreagents, Inc. (Golden, CO). Peroxidase-conjugated secondary antibody was from Calbiochem. The chemiluminescence detection kit was from Boehringer. Premixed linear gradient polyacrylamide gels were from Bio-Rad. All other reagents were of highest purity reagent grade.

Recombinant Sorcin—Preparation of the sorcin bacterial expression vector and purification of recombinant sorcin have been described (18). For the studies reported here, bacteria were lysed in 10 mM Tris (pH 7.4) containing 1 mM EDTA, and supernatants were applied to an ion exchange columns equilibrated with that buffer. Columns were eluted with buffers containing 0–5 mM NaCl, and fractions were analyzed by gel electrophoresis and Western blot with sorcin antibody. Sorcin, as a single 22-kDa band, was found in fractions containing 0.14–0.18 mM NaCl. Aliquots of the buffer solutions used for sorcin elution were used as control buffers. All binding and planar bilayer solutions contained millimolar EGTA to attenuate any changes in \(\text{Ca}^{2+}\) which micromolar additions of sorcin could otherwise induce.

Preparation of SR Microsomes—Cardiac SR-enriched microsomes were isolated from combined left and right ventricles of adult pigs by differential centrifugation as described previously (19). Skeletal muscle SR-enriched microsomes were also isolated by this technique from the hind leg muscles of adult Yorkshire pigs. Briefly, tissues were excised rapidly, placed in a solution containing 0.9% NaCl, 10 mM MOPS (pH 7.2), and homogenized in a Waring blender at high speed for 2 min. Unlysed tissue remaining in the homogenate was further treated with a Brinkmann Polytron (20–μm probe, three times for 15 s each at low speed). The Polytron homogenate was spun at 4,000 g for 20 min and the supernatant filtered through four layers of cheesecloth and spun further at 8,000 g for 20 min. The 8,000 g pellet was kept on ice and the supernatant centrifuged at 40,000 g for 30 min. The 8,000 g and the 40,000 g pellets were resuspended in a solution containing 0.9% NaCl, 0.3 M sucrose, and protease inhibitors to a final protein concentration of 20–30 mg/ml. The 40,000 g pellet invariably yielded higher Bmax (maximal receptor density) in \(^{3}H\)ryanodine binding assays than the 8,000 g pellet and was used for all subsequent experiments.

\(^{3}H\)Ryanodine Binding Assays—High affinity \(^{3}H\)ryanodine binding (Kd, 5–10 nM) to pig cardiac and skeletal muscle microsomes was measured as described previously (10, 19) with minor modifications. Aliquots of 60 μg of microsomal protein were added to an incubation medium containing 7 nM \(^{3}H\)ryanodine in 0.2 M KCl, 20 mM MOPS (pH 7.2), 1 mM EGTA, and different amounts of CaCl2 to set \(\text{[free Ca}^{2+}\)] in the range of 0.08–100 μM. The stability constants for \(\text{Ca}^{2+}\) were calculated from the slope of a double reciprocal plot of bound versus free \(^{3}H\)ryanodine. To monitor sorcin phosphorylation, an aliquot of \(^{32}\text{P}\)ATP was added to some reaction mixtures. After 10 min, the reaction was terminated by the addition of a 4 × Laemmli buffer (0.25 M Tris (pH 6.8), 0.4 M dithiothreitol, 8% SDS, 40% glycerol, 0.04% bromophenol blue). Samples were analyzed by SDS-PAGE on linear gradient acrylamide gels (4–15%) followed by Coomassie Blue staining and exposure of the dried gels to x-ray film for 2 days.

Phosphorylation of Sorcin—Sorcin and the activated PKA catalytic subunit (2:1, w/w) were incubated at 30 °C for 5–10 min in 140 mM NaCl, 50 mM MOPS (pH 7.2), 1 mM ATP, 2 mM MgCl2, and 1 mM EGTA in a total volume of 100 μl. After completion of the phosphorylation reaction, the mixture was diluted 2-fold into the \(^{3}H\)ryanodine binding reactions for analysis of the effect of sorcin phosphorylation on \(^{3}H\)ryanodine binding. To monitor sorcin phosphorylation, an aliquot of \(^{32}\text{P}\)ATP was added to some reaction mixtures. After 10 min, the reactions were terminated by the addition of a 4 × Laemmli buffer (0.25 M Tris (pH 6.8), 0.4 M dithiothreitol, 8% SDS, 40% glycerol, 0.04% bromophenol blue). Samples were analyzed by SDS-PAGE on linear gradient acrylamide gels (4–15%) followed by Coomassie Blue staining and exposure of the dried gels to x-ray film for 2 days.

RESULTS

Localization of Sorcin in the Heart—Sorcin is a 22-kDa protein normally expressed in cardiac myocytes, where it localizes preferentially to the SR (15). However, sorcin may translocate from membrane to soluble compartments in a Ca\(^{2+}\)-dependent manner (18). To investigate if sorcin remained associated with the SR-enriched microsomes used in these experiments, we carried out Western blot analysis of total cardiac homogenate and of SR microsomes. Fig. 1 shows that a monoclonal antibody against the NH2 terminus of sorcin recognized recombinant sorcin (lane 1) and a 22-kDa protein in total cardiac homogenate (lane 2), but this band was absent in SR microsomes (lane 3). Conversely, RyRs were heavily detected in the total homogenate but enriched in SR microsomes (lanes 2 and 3, respectively). Thus, little endogenous sorcin remained associated with the SR microsomes in the last step of isolation, and a systematic study of the effect of sorcin on RyRs was possible by adding exogenous sorcin to sorcin-depleted functional RyR.

Sorcin Inhibits RyR Activity—To determine if sorcin was...
capable of modifying RyR activity, we reconstituted swine cardiac RyRs in planar bilayers as described (19). Fig. 2 shows traces of steady-state activity from a single RyR recorded at −30-mV holding potential in the absence (Control) and the presence of the indicated concentrations of sorcin. Channel activity was monitored for more than 60 s in each condition, and histograms of open and closed events were constructed from the binned events. The most significant kinetic effects of sorcin were a decrease in the bursting frequency and an increase in the mean closed time. In the absence of sorcin, closing events could be fitted with two exponentials with mean closed time (τ), \( t_{\text{close}1} = 0.37 \text{ ms (74%)} \) and \( t_{\text{close}2} = 2.65 \text{ ms (26%)} \). In the presence of 800 nM sorcin, \( t_{\text{close}1} \) and \( t_{\text{close}2} \) values remained essentially unchanged, but the majority of events were fitted with a third, longer τ (\( t_{\text{close}3} = 55.3 \text{ ms, 52%} \)). The appearance of longer periods of silence contributed significantly to lower the mean open probability (\( P_o \)). For the particular channel shown in Fig. 2, \( P_o \) was 0.284 in control and 0.310, 0.102, and 0.017 at 200, 600, and 800 nM sorcin, respectively. No significant change in unitary conductance was detected. The bottom panel of Fig. 2 shows the cumulated dose-response relation of this sorcin effect from four independent experiments. The concentration of sorcin necessary for half-maximal inhibition (IC_{50}) of RyR activity was 480 nM. Thus, sorcin at submicromolar concentrations interacts with RyRs or a closely associated regulatory protein to depress channel activity.

Comparison of Sorcin and Calmodulin Effects on RyRs—Sorcin cDNA predicts a structure with two EF-hand Ca^{2+} binding domains, homologous to those of calmodulin (16), which bind Ca^{2+} with high affinity (apparent \( K_d \) for the sorcin-Ca^{2+} complex = \(-1 \mu M \)) (18). We thus compared the effect of sorcin with that of calmodulin (Fig. 3). In this and subsequent experiments, we used the [3H]ryanodine binding assay to assess the effect of modulators in a large population of receptors. [3H]Ryanodine binds with high affinity to a conformationally sensitive domain on the RyR (8). Therefore, experimental conditions that decrease or increase channel activity also modify [3H]ryanodine binding in the same manner (6–8, 10). Fig. 3A shows the effect of sorcin and calmodulin on cardiac RyRs. Experiments were conducted at a [free Ca^{2+}] = 10 μM, a concentration at which the Ca^{2+} binding domains of sorcin are essentially saturated. Sorcin was capable of inhibiting [3H]ryanodine binding completely, with an IC_{50} = 760 nM. The value was reasonably close to that calculated from bilayer experiments (Fig. 2). In contrast, the interaction of calmodulin with cardiac RyR, albeit of higher affinity (IC_{50} = 200 nM), resulted in a maximum of 20% inhibition of [3H]ryanodine binding. When 1 μM sorcin and 10 μM calmodulin were added in tandem, the level of binding was reduced to 31 ± 11% of control (Fig. 3A, filled diamond). This percent of inhibition was the sum of each inhibitor acting separately.
Both the relatively modest effect of calmodulin and the inhibitory effect of sorcin were dramatically changed when skeletal RyR, instead of cardiac RyR, was used in $[^3H]$ryanodine binding assays (Fig. 3B). In agreement with previous results (21, 22), calmodulin inhibited $\approx 70\%$ $[^3H]$ryanodine binding with an IC$_{50}$ $\approx 1200$ nM. Surprisingly, $\approx 1$ $\mu M$ sorcin increased $[^3H]$ryanodine binding more than 200% with respect to control, although the percent of increase varied widely among different SR preparations. These results are consistent with sorcin and calmodulin exerting a different mechanism of action for modulation of RyRs.

**Integrity of Sorcin-treated RyR—Digestion of skeletal RyR by calpain, a $Ca^{2+}$-dependent protease with an amino acid sequence homologous to that of sorcin (14, 16), has been demonstrated by several groups (23, 24). To examine whether sorcin inhibition of RyR activity was caused by sorcin acting as a $Ca^{2+}$-dependent protease, we compared sorcin-treated and untreated RyR by immunoblot analysis (Fig. 4). Cardiac SR microsomes were incubated with several concentrations of sorcin, subjected to SDS-PAGE, transferred to a nitrocellulose membrane, and probed with a cardiac RyR antibody. Quantification of the Western blot (Fig. 4A) by densitometric analysis revealed that the presence of sorcin did not decrease the amount of protein recognized by the RyR antibody (Fig. 4B). These results are incompatible with the possibility that the inhibition of $[^3H]$ryanodine binding and channel activity caused by sorcin was the result of RyR protein degradation.

**$Ca^{2+}$ Dependence of RyR Activity and Sorcin Effect—**We next investigated the effect of $Ca^{2+}$ on sorcin-induced inhibition of $[^3H]$ryanodine binding. $Ca^{2+}$ is essential for $[^3H]$ryanodine binding (6–9), and an increase of $[Ca^{2+}]$ from pCa 8 to pCa 6 decreases the intrinsic fluorescence of sorcin (18), indicative of a $Ca^{2+}$-dependent conformational change. Fig. 5A shows the $Ca^{2+}$ dependence of $[^3H]$ryanodine binding to cardiac RyRs (open circles). Binding was minimal at low $[Ca^{2+}]$ (pCa 7) and increased proportionally with $[Ca^{2+}]$. In the presence of 1 $\mu M$ sorcin (filled circles), binding decreased at all $[Ca^{2+}]$ values tested. For the experiment shown in Fig. 5A, specific binding in the absence and presence of sorcin was (in pmol/mg) 0.012 and 0.004 (pCa 7), 0.05 and 0.031 (pCa 6), 0.10 and 0.06 (pCa 5), and 0.091 and 0.04 (pCa 4), respectively. Thus, sorcin inhibition occurred over a wide range of $[Ca^{2+}]$. The normalized depression of binding induced by sorcin in four independent
determinations was 63.8 $\pm$ 11.6 (pCa 7), 37.6 $\pm$ 17.2% (pCa 6), 40.0 $\pm$ 7.6% (pCa 5), and 55.2 $\pm$ 5.4% (pCa 4).

Fig. 5B shows the effect of sorcin on caffeine-activated RyR. At pCa 7, a $[Ca^{2+}]$ insufficient to open RyR (10) or to saturate the $Ca^{2+}$ binding domains of sorcin (18), binding of $[^3H]$ryanodine to SR-enriched cardiac microsomes was low, but it increased with caffeine concentration (open circles). This stimulating effect reflects binding of caffeine to a specific receptor site in the channel protein that “sensitizes” RyR to $Ca^{2+}$ (9, 10). In the presence of sorcin (filled circles), binding decreased at caffeine concentrations $\approx 1$ mM, with $p < 0.05$ at [caffeine] $\approx 3$ mM (asterisks). These results, along with those of Fig. 5A, suggest that $Ca^{2+}$ was not a necessary cofactor for sorcin inhibition of RyR.

**Phosphorylation Prevents Sorcin from Modulating RyR Activity—**The complementary DNA for sorcin predicts two PKA recognition sites near the COOH terminus. Furthermore, sorcin has been demonstrated to be a substrate for PKA both in vitro and in intact drug-resistant cells (25). However, the functional significance of sorcin phosphorylation has yet to be defined. We therefore investigated whether phosphorylation of
sorcin influenced its capacity to modulate RyR. In prior experiments, sorcin was incubated with [γ-32P]ATP and the catalytic subunit of PKA in the absence and presence of SR microsomes. The proteins were then subjected to SDS-PAGE. Fig. 6A shows a Coomassie-stained gel containing lanes 1–4, recombinant sorcin (1.5 μg, asterisks) and [γ-32P]ATP (1 μM); in lanes 2–4, the catalytic subunit of PKA (0.75 μg); and in lane 4, SR microsomes (30 μg). The autoradiogram of this gel (Fig. 6B) shows that a 10-min incubation of sorcin with PKA results in phosphorylation of sorcin (lane 2). Omission of PKA yields no labeled protein bands (lane 1), suggesting a specific kinase-driven incorporation of [γ-32P]ATP. After 90 min at 36 °C (the time and temperature of our standard [3H]ryanodine binding reaction), phosphorylation of sorcin increases (lane 3) with little phosphorylation of the RyR (lane 4, arrow). Thus, under our experimental conditions, sorcin is more readily phosphorylated by PKA than RyR. Fig. 6C shows that phosphorylated sorcin inhibits only marginally the binding of [3H]ryanodine to cardiac RyRs. In the presence of 800 nM nonphosphorylated sorcin (sorcin incubated with phosphorylation buffer without PKA), binding decreased to 54 ± 11% of control, consistent with the potency observed for the native sorcin (Fig. 3A). In the presence of an identical amount of PKA-treated sorcin, binding decreased only to 88 ± 12%. Furthermore, in phosphorylation reactions where ATP was replaced by its nonhydrolyzable analog AMP-PCP, PKA-treated sorcin retained its capacity to inhibit RyRs (not shown). These results strongly suggest that phosphorylation of sorcin reduces its potency to modulate RyR.

**DISCUSSION**

An influx of extracellular Ca2+ is the triggering stimulus that initiates cardiac muscle contraction (1, 2). The release of Ca2+ from intracellular stores requires the interaction of a number of well characterized components (26) and, presumably, unidentified constituents. Sorcin, a 22-kDa Ca2+-binding protein originally isolated from multidrug-resistant cells, was recently localized to the dyadic junctions of transverse tubules and SR (15). Furthermore, sorcin biochemically associated with the RyR, and its forced expression in nonexcitable cells led to the acquisition of caffeine-dependent intracellular Ca2+ release (15). Together, these observations suggested a functional interaction between sorcin and the RyR, a hypothesis that we have now tested in [3H]ryanodine binding assays and single channel recordings of RyR in the absence and presence of sorcin.

Single channel studies indicated that sorcin inhibits RyR activity in a dose-dependent fashion by prolonging the mean close time without modifying single channel conductance. The IC50 for sorcin inhibition of RyRs obtained from these studies (480 nM, Fig. 2) was approximately the same as that obtained in [3H]ryanodine binding experiments (~700 nM, Fig. 3), indicating that the two methods provided convergent descriptions of the effect of sorcin on RyRs, a convergence that supports the assumption that [3H]ryanodine binding is proportional to P o (6–8, 10). Sorcin depressed cardiac RyR activity whether the channel was activated by an increase in cytoplasmic [Ca2+] or by application of caffeine (Fig. 5), suggesting that Ca2+ was not an obligatory cofactor for the sorcin effect. In addition, no leftward shift in the Ca2+ dependence of [3H]ryanodine binding curve was detected, indicating that sorcin did not significantly alter the affinity of RyRs for activating Ca2+.

Sorcin cDNA sequence predicts a structure with two EF-hand Ca2+-binding domains homologous to those in calmodulin (18). Sorcin binds Ca2+ (Kd ~1 μM) and undergoes both a Ca2+-dependent decrease in intrinsic fluorescence and Ca2+-mediated intracellular translocation from soluble to membranous compartments (25). Comparison of the mechanism of action of sorcin with that of calmodulin was, therefore, of interest. In solutions containing 10 μM Ca2+, calmodulin decreased [3H]ryanodine binding to both cardiac and skeletal RyRs, although in neither case was the inhibition complete (Fig. 3 and Refs. 21 and 22). On the other hand, sorcin totally inhibited binding to cardiac RyR. When added in tandem, the inhibitory effect of sorcin and calmodulin on RyR was additive (Fig. 3A, filled diamond). Unlike calmodulin, which activates RyRs at low [Ca2+] and inhibits them at micromolar [Ca2+] (22), sorcin inhibited RyRs in a Ca2+-independent manner (Fig. 5). In contrast to the effect of sorcin on cardiac RyR, [3H]ryanodine binding to skeletal RyRs was not inhibited. In fact, an increase in specific binding of the alkaloid in the presence of sorcin was observed in those preparations (Fig. 3B). Whether this directionally opposite response reflects structural differences between the cardiac and skeletal RyR isoforms or is a consequence of differences in other components of the Ca2+ release channel complex remains to be determined. Thus, by several criteria, sorcin appears to have a different mode of RyR modulation compared with that of calmodulin.

An important characteristic of the sorcin effect was that it could be relieved by phosphorylation with the catalytic subunit of PKA (Fig. 6). Two consensus sites for PKA phosphorylation...
near the carboxyl terminus of sorcin (18) predicted that phosphorylation was a potential mechanism for modulation of sorcin activity, but no functional consequences of this reaction had been determined. Inactivation of sorcin by phosphorylation is reminiscent of the role that phosphorylation exerts on phospholamban, a protein that controls the activity of the Ca\(^{2+}\)-ATPase pump of SR (26). Phosphorylation of phospholamban by either PKA or Ca\(^{2+}\)/calmodulin-dependent kinase II causes dissociation of phospholamban from the pump, thus relieving the sustained inhibition by phospholamban and increasing the rate of Ca\(^{2+}\) uptake by the SR (27). Like phospholamban, sorcin possesses a phospholipid binding domain (18) which enables it to embed into the SR membrane, and, like phospholamban, sorcin controls the activity of a Ca\(^{2+}\) transport protein of SR to levels that are determined by signaling pathways that increase PKA activity.

The inhibition of RyR activity by sorcin raises the possibility that sorcin may contribute to counter the explosive nature of CICR in cardiac cells. Because both the trigger for Ca\(^{2+}\) release (the inward Ca\(^{2+}\) current) and Ca\(^{2+}\) release itself result in elevated [Ca\(^{2+}\)]. CICR is expected to elicit a strong positive feedback and lead to all-or-none tension development. However, flux measurements in intact cells indicate that RyRs released Ca\(^{2+}\) rapidly in response to triggering signals and then spontaneously stop releasing Ca\(^{2+}\) (28). This inactivation of RyRs avoids depletion of SR Ca\(^{2+}\) content and prevents over-flowing of the myoplasm with Ca\(^{2+}\), an effect that would produce deleterious effects in virtually every aspect of cell function. In this context, sorcin may act as an accessory protein of RyR which helps prevent an excessive release of Ca\(^{2+}\) by inhibiting RyR activity. The Ca\(^{2+}\)-mediated translocation of sorcin from soluble to membrane cellular locations (18) (and the preferential access of sorcin to RyRs (15)) are consistent with this hypothesis. Under low Ca\(^{2+}\) concentrations, sorcin would be present in cytosolic compartments and outside the critical distance in which a physical interaction with RyRs may occur. However, a local elevation of [Ca\(^{2+}\)], produced by activation of RyRs would cluster sorcin in the SR membrane and in the microenvironment of RyRs to inhibit further Ca\(^{2+}\) release. This Ca\(^{2+}\)-dependent, dynamic sorcin-RyR interaction would ensure that after dissipation of the Ca\(^{2+}\) gradient produced by Ca\(^{2+}\) release, sorcin would dissociate from RyRs to leave the channels available for subsequent triggering signals. This hypothesis remains to be tested in intact cardiac myocytes.

Although the present data provide ground to postulate a functional association of sorcin to RyR, it is important to remark that the exact biological function of sorcin is unknown. Sorcin was originally isolated as an abundant 22-kDa protein overexpressed in multidrug-resistant cells as a result of amplification of the locus encompassing both the F-glycoprotein (mdr1) and sorcin genes (14). However, although F-glycoprotein overexpression correlates with resistance development, increased sorcin expression is not required, and its abundance does not correlate with the degree of resistance (14). Hence, sorcin expression in multidrug-resistant cells may reflect fortuitous genetic amplification. A broader biological role for sorcin is suggested by its wide distribution in normal mammalian tissues and its highly conserved amino acid sequence among species. It is possible then that the biological function of sorcin transcends its potential role as a protein involved in multidrug resistance. It has been recently established that sorcin not only interacts with RyRs, but with the L-type Ca\(^{2+}\) channel \(\alpha_1\) subunits of cardiac and skeletal muscle as well.\(^2\) Although the mechanisms regulating cross-talk between the plasma membrane L-type Ca\(^{2+}\) channel and the SR Ca\(^{2+}\) release channel remain incompletely characterized, these new data further implicate sorcin in muscle Ca\(^{2+}\) release regulation. Efforts to examine alterations in contractile function resulting from modulation of sorcin expression should be revealing.

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\(^2\) M. B. Meyers, in preparation.