Calmodulin and S100A1 fine tune skeletal muscle E-C coupling. Focus on “Modulation of sarcoplasmic reticulum Ca\textsuperscript{2+} release in skeletal muscle expressing ryanodine receptor impaired in regulation by calmodulin and S100A1”

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THE FORCE OF SKELETAL MUSCLE contraction is controlled by the amount of Ca\textsuperscript{2+} release from the sarcoplasmic reticulum (SR) via the ryanodine receptor (RyR1). The RyR1 is activated during excitation-contraction coupling (ECC) by plasmalemmal voltage-dependent Ca\textsuperscript{2+} channel activation, via a mechanical voltage-induced Ca\textsuperscript{2+} release (without requiring Ca\textsuperscript{2+} influx). RyR1 is also activated by Ca\textsuperscript{2+}, and SR Ca\textsuperscript{2+} release via one RyR1 can activate neighboring RyR1 that are not directly coupled to voltage-dependent Ca\textsuperscript{2+} channels (1). The termination of SR Ca\textsuperscript{2+} release is caused by repolarization of the plasma membrane, but there is also Ca\textsuperscript{2+}-dependent inactivation that is apparent during prolonged voltage-clamp depolarizations. The RyR1 has numerous protein partners, including calmodulin (CaM) and S100A1, which can modulate the gating of the RyR and hence contraction, but how these accessory proteins really function in the cell is not well understood.

In this issue of American Journal of Physiology-Cell Physiology, Yamaguchi et al. (12) studied a novel knock-in mouse that expresses only a point mutant RyR1 (L3625D) that lacks CaM and S100A1 binding (Ryr\textsuperscript{D/D}). Prior work had shown that these two proteins bind competitively to a site near residue 3625 on RyR1 (4, 10). It was also known that CaM binding activates RyR1 at low (submicromolar) Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) but inhibits RyR1 opening at higher [Ca\textsuperscript{2+}]. Notably, this differs in cardiac myocytes where CaM inhibits RyR2 opening at all [Ca\textsuperscript{2+}], S100A1 activates RyR1 at low [Ca\textsuperscript{2+}] with little effect at high [Ca\textsuperscript{2+}] (9). Moreover, S100A1-knockout mice (S100A1\textsuperscript{−/−}) exhibit reduced SR Ca\textsuperscript{2+} release and force during the skeletal muscle twitch as well as during tetanic contractions (4, 5). This has led to the conclusion that S100A1 is a physiologically important activator/sensitizer of RyR1 release in skeletal muscle.

Yamaguchi et al. (12) now show that RyR1 channels isolated from the Ryr\textsuperscript{D/D} mouse show impaired regulation by both CaM and S100A1. Key results are that RyR1\textsuperscript{D/D} muscle exhibits reduced twitch Ca\textsuperscript{2+} transients, SR Ca\textsuperscript{2+} release, and twitch force, and these effects are quantitatively similar to those seen in S100A1\textsuperscript{−/−} mice. This suggests that, in wild-type (WT) mice, it is primarily the activating effect of S100A1 (and not of CaM) that sensitizes the resting RyR1 to activation by either voltage- or Ca\textsuperscript{2+}-dependent activation. They also show that, upon repetitive high-frequency stimulation (tetanus), the progressive buildup of intracellular [Ca\textsuperscript{2+}] ([Ca\textsuperscript{2+}]i) and force compared with the twitch is dramatically enhanced in Ryr\textsuperscript{D/D} versus WT mice. This is opposite to the S100A1\textsuperscript{−/−} mouse where ratio of tetanus to twitch activation was reduced compared with WT mice. This suggests that abolishing CaM effects on RyR1 allows a stronger tetanic enhancement of [Ca\textsuperscript{2+}]i and force (i.e., the inhibitory effect of Ca\textsuperscript{2+}-CaM on SR Ca\textsuperscript{2+} release may be importantly removed).

This work leads to the following working model concerning how S100A1 and CaM modulate RyR1 activity in skeletal muscle (Fig. 1). S100A1 binding to the amino side of the CaM-binding domain of RyR1 activates or sensitizes the RyR1 to activating signals (both elevated [Ca\textsuperscript{2+}] and depolarization; Fig. 1B). CaM at low resting [Ca\textsuperscript{2+}], (where it may have no

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Fig. 1. Calmodulin (CaM) and S100A1 effects on skeletal muscle ryanodine receptor (RyR1). A: schematic drawing of approximate position of CaM binding in the three-dimensional reconstruction RyR1 showing both Ca-CaM and Apo-CaM loci (based on Ref. 8, but see Ref. 2). B-G: CaM-binding domain of RyR1 [residues 3614–3643; (6, 11)] showing functional interactions with S100A1 and CaM under different situations with respect to conclusions at right. WT, wild type; KO, knockout.
bound Ca\(^{2+}\); Apo-CaM) exerts an activating effect on RyR1 that is weaker than that of S100A1 (Fig. 1C) and when both are present, the S100A1 effect dominates (Fig. 1D). When [Ca\(^{2+}\)] is elevated, repeated action potentials or depolarizations, S100A1 might limit the inhibitory effect of Ca\(^{2+}\)-CaM on RyR1 (Fig. 1E). If the [Ca\(^{2+}\)] elevation is prolonged, Ca\(^{2+}\)-CaM may overcome or displace S100A1 and produce a more tonic inhibition of the RyR1 (Fig. 1F). Indeed, this may be the situation in the S100A1–/– mice, where tetanic force and [Ca\(^{2+}\)] are suppressed (4, 5). In the RyR\(^{DD}\) mouse where both the activating effects of S100A1 (and CaM) and the inhibitory effects of Ca\(^{2+}\)-CaM are abolished (Fig. 1G), the twitch Ca\(^{2+}\) transient and force are depressed (loss of S100A1 activation) and tetanic summation is enhanced (loss of Ca\(^{2+}\)-CaM-dependent inactivation). Detailed testing and molecular analysis of some aspects of this framework are still needed, but this provides a simple working hypothesis for such testing.

Inactivation of RyR1 during sustained depolarization is slowed in the RyR\(^{DD}\) mouse, but the difference from WT is rather modest (see their Fig. 10B). While this is consistent with CaM contributing to RyR1 inactivation, it indicates that it may not be the dominant pathway for RyR1 inactivation. On the other hand, it is possible that this Ca\(^{2+}\)-CaM dependent inactivation is a more absorbing kind of inactivation that is long-lasting between activations. That may explain the more pronounced increase in [Ca\(^{2+}\)], and force during tetanic stimulation (treppe) in the RyR\(^{DD}\) versus WT mice. That is, Ca\(^{2+}\)-CaM may limit the normal tetanic SR Ca\(^{2+}\) release in WT, and that effect is relieved in the RyR\(^{DD}\) mouse. Notably, the S100A1-knockout mouse (S100A1–/–) has an even more profound limitation in the tetanic treppe than WT mice (5). This may mean that S100A1 in WT mice limits the ability of Ca\(^{2+}\)-CaM to inactivate RyR1. That would be consistent with a role of S100A1 to both activate RyR1 and prevent the Ca\(^{2+}\)-CaM inhibitory effect (Fig. 1, B, D, and E), and both effects could be related to the competition between S100A1 and CaM for binding to the RyR1 (10). This suggests an additional experimental test. If in the WT mouse substantial RyR1 inhibition develops during tetany via Ca\(^{2+}\)-CaM displacement of S100A1 (Fig. 1F), then a posttetanic twitch may be more depressed than one after a longer pause where S100A1 might reinsinuate itself (Fig. 1D). This effect would not be expected in the RyR\(^{DD}\) mice and might be reduced in S100A1–/– mice.

The cardiac muscle RyR2 also binds both CaM and S100A1, but the effects are a bit different. Both CaM and S100A1 appear to exhibit mainly inhibitory effects on RyR2 at diastolic [Ca\(^{2+}\)], and are thought to limit diastolic SR Ca\(^{2+}\) release which can otherwise be arrhythmogenic and also can reduce SR Ca\(^{2+}\) content available for release (e.g., Refs. 3 and 7). Both CaM and S100A1 inhibit resting Ca\(^{2+}\) sparks (diastolic leak) in cardiac myocytes, but S100A1 also enhances the fractional SR Ca\(^{2+}\) release during ECC. So there may be some degree of similarity regarding the CaM/S100A1 effects on RyR2 versus RyR1. S100A1 also interacts with the SERCA2a-phospholamban complex (possibly relieving phospholamban inhibition), the myofilament protein titin (it may reduce myofilament stiffness), and mitochondria (it may also enhance energy production). Moreover, S100A1 expression declines in heart failure and adenoviral expression of S100A1 in failing cardiac myocytes can exhibit beneficial effects (7).

This study has helped to clarify the interaction of CaM and S100A1 signaling at the RyR1, but there is key fundamental information still missing in terms of understanding the dynamics of this interplay in intact skeletal (and cardiac) muscle. The absolute concentrations of both CaM and S100A1 at transverse tubule-SR junctions in skeletal muscle are not known, nor are the fractional occupancies of RyR1 sites by these two modulatory ligands under physiological conditions. There may also be conditions where this occupancy and competition changes (e.g., during exercise, oxidative stress, fatigue, disease). Regardless, the study here shows how the interplay of CaM and S100A1 effects can fine tune skeletal muscle ECC.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**


