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New Insights on Sarcoplasmic Reticulum Calcium Regulation in Muscle Fatigue

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A consistent observation with fatigue in skeletal muscle is a decline in the amplitude of the myoplasmic Ca^{2+} transient, which is thought to result primarily from a reduced Ca^{2+} flux through the ryanodine receptor (RyR1) of the sarcoplasmic reticulum (SR) ([Fig. 1](#)). This in turn is thought to contribute to the loss in muscle force and power ([2](#)). In the past 20 years, the important proteins at the t-tubule SR junction have been identified ([Fig. 1](#)), and considerable progress has been made in understanding the molecular mechanism by which t-tubular charge induces SR Ca^{2+} release. However, the cellular nature of the disturbance(s) in excitation-contraction coupling (ECC) responsible for the reduced Ca^{2+} release with fatigue have yet to be elucidated ([2](#)). Possibilities include t-tubular dihydropyridine receptor (DHPR) inactivation, a disturbance in the linking process between the DHPR and the RyR1, factors that reduce the open probability or conductance of the RyR1, and/or a decline in SR lumen Ca^{2+} that reduces the chemical driving force (ΔC) for Ca^{2+} release. It seems likely that more than one factor is involved. For example, high-intensity contractile activity increases extracellular K^+ depolarizing the t-tubular membrane, which can at values less negative than -60 mV inhibit the DHPR. Concurrently, a drop in cell ATP and increase in Mg^{2+} directly inhibits the RyR1.

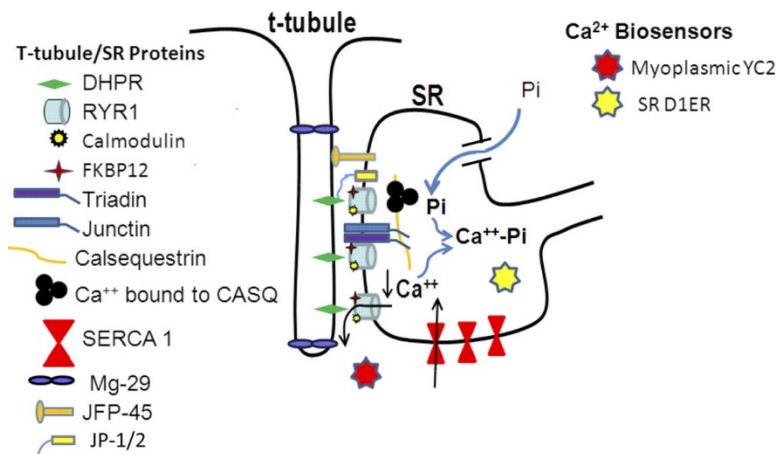


Fig. 1. Schematic of t-tubular/sarcoplasmic reticulum (SR) junction showing Ca^{2+} biosensors and path for inorganic phosphate (P_i) movement into SR. A schematic showing the t-tubular SR interface with the proteins involved in excitation-contraction coupling (ECC). In the study of Allen et al. (1) the myoplasmic and SR lumen Ca^{2+} content was determined with the biosensors yellow cameleon 2 (YC2) and D1ER, respectively. The authors hypothesize that fatigue with contractile activity was in part caused by increased P_i that moved from the myoplasm via anion channels in the SR membrane into the SR lumen where a $\text{Ca}^{2+}\text{-P}_i$ precipitate was formed. This decreased the SR lumen Ca^{2+} and likely contributed to the observed decrease in the amplitude of the myoplasmic Ca^{2+} transient. Alterations in other proteins associated with the RYR1 [calmodulin and FK506-binding protein 12 kDa (FKBP12)] or the t-tubular/SR junction [mitsugumin-29 (Mg-29), junctophilin 1,2 (JP-1/2), and junctional face protein (JFP-45)] might also contribute to the decline in the SR Ca^{2+} release in fatigued fibers (3).

An important unanswered question addressed by Allen et al. (1) in a study published in this issue of the *Journal of Applied Physiology* is does free SR lumen Ca^{2+} decline with fatigue. To study this question, the authors transfected mouse tibialis anterior muscles with yellow cameleon 2 (YC2), and the cameleon biosensor D1ER to monitor myoplasm and SR Ca^{2+} , respectively (6, 8). Cameleons are calmodulin with cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) added to the ends. When Ca^{2+} increases and binds to calmodulin, the molecule folds and allows fluorescent resonance energy transfer (FRET) between CFP and YFP. While others have used this technology to measure SR lumen Ca^{2+} (6, 8), Allen et al. (1) are the first to use the D1ER to monitor SR lumen Ca^{2+} during the development of fatigue. The primary observation was that both myoplasmic and SR Ca^{2+} declined steadily during a 4-min fatigue protocol, and that the decline was correlated with an increase in intracellular inorganic phosphate (P_i) concentration. The data support the hypothesis that P_i enters the SR through anion channels in the SR membrane and precipitates with Ca^{2+} , reducing free SR lumen Ca^{2+} and thus the driving force for release (Fig. 1 and Ref. 4). Unlike the SR Ca^{2+} binding protein calsequestrin that releases Ca^{2+} as SR lumen Ca^{2+} declines, thus facilitating a maintenance of release, the Ca^{2+} remains precipitated with P_i (Fig. 1). The whole muscle data are distinctively different from previous work in single fibers by Westerblad and Allen (10) where the amplitude of the Ca^{2+} transient initially increased as force declined, after which both force and the Ca^{2+} signal declined. The general conclusion was that in single fibers fatigue was initially elicited by metabolic factors (such as direct inhibition of the cross bridge by high P_i and H^+), while alterations in ECC were important in the latter stages of fatigue. In the whole muscle study (1), the authors suggest that the higher temperature of the in situ preparation ($30.5 \pm 0.6^\circ\text{C}$) accelerated fatigue of the fatigue-sensitive fibers (which they

identify as IIb and IIa/IIb but surely some were IIx or IIb/IIx), such that the amplitude of the myoplasmic Ca^{2+} transient and force fell monotonically eliminating the phase 2 period of steady force observed in single fibers. Thus fatigue throughout the stimulation period was due to both less Ca^{2+} release by the SR and direct inhibition of the cross bridges by P_i .

This work is important not only because it is the first to demonstrate a reduced free Ca^{2+} in the SR lumen with fatigue, but also for the methodology demonstrating the ability to monitor both myoplasmic and SR lumen Ca^{2+} during fatigue produced in situ. The in situ preparation has important advantages over single-fiber studies in that muscles can be studied at temperatures the same or similar to those observed in vivo, and extracellular factors such as increased K^+ , and blood-borne factors (hormones, changes in Po_2 and Pco_2) known to effect muscle fatigability are present and can be manipulated to assess their role in eliciting deleterious changes in ECC. While the methodology presented is novel and shows great potential for elucidating important fatigue factors, the authors recognize certain limitations. In this work, the authors were unable to synchronize the Ca^{2+} imaging with the tetanic contraction, and thus the Ca^{2+} measurements were not always made at the time of peak force. This problem, plus muscle movement during the image collection, made it difficult to make continuous measurements relating the myoplasmic free Ca^{2+} to force production. Additionally, only 10–20% of fibers in the region of the transfection expressed the cameleon at levels high enough to record the Ca^{2+} signal. Nonetheless, the work represents an important first step in understanding the role of SR lumen Ca^{2+} in altering SR Ca^{2+} release in muscle fatigue.

It remains to be determined whether or not the drop in SR Ca^{2+} content in skeletal muscle with activity actually contributes to fatigue by reducing the release flux. In heart cells it is well established that this is the case as low SR Ca^{2+} working through calsequestrin and other junctional complex proteins triadin and junctin ([Fig. 1](#)) decreases RyR2 gating and thus release flux ([5](#)). Recently, Rios and colleagues ([9](#)) found evidence to support a similar mechanism in skeletal muscle. They observed SR permeability (P) to decrease significantly during long-lasting voltage-clamp depolarizations, and as a result SR Ca^{2+} never fell below 40% of control. Like heart cells, the P decrease required calsequestrin as muscle fibers from calsequestrin-null mice showed greater than 90% depletion. A question remains as to whether SR Ca^{2+} decline observed with fatigue is primarily due to high P_i precipitating Ca^{2+} or if inhibition of the SERCA pump and/or SR leak (through the RyR1 or SERCA pump) contributes to the low SR Ca^{2+} . Additionally, does any fall in SR Ca^{2+} reduce release or are there no effects above a certain threshold level? There is evidence that fibers can sustain some decline in ΔC without a change in Ca^{2+} release ([7](#)). Finally, the relative importance of the decline in SR lumen Ca^{2+} vs. other factors including alteration in proteins associated with the t-tubular/SR interface or known to regulate the RYR1 in mediating the decline in SR Ca^{2+} release in fatigued fibers is unknown ([Fig. 1](#)). Future studies need to sort out these questions and determine the relative importance of the various factors contributing to low SR lumen Ca^{2+} , and establish the threshold level of SR Ca^{2+} required to maintain robust release.

DISCLOSURES

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

AUTHOR NOTES

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