S100A1 decreases calcium spark frequency and alters their spatial characteristics in permeabilized adult ventricular cardiomyocytes

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Abstract

S100A1, a Ca2+-sensor protein of the EF-hand type, exerts positive inotropic effects in the heart via enhanced cardiac ryanodine receptor (RyR2) activity. Here we report that S100A1 protein (0.1 μM) interacts with the RyR2 in resting permeabilized cardiomyocytes at free Ca2+-levels comparable to diastolic Ca2+-concentrations (∼150 nM). Alterations of RyR2 function due to S100A1 binding was assessed via analysis of Ca2+-spark characteristics. Ca2+-spark frequency, amplitude and duration were all reduced upon perfusion with 0.1 μM S100A1 protein by 38%, 14% and 18%, respectively. Most likely, these effects were conveyed through the S100A1 C-terminus (S100A1-ct; amino acids 75–94) as the corresponding S100A1-ct peptide (0.1 μM) inhibited S100A1 protein binding to the RyR2 and similarly attenuated frequency, amplitude and duration of Ca2+-sparks by 52%, 8% and 26%, respectively. Accordingly, the sarcoplasmic reticulum (SR) Ca2+-content was slightly increased but the stoichiometry of other accessory RyR2 modulators (sorcin/FKBP12.6) remained unaltered by S100A1. Hence, we propose S100A1 as a novel inhibitory modulator of RyR2 function at diastolic Ca2+-concentrations in rabbit ventricular cardiomyocytes.

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1. Introduction

S100A1, a Ca2+-sensor protein of the EF-hand type, is predominantly expressed in striated muscle [1–3]. Recent studies have shown that S100A1 colocalizes and coimmunoprecipitates both with the cardiac and skeletal muscle sarcoplasmic reticulum (SR) Ca2+-release channel/ryanodine receptor (RyR) isoform in a Ca2+-dependent manner [4–8].

Moreover, functional studies revealed that S100A1 enhances Ca2+-release from the SR in cardiac and skeletal muscle preparations [4,6,7,9,10] and this effect was found to be maximal at 0.1 μM S100A1 [4,10]. Hence, these findings support the notion of a physical interaction between S100A1 and the SR Ca2+-release channel in cardiac (RyR2) and skeletal muscle (RyR1) that causes increased open probability of the channel. However, in the presence of low cytosolic Ca2+-concentrations ([Ca2+]i) occurring in contracting cardiomyocytes during diastole, S100A1 decreased [3H]-ryanodine binding [4,6] indicating an additional Ca2+-dependent inhibitory action on RyR2 under resting conditions.

The current study therefore aimed to explore the effect of S100A1 on spontaneous SR Ca2+-release in quiescent...
ventricular cardiomyocytes and assessed the active domains of the protein involved in this effect. Taking advantage of access to the cytoplasmic space facilitated by β-escin permeabilization of adult rabbit ventricular cardiomyocytes, we explored S100A1 modulation of RyR2 activity at rest using Ca$^{2+}$-sparks as an index of spontaneous openings of clusters of RyR2 channels. We describe here for the first time that S100A1 can decrease Ca$^{2+}$-spark frequency and their spatial characteristics in quiescent cardiomyocytes at low [Ca$^{2+}$]~150 nM. Moreover, we provide evidence that this effect is most likely conveyed through a direct interaction of C-terminus of the native protein with the RyR2. Hence, S100A1 is not only capable of enhancing the gain during cardiac excitation–contraction coupling but also seems to be involved in modulation of RyR2 activity at rest.

2. Materials and methods

2.1. Generation and labeling of recombinant S100A1 protein and synthesis of S100A1 C-terminal peptide (S100A1-ct)

Recombinant human S100A1 protein was expressed and purified as previously reported [11]. Synthesis and purification of the S100A1 C-terminal peptide (S100A1-ct, amino acid 75–94) and N-terminal labeling of recombinant S100A1 protein with tetramethyl-rhodamine (rhod-S100A1) was custom-made by Eurogentec (Seraing, Belgium) as reported elsewhere [10,12].

2.2. Rabbit ventricular cardiomyocyte isolation and Ca$^{2+}$-spark measurements

Adult ventricular rabbit cardiomyocytes were isolated from four different animals as previously described [9] and Ca$^{2+}$-sparks in permeabilized cells were monitored using a Bio-Rad 2000 laser scanning confocal microscope (LSCM) as detailed elsewhere [13]. In brief, isolated cardiomyocytes were perfused with a mock intracellular solution and permeabilized using β-escin (0.1 mg/ml). Fluo-3 free acid (10 µM) present in the perfusing solution was excited at 488 nm (Kr laser) and measured at >515 nm applying epifluorescence optics of an inverted microscope with a ×60,1.2 NA water-immersion objective lens. Fluorescence was acquired in line-scan mode at 2 ms line$^{-1}$; pixel dimension was 0.3 µM (512 pixels scan$^{-1}$; zoom = 1.4). The scanning laser line orientated parallel with the long axis and placed approximately equidistant between the outer edge of the cell and the nucleus/nuclei, to ensure the nuclear area was not included in the scan line. To enable this trace to be converted to free calcium concentration ([Ca$^{2+}$]) a series of calibration solutions were used at the end of each Ca$^{2+}$-spark measurement period incorporating 10 mM EGTA as previously described [13]. In all experiments concerning Ca$^{2+}$-sparks, the [Ca$^{2+}$] in the test solution was 145–160 nM. Ca$^{2+}$-sparks recorded in Fluo-3-containing solutions were quantified using an automated detection and measurement algorithm adapted from a previously published method [14]. All Ca$^{2+}$-spark measurements were made within 7–8 min of cell permeabilization. This time was standardized to minimize loss of soluble proteins. S100A1 protein or S100A1-ct peptide was applied in mock solution using a gravity-fed perfusion system. Effects were compared to permeabilized control cardiomyocytes perfused with mock-solution without addition of S100A1. Up to four different cells from each animal were used for Ca$^{2+}$-spark measurements.

2.3. Estimation of sarcoplasmic reticulum (SR) total calcium content

Total sarcoplasmic reticulum (SR) Ca$^{2+}$-content was immediately assessed after termination of Ca$^{2+}$-spark measurements by rapidly switching to mock solution containing 10 mM caffeine to completely release SR Ca$^{2+}$ as described elsewhere [13]. Up to four different cells from each animal were used for SR Ca$^{2+}$-content.

2.4. Western blotting and coimmunoprecipitation

Coimmunoprecipitation and Western blotting for RyR2 (anti-RyR2; MA-3-925, ABR) with S100A1 (anti-S100A1; SP5355P, Acris), sorcin (anti-sorcin; 33–8000, Zymed laboratories) and FKBP12.6 (anti-FKBP12; 554091, BD Bioscience) was performed in β-escin permeabilized isolated cardiomyocytes either in the presence of 150 nM [Ca$^{2+}$] or in EGTA (2 mM) containing mock intracellular solution as described in details elsewhere [6,15].

2.5. Confocal localization of rhod-S100A1 and BODIPY-FL X ryanodine

To evaluate access of S100A1 protein to the cytoplasmic space of permeabilized cells, 0.1 µM rhod-S100A1 protein was applied to β-escin permeabilized cells in mock solution (mM: 100 KCl, 5 Na2ATP, 10 disodium creatine phosphate, 5.5 MgCl2, 25 HEPES, 0.05 K2EGTA, pH 7.0 (20–21 °C)) in the presence of 145–160 nM [Ca$^{2+}$] for 1 min. BODIPY-FL X ryanodine (B-7507) and unlabeled ryanodine (R7478) were purchased from Molecular Probes. Permeabilized cells were incubated with BODIPY-FL X ryanodine (0.1 µM) in mock solution (20–21 °C) in the presence of 145–160 nM [Ca$^{2+}$] for 1 min. To evaluate specific binding of the fluorescent probe, permeabilized cells were pretreated with 1 mM ryanodine. Cells were gently washed three times before confocal imaging. Coverslips were mounted on the stage of an inverted confocal microscope (Leica SP2, Mannheim, Germany) equipped with a 40×1.2NA water immersion objective. Fluorescence was excited using the 488 nm (BODIPY-FL X ryanodine) and the 543 nm (rhod-S100A1) lines of an Ar$^{+}$ and a HeNe laser, respectively. The laser power was attenuated to 13% (488 nm) and 30% (543 nm) using neutral density
filters. Emission was detected between 505 and 530 nm for the BODIPY-FL X fluorescence and between 550 and 600 nm for rhod fluorescence. To exclude channel crosstalk, images (1024 × 1024, 8 bit) were taken sequentially using only one of the two aligned laser lines at a time. Corresponding images of both channels were overlaid for signal colocalization using the open-source software package ImageJ (NIH software, available at http://rsb.info.nih.gov/ij). Signals are considered as colocalized if their respective pixel values are higher than a fixed threshold. For further information see the description details of the ImageJ colocalization plugin. Pixel values were rescaled for visualization purposes using a custom-made procedure written in IDL6.0 (RSI Systems, Boulder, CO, USA).

2.6. Statistics

Data was expressed as means ± S.E.M. An unpaired two-tailed Student’s t-test and two-way repeated ANOVA were performed for statistical comparisons. For all tests, a P value <0.05 was considered to be significant.

3. Results

3.1. Assessment of entry of S100A1 in permeabilized ventricular cardiomyocytes using rhod-S100A1 protein

Prior to Ca\(^{2+}\)-spark assessment we investigated whether homodimeric S100A1 protein (20.830 M\(_{r}\)) can access the SR in permeabilized cardiomyocytes. We therefore incubated β-escin treated cardiomyocytes with tetramethyl-rhodamine-conjugated S100A1 protein (rhod-S100A1; 0.1 μM) in the presence of 150 nM free calcium concentration ([Ca\(^{2+}\)]). As depicted by a representative confocal fluorescent image (Fig. 1B and B'), rhod-S100A1 protein shows mostly a striated pattern of high intensity staining interspaced at about ∼2 μm running along the length of the cell. The periodic interruption of rhod-S100A1 staining pattern apparently overlaps with Z-lines in the corresponding transmission image (Fig. 1A and A'). No further staining was observed with longer incubation times and control cells incubated with 0.1 μM fluorescent dye lacked this characteristic staining pattern (data not shown).

Hence, this approach confirms free access of native S100A1 protein to the intracellular space of permeabilized rabbit ventricular cardiomyocytes in our experimental setting and suggests specific binding to structures within the cytosol.

3.2. Colocalization of rhod-S100A1 protein and RyR2 at diastolic Ca\(^{2+}\)-concentrations

Subcellular binding sites of rhod-S100A1 protein were further examined by the use of BODIPY-FL X ryanodine to visualize RyR2 distribution in β-escin permeabilized cardiomyocytes. As shown by a representative confocal fluorescent image (Fig. 1C and C', green) RyR2 labeling with BODIPY-FL X ryanodine (0.1 μM) in mock-solution (150 nM [Ca\(^{2+}\)]) resulted in a longish striated and periodically

Fig. 1. Access of S100A1 protein to the intracellular space in β-escin permeabilized rabbit ventricular cardiomyocytes and Ca\(^{2+}\)-dependent colocalization of S100A1 protein with the RyR2 at diastolic [Ca\(^{2+}\)]. (A and B): Shown are representative confocal images of the intracellular distribution of rhod-S100A1 protein (0.1 μM) in a permeabilized cardiomyocyte after 1 min incubation (B and B', red) and the corresponding transmission image (A and A'). (C) Depicted are representative confocal images of the same cell co-stained with BODIPY-FL X ryanodine (0.1 μM) after 1 min (C and C', green) targeting RyR2. (D) Corresponding overlay of B and C (D and D', colocalization is indicated by yellow pseudo-coloring) illustrates colocalization of rhod-S100A1 protein and RyR2 at diastolic [Ca\(^{2+}\)]. Scale bar = 35 μm (A). A'–D': 4 × magnification of the indicated inlet (white boxes) in A–D.
interrupted SR-like staining pattern similar to rhod-S100A1 (Fig. 1B and B’, red). Preincubation with unlabeled ryanodine (10 mM) completely abolished BODIPY-FL X dependent RyR2 staining indicating specific binding of SR Ca\(^{2+}\)-release channel by the fluorescent probe (data not shown). Overlay of rhod-S100A1 (Fig. 1B) and RyR2 (Fig. 1C) images revealed significant colocalization of both molecules as indicated in Fig. 1D and D’ (yellow).

Since subcellular location of rhod-S100A1 protein clearly corresponds with intracellular RyR2 distribution, this finding strongly supports the notion of an specific interaction between the Ca\(^{2+}\)-sensor protein and the SR Ca\(^{2+}\)-release channel in permeabilized ventricular cardiomyocytes at diastolic Ca\(^{2+}\)-concentrations.

3.3. Ca\(^{2+}\)-dependent interaction of S100A1 protein with RyR2 at diastolic Ca\(^{2+}\)-concentrations

Taking advantage of coimmunoprecipitation, we sought to further corroborate the interaction of S100A1 protein with the macromolecular RyR2 Ca\(^{2+}\)-release complex in our experimental setting. Fig. 2A shows a representative coimmunoprecipitation for RyR2 and added S100A1 protein (0.1 \(\mu\)M) in permeabilized cardiomyocytes. S100A1 protein coimmunoprecipitates with the RyR2 in the presence of calcium (\(~\sim 150\) nM [Ca\(^{2+}\)]) but not in its absence (EGTA 2 mM) (Fig. 2A, lane 1/2). The hydrophobic C-terminus of S100A1 (amino acids 75–94) has been recognized as a crucial domain for binding and altering function of various target proteins and is exposed upon Ca\(^{2+}\)-binding [16–20]. The effect of the corresponding S100A1-ct peptide (amino acids 75–94) (Fig. 2B) on S100A1 interaction with the RyR2 was therefore evaluated. Preincubation of permeabilized cells with 10 \(\mu\)M S100A1-ct peptide for 15 min completely inhibited the Ca\(^{2+}\)-dependent coimmunoprecipitation of the protein with the RyR2 Ca\(^{2+}\)-release complex (Fig. 2A, lane 3/4) indicating a crucial role of the C-terminus for S100A1-RyR2 interaction. Moreover, the S100A1-ct peptide almost completely blocked coimmunoprecipitation of endogenous S100A1 protein with the RyR2 in the presence of 150–200 nM calcium (data not shown) suggesting that the S100A1-ct peptide also competes with the native protein for RyR2 binding sites.

Hence, these data suggest that S100A1 protein can interact with the RyR2 in our experimental setting in the presence of diastolic [Ca\(^{2+}\)] and this effect is likely conveyed by the hydrophobic C-terminus of the native protein.

3.4. Impact of S100A1 protein and S100A1-ct peptide on resting calcium sparks and SR Ca\(^{2+}\)-content in permeabilized ventricular cardiomyocytes

As shown by representative original tracings (Fig. 3A), Ca\(^{2+}\)-sparks were recorded under control conditions (\(n = 336\) sparks, \(n = 10\) myocytes) and in the presence of S100A1 protein (0.1 \(\mu\)M) (\(n = 350\) sparks, \(n = 12\) myocytes) in a mock intracellular solution containing 150 nM [Ca\(^{2+}\)] and 10 \(\mu\)M Fluo-3 salt. S100A1 protein significantly reduced Ca\(^{2+}\)-spark mean frequency (events s\(^{-1}\); control 0.045 ± 0.005 versus S100A1 0.028 ± 0.006, \(P < 0.05\)) (Fig. 3C) and mean amplitude (\(F/F_0\); control 1.93 ± 0.07 versus S100A1 1.89 ± 0.04).
Fig. 3. S100A1 protein decreases Ca\(^{2+}\)-spark frequency and alters their characteristics in permeabilized rabbit ventricular cardiomyocytes. (A) Representative line-scan images recorded from permeabilized control and S100A1 protein treated cells perfused with Fluo-3-containing mock solution for 420 s. (B) Time-dependent decrease in Ca\(^{2+}\)-spark frequency in permeabilized control (n = 10 cells) and S100A1 protein (0.1 μM) (B, n = 12 cells) perfused cardiomyocytes. T\(_0\) indicates addition of S100A1 protein. Note that control cells exhibit unchanged Ca\(^{2+}\)-spark frequency within the time frame of the study. (C–F) Mean values given for Ca\(^{2+}\)-spark frequency, amplitude, duration and width under control conditions and in response to S100A1 protein after 420 s. * P < 0.05 compared with control.

1.67 ± 0.02, P < 0.05) (Fig. 3D) in a time-dependent manner (Fig. 3B). In addition, S100A1 abbreviated the mean spark duration (ms; control 27.99 ± 2.16 versus S100A1 22.97 ± 0.04, P < 0.05) (Fig. 3E) of Ca\(^{2+}\)-sparks while mean spark width (μm; control 3.12 ± 0.04 versus S100A1 2.99 ± 0.18, P = 0.08) (Fig. 3F) remained unaltered.

As S100A1-ct inhibited the Ca\(^{2+}\)-dependent interaction of S100A1 protein with the RyR2, the effect of S100A1-ct (amino acids 75–94) on Ca\(^{2+}\)-spark frequency and characteristics was therefore explored. As shown by representative original tracings obtained under control conditions (n = 368 sparks, n = 10 myocytes) and in the presence of S100A1 protein (0.1 μM) (n = 374 sparks, n = 12 myocytes) (Fig. 4A), application of 0.1 μM S100A1-ct resulted in a significant time-dependent attenuation of Ca\(^{2+}\)-spark mean frequency (events μm\(^{-1}\) s\(^{-1}\); control 0.050 ± 0.004 versus S100A1-ct...
Fig. 4. The hydrophobic C-terminus of S100A1 protein (S100A1-ct) attenuates Ca\(^{2+}\)-spark frequency and alters their characteristics in permeabilized rabbit ventricular cardiomyocytes. (A) Representative line-scan images recorded from permeabilized control and S100A1-ct treated cells perfused with Fluo-3-containing mock solution for 240 s. (B) Time-dependent decrease in Ca\(^{2+}\)-spark frequency in permeabilized control (\(n=10\) cells) and S100A1-ct (0.1 \(\mu\)M) (B, \(n=12\) cells) perfused cardiomyocytes. \(T_0\) indicates addition of S100A1-ct peptide. Note that control cells exhibit unchanged Ca\(^{2+}\)-spark frequency within the time frame of the study. (C–F) Mean values given for Ca\(^{2+}\)-spark frequency, amplitude, duration and width under control conditions and in response to S100A1-ct after 240 s. \(* P<0.05\) compared with control.

0.024±0.003, \(P<0.05\) (Fig. 4B and C) and alterations of Ca\(^{2+}\)-spark characteristics: mean amplitude (\(F/F_0\)) control 1.78±0.04 versus S100A1-ct 1.65±0.02, \(P<0.05\) (Fig. 4D), mean spark duration (ms) control 29.09±2.56 versus S100A1-ct 22.6±0.08, \(P<0.05\) (Fig. 4E), Mean Ca\(^{2+}\)-spark width (\(\mu\)m) control 3.03±0.08 versus S100A1-ct 2.93±0.07, \(P=0.12\) was not changed by S100A1-ct (Fig. 4F).

As SR Ca\(^{2+}\)-content critically affects Ca\(^{2+}\)-sparks activity [21,22], we assessed whether S100A1 protein and S100A1-ct peptide effects the SR Ca\(^{2+}\)-levels under the experimental settings. Both S100A1 protein (\(F/F_0\)) control 3.11±0.12 versus S100A1 protein 3.44±0.15, \(P<0.05\), \(n=15\) myocytes) and S100A1-ct (\(F/F_0\)) control 3.09±0.24 versus S100A1-ct 3.83±0.22, \(P<0.05\), \(n=15\) myocytes) exerted a slight but significant increase on SR Ca\(^{2+}\)-content in permeabilized cardiomyocytes in the presence of 150 nM [Ca\(^{2+}\)]. This finding is in line with the notion that a decrease in diastolic SR Ca\(^{2+}\)-release can result in enhanced SR Ca\(^{2+}\)-content [23].

Thus, our data indicate that S100A1 protein can decrease spontaneous activity of the SR Ca\(^{2+}\)-release channel most
Fig. 5. S100A1 protein and S100A1-ct do not alter sorcin/FKBP12.6 stoichiometry with the RyR2. (A) As shown by a representative Western blot, neither addition of 0.1 μM S100A1 protein nor 0.1 μM S100A1-ct peptide alters the amount of sorcin and FKBP12.6 coimmunoprecipitating with the RyR2. (B and C) Mean values for the densitometric ratio for sorcin/RyR2 and FKBP12.6/RyR2 in the presence of 0.1 μM S100A1 protein and 0.1 μM S100A1-ct compared to control (n = 3).

likely through its C-terminus. Moreover, our results show that the inhibitory effect of S100A1 on spontaneous RyR2 activity does not rely on decreased SR Ca2+-content.

3.5. Impact of Ca2+-dependent interaction of S100A1 protein and S100A1-ct with the RyR2 on stoichiometry of other RyR2 modulators

As other EF-hand Ca2+-binding proteins such as sorcin or immunophilins like FKBP-12.6 are known to modulate spontaneous RyR2 activity [24,25], we further investigated whether the effect of S100A1 on Ca2+-sparks might be caused by an altered stoichiometry of sorcin and FKBP12.6. However, as depicted by representative coimmunoprecipitation (Fig. 5A), neither addition of S100A1 protein nor S100A1-ct peptide altered the amount of coimmunoprecipitated sorcin (Fig. 5B) and FKBP 12.6 (Fig. 5C) with the RyR2 in the presence of 150 nM [Ca2+]. Thus, our results support the notion that S100A1 affects on Ca2+-spark frequency are accomplished without changing sorcin and FKBP12.6 binding to the cytosolic portion of the RyR2.

Thus, our results support the notion that S100A1 effects on Ca2+-spark frequency are accomplished without changing sorcin and FKBP12.6 binding to the cytosolic portion of the RyR2.

4. Discussion

In the present study we report two novel findings: first, at low [Ca2+] occurring during diastole, S100A1 protein can decrease spontaneous Ca2+-sparks in β-escin permeabilized resting ventricular cardiomyocytes; second, most likely, this effect is conveyed through its C-terminal domain as the corresponding S100A1-ct peptide completely mimics the effect of S100A1 protein. This notion is further supported by the finding that S100A1-ct peptide effectively inhibits the Ca2+-dependent interaction of S100A1 protein with the RyR2 and points to the functional importance of the C-terminal domain in this process.

The change in the S100A1 tertiary structure occurring upon Ca2+-binding [19,20] might provide a reasonable explanation for the differential effect of the S100A1-ct peptide. The hydrophobic S100A1 C-terminus, hitherto recognized as a crucial domain for binding and modulation of target proteins [16,17,20], is buried in the apoform and only exposed to the surface of the molecule in the Ca2+-bound form resulting in the Ca2+-activated state [19]. Based on previously published Ca2+-binding constants [19,26–28], diastolic [Ca2+] (∼150 nM) used in our experimental setting would not expect to fully saturate S100A1 protein. Hence, our results are consistent with the notion that the S100A1-ct peptide alone should exert a superior bioactivity than an equimolar concentration of the protein in the context of our experimental setting. This finding is in line with several previous reports showing an equal or even superior bioactivity of the C-terminus-like S100A1-ct peptide in modulation of various S100A1 target proteins [6,10,17]. Moreover, the smaller size of the peptide (∼10-fold) might facilitate the access to the RyR2 and may account, at least in part, for its accelerated effect.

Assessment of total SR Ca2+-content excluded that the S100A1 mediated attenuation of spontaneous RyR2 activity might be due to a decrease in SR Ca2+-load. Mechanistically, the slight increase in total SR Ca2+-content can be explained through the S100A1-mediated decrease in diastolic Ca2+-sparks activity. However, S100A1 protein and S100A1-ct peptide have recently been shown to enhance Ca2+-dependent...
SERCA2 activity [4–6, 17, 29] and this effect might also contribute, at least in part, for the slightly increased total SR Ca²⁺-content in our experimental setting although SR Ca²⁺-uptake, at least in part, for the slightly increased total SR Ca²⁺-release in cardiac and activated by Ca²⁺, S100A1 apparently serves as an endogenous enhancer of Ca²⁺-induced SR Ca²⁺-release in cardiac and skeletal muscle fibers [10]. However, once the RyR2 is activated through facilitated SR Ca²⁺-fluxes [4–7, 29, 31], our novel results support the notion of S100A1 as a Ca²⁺-dependent biphasic modulator of RyR2 activity at rest. Mechanistically, neither increased total SR Ca²⁺-content nor altered stoichiometry of accessory inhibitory modulators of the RyR2 Ca²⁺-release appears to influence the S100A1-mediated attenuation of spontaneous SR Ca²⁺-release. Therefore, on the basis of our results, it appears feasible that the inhibitory action of S100A1 protein and S100A1-ct peptide on RyR2 is the dominant cause of attenuated spontaneous RyR2 activity in our experimental setting.

Thus, regarding the established role of S100A1 as a positive inotropic regulator of heart function mainly acting through facilitated SR Ca²⁺-fluxes [4–7, 29, 31], we evaluated whether S100A1 might enhance the binding of the peptide-prolyl isomerase FKBP12.6 to the cardiac SR Ca²⁺-release channel that might account for the S100A1-mediated decrease of spontaneous RyR2 activity. However, coimmunoprecipitation carried out for FKBP12.6 and RyR2 in the presence of S100A1 protein and S100A1-ct excluded a significant effect of the EF-hand Ca²⁺-binding protein on FKBP12.6/RyR2 stoichiometry. In addition, our results also excluded enhanced binding of sorcin, another EF-hand Ca²⁺-binding protein family that exerts inhibitory actions on RyR2 activity [25], as the underlying mechanism for the inhibitory effect of S100A1 on RyR2 activity at rest. Mechanistically, neither decreased total SR Ca²⁺-content nor altered stoichiometry of accessory inhibitory modulators of the RyR2 Ca²⁺-release complex apparently underlies the S100A1-mediated attenuation in spontaneous SR Ca²⁺-release. Therefore, on the basis of this study, it appears feasible that the inhibitory action of S100A1 protein and S100A1-ct peptide on RyR2 is the dominant cause of attenuated spontaneous RyR2 activity in our experimental setting.

In summary, our study shows for the first time that S100A1 can decrease spontaneous SR Ca²⁺-release in form of decreased frequency and altered characteristics of Ca²⁺-sparks under resting conditions. Furthermore, our results strongly support the notion that this effect and binding of S100A1 to the RyR2 under these conditions is likely conveyed by the C-terminus of the native protein. Under pathophysiological conditions, diminished levels of S100A1, occurring in failing myocardium [6,32], may therefore contribute to enhanced diastolic SR Ca²⁺-leak in heart failure [23,33]. This notion is in line with recent observations showing that reconstitution and preservation of normal S100A1 protein levels in experimental heart failure models through adenoviral S100A1 gene transfer and transgenic manipulation, respectively, decreased enhanced diastolic SR Ca²⁺-leak in failing myocardium [6,34]. Hence, we propose a model of S100A1 as a Ca²⁺-dependent biphasic modulator of RyR2 activity that may contribute to its inhibition at rest, and, in some cases, may enhance RyR2 opening during CICR.

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**References**


FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death, Cell 113 (2003) 829–840.

The C-terminal (aa 75–94) and the linker region (aa 42–54) of the Ca²⁺ binding protein S100A1 are essential for target protein binding and Ca²⁺ cycling in rat cardiac myocytes, Basic. Res. Cardiol. 97 (2002), I/56–I/62.


