Leaky ryanodine receptors delay the activation of store overload-induced Ca\textsuperscript{2+} release, a mechanism underlying malignant hyperthermia-like events in dystrophic muscle

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Cully TR, Launikonis BS. Leaky ryanodine receptors delay the activation of store overload-induced Ca\textsuperscript{2+} release, a mechanism underlying malignant hyperthermia-like events in dystrophic muscle. Am J Physiol Cell Physiol 310: C673–C680, 2016. First published January 28, 2016; doi:10.1152/ajpcell.00366.2015.—The mouse model of Duchenne muscular dystrophy, the mdx mouse, displays changes in Ca\textsuperscript{2+} homeostasis that may lead to the pathology of the muscle. Here we examine the activation of store overload-induced Ca\textsuperscript{2+} release (SOICR) in mdx muscle. The activation of SOICR is associated with the depolymerization of the sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} buffer calsequestrin and the reduction of SR Ca\textsuperscript{2+} buffering power (B\textsubscript{Sr}). The role of SOICR in healthy and dystrophic muscle is unclear. Using skinned fibers we show that lowering the Mg\textsuperscript{2+} concentration can activate discrete Ca\textsuperscript{2+} release events that did not necessarily lead to activation of SOICR. However, SOICR waves could propagate into these fiber segments. The average delay to activation of SOICR in mdx fibers was longer than in wild-type (WT) fibers. In the lowered Ca\textsuperscript{2+}-buffered environment following large SOICR events, brief waves in mdx fibers displayed a low amplitude and propagation rate, in contrast to WT fibers that showed a range of amplitudes correlated with wave propagation rate. The distinct properties of SOICR in mdx fibers were consistent with a ryanodine receptor (RyR) that was leakier to Ca\textsuperscript{2+} than in WT. The consequence of delayed SOICR and leaky RyRs is prolonged high B\textsubscript{Sr} and a reduction in free Ca\textsuperscript{2+} concentration inside the SR as total SR calcium drops. We present a hypothesis that SOICR activation is required in healthy muscle and that this mechanism works suboptimally in mdx fibers to fail to limit the activation of store-operated Ca\textsuperscript{2+} entry.

Calcium; sarcoplasmic reticulum; mdx; dystrophic; malignant hyperthermia; skeletal muscle; store-operated Ca\textsuperscript{2+} entry; RyR; store overload-induced Ca\textsuperscript{2+} release; skinned fiber

Importantly, the activation of propagating SOICR is associated with chronic or acute modifications of the RyR (6, 8, 17). The proteins that have been implicated in the changes in Ca\textsuperscript{2+} homeostasis in mdx muscle are stretch-activated channels (2) on the plasma membrane; the ryanodine receptor (RyR) on the SR membrane (3); and the store-operated Ca\textsuperscript{2+} entry (SOCE) mechanism that is dependent on depletions in SR Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{SR}) among other pathways (5). We note that SOCE is dependent on [Ca\textsuperscript{2+}]\textsubscript{SR} and has been demonstrated to rapidly activate and deactivate in response to changes in [Ca\textsuperscript{2+}]\textsubscript{SR} in mdx muscle and so should not be considered dysfunctional (11). It is the activity of the RyRs and the SR Ca\textsuperscript{2+} pump or related proteins within the SR that may change with deleterious results (3, 13) and are probably responsible for inducing SOCE to be active for extended periods, which may then contribute to Ca\textsuperscript{2+}-dependent damage inside the fiber.

We have recently shown that RyRs made leaky to Ca\textsuperscript{2+} by lowering the cytoplasmic Mg\textsuperscript{2+} concentration ([Mg\textsuperscript{2+}]\textsubscript{cyto}) result in a large release of Ca\textsuperscript{2+} from the SR that can propagate along mammalian skeletal muscle fibers. This type of Ca\textsuperscript{2+} release was deduced to be store overload-induced Ca\textsuperscript{2+} release (SOICR) and suggested to be an important component of the dysregulation of Ca\textsuperscript{2+} in an MH episode (8). SOICR is Ca\textsuperscript{2+} release from the SR induced by activation of the RyRs by high levels of luminal Ca\textsuperscript{2+}. Shifts in the threshold for SOICR occur through chronic or acute modifications of the RyR (6, 8, 17). Importantly, the activation of propagating SOICR is associated with a depolymerization of calsequestrin (CSQ), the SR Ca\textsuperscript{2+} buffer, and a subsequent reduction in the power of the SR to
buffer Ca$^{2+}$ (B$_{SR}$) (8, 25, 27, 31). Little is known of the activation of SOICR and associated changes in B$_{SR}$ in skeletal muscle under physiological conditions. However, one should assume the ability to regulate the [Ca$^{2+}$]$_{SR}$ by lowering B$_{SR}$ as total calcium in the SR varies would be an important aspect of maintaining Ca$^{2+}$ homeostasis in the active muscle.

To examine the role of SOICR in mdx muscle fibers we imaged [Ca$^{2+}$]$_{cyto}$ ($t$) in mechanically skinned fibers from wild-type (WT) and mdx mouse muscle with the Ca$^{2+}$-sensitive dye rhod-2 on a confocal microscope while changing [Mg$^{2+}$]$_{cyto}$ (12). In low Mg$^{2+}$, we observed the mdx fiber to average longer periods of delay to activation of SOICR compared with WT and we also report novel aspects of Ca$^{2+}$ release in mdx muscle under these conditions. From the differences observed in Ca$^{2+}$ release in WT and mdx fibers we present a novel hypothesis where prompt SOICR activation is required to reduce B$_{SR}$ for the maintenance of [Ca$^{2+}$]$_{SR}$ and thus the appropriate restriction of SOCE activity.

**METHODS**

All experimental methods using rodents were approved by the Animal Ethics Committee at The University of Queensland. Eight male C57/BL6j (University of Queensland Biological Resources, Brisbane, Australia), four C57/BL10j, and nine mdx mice (Animal Resources Centre, Perth, Australia) between the ages 2 and 4 mo were used in this study. Mice were euthanized by cervical dislocation and the extensor digitorum longus (EDL) muscles rapidly excised. EDLs were placed in a Petri dish under paraffin oil above a layer of Sylgard.

Segments of individual fibers were then isolated and mechanically skinned to completely remove the surface membrane and seal the t-system. Skinned fibers were transferred to a custom-built experimental chamber with a coverslip bottom, where they were bathed in an "internal solution" containing 10 µM of dissolved rhod-2 salt (Molecular Probes).

**Compositions.** The standard internal solution used to bath the skinned fibers contained the following (in mM): 48.5 HDTA$^{2-}$/H$_{11001}$ (Fluka, Buchs, Switzerland), 8 total ATP, 10 creatine phosphate, 36 Na$^{+}$, 126 K$^{+}$, 6.5 total Mg$^{2+}$, 1 total EGTA, 90 HEPES, 0.0001 Ca$^{2+}$, and pH 7.1. This solution had an osmolality of 295 ± 5 mosmol/kg H$_2$O, a calculated free [Mg$^{2+}$] of 1 mM, and high [K$^+$] to keep the sealed t-system polarized. Internal solutions with [Ca$^{2+}$] raised to 800, 200, and 100 nM were used to load the SR and t-system with Ca$^{2+}$ and to prequilibrate the preparations before Ca$^{2+}$ release, respectively. "Low Mg$^{2+}$ solution" was similar to the standard internal solution but free [Mg$^{2+}$] was reduced to 10 µM and was nominally free of Ca$^{2+}$. This solution was designed to induce cell-wide SR Ca$^{2+}$ release (21) which was followed by release inactivation and resequstration of SR Ca$^{2+}$ (25). n-Benzyl-p-toluene sulphonamide (BTS; 50 µM) was added to all solutions to inhibit contraction. All chemicals were from Sigma (Sydney, Australia) unless otherwise stated.

**Confocal imaging.** The experimental chamber containing a skinned fiber was placed above a water immersion objective (×40, NA 0.9) of the confocal laser scanning system (FV1000; Olympus, Tokyo, Japan). Acquisition of rhod-2 fluorescence was achieved using excitation and emission ranges of 543 and 562–666 nm, respectively. Imaging was performed at 21–24°C.

**Confocal image analysis.** All imaging in this study was performed in xy mode, in most cases at 0.2056-µm pixel distance and 2.162 ms line interval. The xy scanning lent itself to a method of image analysis illustrated in Fig. 4 and is outlined in detail in Refs. 8 and 23.

**RESULTS**

**Increase in local RyR Ca$^{2+}$ leak may not trigger SOICR.** Figure 1 shows an mdx skeletal muscle fiber exposed to a low Mg$^{2+}$ solution to induce Ca$^{2+}$ release from the SR. A significant and prolonged leak of Ca$^{2+}$, indicated by a slow increase and subsequent decrease in rhod-2 fluorescence, occurred over a period of 30 s but did not result in a cell-wide transient. Riding this increased leak of Ca$^{2+}$ were discrete release events (Fig. 1). The events increased in frequency and then fell silent (across images marked 13 to 44 s; Fig. 1). These events are best observed in Supplemental Movie S1 (Supplemental Material for this article is available online at the Journal website). However, after the image marked 44 s a propagating SOICR wave entered the field of view and moved from left-to-right, passing the imaging field in 6–7 s (images marked 45 to 51 s; Fig. 1, "b"). It is important to note that the whole skinned fiber segment was exposed to the same ionic conditions but Ca$^{2+}$ release (SOICR) must have been initiated elsewhere in the preparation, outside the field of view, to generate the propagating wave observed (8). This result shows that although the discrete events subsided after failing to activate SOICR, a SOICR wave triggered elsewhere in the fiber could still propagate through this region.

**Discrete Ca$^{2+}$ release events reach a critical mass to trigger SOICR in dystrophic muscle.** Discrete Ca$^{2+}$ release events leading to SOICR initiation were captured and are displayed in Fig. 2. The images in Fig. 2 and Supplemental Movie S2 show progressive xy images of rhod-2 fluorescence in the cytoplasm of a skinned fiber from mdx mouse muscle after the internal bathing solution had been changed from the standard resting one containing 1 mM EGTA, 100 nM Ca$^{2+}$, and 1 mM Mg$^{2+}$ to one containing 1 mM EGTA, no added Ca$^{2+}$, and 0.01 mM Mg$^{2+}$. Discrete and brief events of Ca$^{2+}$ release through RyRs in low Mg$^{2+}$ can be observed in the images marked 11 to 12 s on a progressively rising background of fluorescence. Some discrete events are observable on the image marked 13 s to the left of the area of increasing Ca$^{2+}$ leak that has become uniform in the central region of the fiber. Images marked 14 and 15 s show SOICR activation (8) with a rate of release sufficient to increase Ca$^{2+}$ levels in the immediately surrounding internal solution bathing the preparation (the "Ca$^{2+}$ halo"; Ref. 8). There is then propagation of the release. The propagation of the SOICR wave in both directions is evident by the concave halo in the fifth image becoming more intense, broader, and concave only at the leftmost area of the image, which is recorded earlier in time than the right (due to the direction of the scanning laser). The green line on the images represents the average rhod-2 fluorescence profile within the borders of the preparation and is temporally aligned with the image. The graph in Fig. 2, bottom, shows the spatially averaged profile of the change in rhod-2 fluorescence intensity through the course of the low Mg$^{2+}$-induced SOICR event.

**Propagating SOICR is delayed in dystrophic muscle.** The observation of discrete events in response to low Mg$^{2+}$ was observed in mdx fibers but not WT (Figs. 1 and 2). However, delays to SOICR activation were observed in both WT and mdx fibers. Frequency distribution data (Fig. 3) shows that 78% of WT fibers imaged responded with a SOICR immediately following exposure to a low Mg$^{2+}$ solution compared with 50% of mdx fibers. Mdx fibers were more likely to have a period of "leak time" or delay to SOICR once exposed to low Mg$^{2+}$ solution. Dystrophic fiber SOICR wave amplitudes are restricted. The lowering of Mg$^{2+}$ in skinned fibers that causes a cell-wide release of Ca$^{2+}$ can also result in further waves of Ca$^{2+}$ release (8, 11, 12). These Ca$^{2+}$ waves are relatively brief as CSQ is in
a reduced state and therefore BSR is low (8). An example of brief SOICR waves in an mdx fiber is shown in Fig. 4. The propagation rate (as described in Ref. 8 and Fig. 4), full duration at half maximum (FDHM), and amplitude of brief SOICR waves were determined. The relationships between these properties are presented on the three-dimensional mesh plot with different color spectrums applied for WT and mdx data (Fig. 4).

The three-dimensional mesh plot shows that the amplitude and FDHM affect the propagation rate of the SOICR brief waves. The grayscale spectrum represents the values for mdx fibers. In mdx fibers the wave release properties for dystrophic fibers were limited to lower amplitudes and longer FDHMs. The low amplitude and longer FDHM in the mdx brief waves are consistent with what has previously been reported in cell-wide Ca\textsuperscript{2+}/H\textsubscript{11001} releases in low [Mg\textsuperscript{2+}]/H\textsubscript{11001] cytol (7, 11).

**DISCUSSION**

Here we have observed discrete and also large, propagating Ca\textsuperscript{2+} release in WT and mdx fibers in the presence of low [Mg\textsuperscript{2+}]\textsubscript{cytol} (Figs. 1–4). This large, propagating release of Ca\textsuperscript{2+} under these conditions has previously been attributed to SOICR (8). The discrete Ca\textsuperscript{2+} release events ob-

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Fig. 1. Lowering Mg\textsuperscript{2+} and inducing Ca\textsuperscript{2+} leak through ryanodine receptors (RyRs) does not necessarily activate store overload-induced Ca\textsuperscript{2+} release (SOICR). Cytoplasmic rhod-2 fluorescence emitted from the bathing solution surrounding a skinned fiber from an mdx mouse muscle was continuous imaged on a confocal microscope during exchange of a standard internal solution containing 1 mM Mg\textsuperscript{2+} for a solution containing 0.01 mM Mg\textsuperscript{2+}. The series of images displayed are in the presence of 0.01 mM Mg\textsuperscript{2+}. Images marked 13 to 44 s show an increased Ca\textsuperscript{2+} leak from the preparation that peaks around image marked 23 s and then declines. A propagating SOICR wave enters the field of view at the imaged marked 45 s and passes through in the remaining frames shown. Note that the first image immediately following solution exchange was used as a background and subtracted from all images (F-F\textsubscript{0}) in order for the Ca\textsuperscript{2+} leak events to be more easily observable. Note that this is not a measure of the amplitude (F/F\textsubscript{0}) of the Ca\textsuperscript{2+} release events. **Bottom left:** graph shows the spatially averaged profile of rhod-2 fluorescence collected from within the borders of the preparation. Note the low amplitude, long duration Ca\textsuperscript{2+} leak immediately following the introduction of low Mg\textsuperscript{2+} (“a”). This leak subsides before the abrupt SOICR wave passes through the field of view (“b”).
In contrast, the brief SOICR waves in WT fibers had a consistent amplitude for a range of FDHM in mdx compared with WT fibers (Fig. 3). Furthermore, the propagation of SOICR events was extended more frequently in mdx fibers compared with WT fibers (Fig. 4). These differences in Ca²⁺-handling properties of the SR in WT and mdx may underlie pathological consequences for the mdx fiber and perhaps in other muscle types and conditions where RyR Ca²⁺ leak may be increased.

The discrete events observed in mdx fibers in lowered [Mg²⁺]ₗₑₘₜ are SOICR events. In mammalian skeletal muscle the discrete Ca²⁺ release events, or Ca²⁺ sparks, and Ca²⁺ waves never display properties consistent with propagation via a cytoplasmic mechanism, such as Ca²⁺-induced Ca²⁺ release (CICR) (8, 38, 43). CICR is a hallmark of Ca²⁺ sparks in cardiac cells and in amphibian skeletal muscle (38). The Ca²⁺ sparks or discrete Ca²⁺ release events in mammalian skeletal muscle can occur under conditions where the RyR inhibition has been potentially lowered by treatment with saponin (18, 24), in the presence of certain Ca²⁺ buffers that may affect Ca²⁺-dependent inactivation of the RyR (42) or via osmotically induced local activation of dihydropyridine receptors (32).

In the majority of cases we observed a homogeneous rise in the cytoplasmic Ca²⁺-dependent fluorescence signal due to RyR Ca²⁺ leak from WT and mdx fibers in the presence of low [Mg²⁺]ₗₑₘₜ, when there was a delay to activation of propagating SOICR (8). Figures 1 and 2 and associated supplemental online movies permitted the observation of discrete events that could also occur under these conditions. The spatiotemporal properties of the events could not be quantified because of the low resolution of the imaging and the density of events and thus made them difficult to isolate in spark detection software (e.g., Ref. 39). However, inspection of the images presented here shows events (best observed in the supplemental online movies) that have a width of more than 1 µm and can last several hundred milliseconds to a second or more, as they span more than one frame in our xyt imaging (see METHODS). Regardless RyRs clusters acting in concert must drive these events (43). As Ca²⁺ sparks in mammalian skeletal muscle occur in the presence of a reduced inhibition of the RyR cytoplasmic sites, and thus also a lowered threshold for SOICR (5, 15, 16), we suggest the discrete Ca²⁺ release events observed in 0.01 mM [Mg²⁺]ₗₑₘₜ did not necessarily lead to the activation of propagating SOICR (Fig. 1; Ref. 8). However, propagating SOICR events could still move through segments of a fiber where the low Mg²⁺ discrete events had already risen and then subsided without triggering a large, local release of Ca²⁺ (Figs. 1 and 2). The delay to activation of propagating SOICR events was extended more frequently in mdx compared with WT fibers (Fig. 3). Furthermore, the brief SOICR waves succeeding the larger propagating waves had a consistent amplitude for a range of FDHM in mdx fibers, which seemed to clamp wave propagation rate. In contrast, the brief SOICR waves in WT fibers had amplitudes that correlated with FDHM and propagation rate.
observed here, and in other studies of mammalian skeletal muscle (18, 32, 42, 43), are discrete SOICR events. That is, they activate where the local $[\text{Ca}^{2+}]_{\text{SR}}$ is high and there is a stochastic activation of a number of RyRs in a cluster that breach the threshold for event occurrence.

**Delay of propagating or cell-wide SOICR activation in mdx fibers.** We have described propagating or cell-wide SOICR (8). This event causes CSQ to depolymerize as its aggregation state is dependent on the level of calcium inside the SR. The shorter chains of CSQ remaining following SOICR have a different affinity for $\text{Ca}^{2+}$ than that of the aggregated state (31). In this study we now present evidence that the activation of such SOICR events can be delayed in mdx fibers (Fig. 3) and thus the consequence is that BSR remains high for arguably longer than it should. The delay to SOICR activation likely occurs because not enough $\text{Ca}^{2+}$ binds simultaneously to the SOICR activation sites in the pore of enough RyRs in a cluster to initiate a large release of $\text{Ca}^{2+}$ (6). We propose that it is the
leakiness of the mdx fiber RyRs that denies the array of SOICR sites of the clustered RyRs sufficient Ca$^{2+}$ for activation. This property of mdx fibers is supported by the observation of depressed amplitudes for both the cell-wide SOICR events (11) and the brief SOICR waves (Fig. 4). SOICR is a SR load-dependent process (19) and the lower amplitude of release compared with WT is consistent with a lower load of Ca$^{2+}$ in the SR or at least a lower local [Ca$^{2+}$] near the SOICR activation site in the pore of the RyR (6).

SOICR appears to be a mechanism that is needed to avoid [Ca$^{2+}$]$_{SR}$ overload, at least in cardiomyocytes (6, 16, 17, 33). In skeletal muscle SOICR activation causing CSQ depolymerization and lowering BSR (8, 25, 31) has different implications for muscle fiber function and this is addressed by the model we present below.

SOICR lowers BSR to maintain [Ca$^{2+}$]$_{SR}$ avoiding excessively active SOCE. SOICR is activated when the [Ca$^{2+}$]$_{SR}$ increases to levels that are too high, presumably to maintain the appropriate level of [Ca$^{2+}$]$_{SR}$, which probably avoid or reduce spontaneous, large releases of Ca$^{2+}$. Under conditions were the cytoplasmic inhibition on the RyR is reduced and the channel becomes leakier than normal, the threshold for SOICR drops (6, 16, 17, 33). If the drop in the threshold for SOICR falls below the endogenous level of [Ca$^{2+}$]$_{SR}$ (8, 19, 20) Ca$^{2+}$ release results. How SOICR is acting physiologically in skeletal muscle is not understood. Changes in BSR under long, depolarizing pulses produce a secondary peak of Ca$^{2+}$ release after the main, initial one (27), consistent with a SOICR mechanism. We can extrapolate that SOICR might be a part of maintaining [Ca$^{2+}$]$_{SR}$ during prolonged tetani. In skeletal muscle SOICR is accompanied by a change in BSR through depolymerization of CSQ (8). The consequence of the lowering of BSR is that the [Ca$^{2+}$]$_{SR}$ can be maintained high in the presence of a leaky RyR and lowered total calcium inside the SR (8, 25). The maintenance of normal levels of [Ca$^{2+}$]$_{SR}$ is critical in controlling the density of Ca$^{2+}$ entering the muscle via SOCE (9, 23). Thus SOICR is critical to controlling SOCE (22).

Figure 5 presents a schematic representation of this idea, which is consistent with our observations in healthy and mdx muscle fibers (Figs. 1–4). Our model combines conventions to describe these phenomena as introduced by MacLennan and Chen (26) and Royer and Rios (35). An important difference between healthy and mdx muscle fibers in regards to Ca$^{2+}$ handling is the leakiness of the RyR to Ca$^{2+}$ (3). Thus such leaky RyRs in mdx could reasonably be expected to lead to a depression in resting [Ca$^{2+}$]$_{SR}$ compared with the healthy fibers, at least at the luminal face and pore of the RyRs where the SOICR activation site is located (6). Alternatively, a difference in the properties in the SR Ca$^{2+}$ pump or in those of CSQ would also affect [Ca$^{2+}$]$_{SR}$ (1, 13). The result for SOICR activation would still be a delay. Regardless, the difference in [Ca$^{2+}$]$_{SR}$ is the starting assumption of the model in Fig. 5. The two important thresholds inside the SR that will affect Ca$^{2+}$ handling by the muscle are the SOICR threshold (dashed line) and the SOCE threshold (dotted line). In both situations, we have set these thresholds to the same levels. However, the lower [Ca$^{2+}$]$_{SR}$ (solid line) in mdx (Fig. 5B, left) will place this value further from the SOICR threshold and closer to the SOCE threshold, respectively, than resting [Ca$^{2+}$]$_{SR}$ is in the healthy fiber (Fig. 5A, left).

The consequence of the longer time required to activate SOICR in mdx fibers is that the BSR will remain high in the presence of significantly increased RyR Ca$^{2+}$ leak for an extended time (Fig. 5B, right) compared with the healthy fibers (Fig. 5A, middle). Only upon SOICR activation is BSR reduced in conjunction with the depolymerization of CSQ (Fig. 5A, right) (8).

As shown in the model (Fig. 5), the activation of SOICR and reduction of BSR will relieve the SR of significant amounts of the total calcium (vertical bars in model). Note that most Ca$^{2+}$ in the SR is bound to calsequestrin (CSQ), CaCSQ, and SR Ca$^{2+}$ concentration ([Ca$^{2+}$]$_{SR}$) (solid line) and SOICR (dotted line) and store-operated Ca$^{2+}$ entry (SOCE) (dashed line) thresholds in WT and mdx fibers following lowering Mg$^{2+}$. See text for details.
In the situation where the reduction in B$_{SR}$ does not occur at all or there are significant delays to SOICR, as in the mdx fiber (Fig. 4), the $[\text{Ca}^{2+}]_{\text{SR}}$ and CaCSQ are decreased by the leaky RyRs in the presence of sustained high B$_{SR}$ (Fig. 5B, right). This is expected from the relationship: $[\text{Ca}^{2+}]_{\text{SR}} = [\text{calcium}]_{\text{SR,total}}/B_{SR}$ (8, 27). We suggest that the lowering of $[\text{Ca}^{2+}]_{\text{SR}}$ will breach the threshold for SOCE and allow this mechanism to be activated for the duration that B$_{SR}$ is not lowered (Fig. 5B, right). Note that SOCE flux will be submaximal under these conditions but, indeed, continuously active (23, 41). We suggest the activation of SOCE for prolonged periods where the RyRs may be made leaky (e.g., following eccentric exercise in mdx fibers; Ref. 3) is likely to contribute to pathology. Note that SOCE cannot be considered dysfunctional in mdx muscle as STIM1 is simply responding appropriately to the level of $[\text{Ca}^{2+}]_{\text{SR}}$ (11).

Implications of leaky RyRs and maintenance of un-physiologically “high” B$_{SR}$ in skeletal muscle. Under any muscle condition where RyRs become too leaky, SOICR may not activate as required. In DMD there are reports of boys who have followed general anesthetics with “MH-like” events (15, 18). This work was supported by the National Health and Medical Research Council (Australia) (GNT1025555), Muscular Dystrophy Queensland (Australia), and Duchenne Foundation (Australia). B. S. Launikonis was a Future Fellow of the Australian Research Council (FT140103109).

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: T.R.C. and B.S.L. conception and design of research; T.R.C. performed experiments; T.R.C. and B.S.L. analyzed data; T.R.C. and B.S.L. interpreted results of experiments; T.R.C. and B.S.L. prepared figures; T.R.C. and B.S.L. drafted manuscript; T.R.C. and B.S.L. edited and revised manuscript; T.R.C. and B.S.L. approved final version of manuscript.

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