Confocal imaging of lumenal and cytosolic [Ca^{2+}] during Ca^{2+} release in skeletal muscle

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Rapid Ca^{2+} release from the sarcoplasmic reticulum (SR) is essential for normal contraction of muscle. To study Ca^{2+} release in muscle we simultaneously imaged mag-indo-1 trapped in SR with cytosolic rhod-2 in skinned skeletal muscle fibres of frog using confocal microscopy. [Ca^{2+}] depletion inside the SR measured during spontaneous Ca^{2+} sparks, termed “skraps”, did not simply follow the Ca^{2+} release time course observed in the cytosolic Ca^{2+} image, but showed a ~20 ms delay between the peak of the spark and the nadir of the skrap. A similar result was observed when Ca^{2+} release was induced by an action potential. This suggests that depletion continues even after Ca^{2+} release channels have closed. A further intriguing observation was an intra-SR Ca^{2+} transient during prolonged Ca^{2+} release induced by lowering $[Mg^{2+}]_{cyto}$. Such an increase in $[Ca^{2+}]_{SR}$ during a decrease in total SR [Ca] would violate mass conservation laws. However, these observations can be explained in the framework of the properties of calsequestrin (CSQ), a Ca^{2+}-buffering protein attached to the lumenal side of Ca^{2+} release channels (Dulhunty *et al.*, 2006): (i) CaCSQ represents a third compartment, a proximate source of Ca^{2+} for release and is invisible to the monitoring dye, thus explaining the apparent delay between cytosolic release and lumenal Ca^{2+} depletion; and (ii) CSQ depolymerizes as the total SR [Ca] falls. Thus as CSQ breaks into dimers and monomers, the capacity of CSQ for Ca^{2+} drops, resulting in a Ca^{2+} transient within the SR during prolonged release. The strategic location and reduction in dimensionality of Ca^{2+}-adsorbed linear polymers of CSQ could deliver Ca^{2+} more efficiently to the release channels than lumenal Ca^{2+}.

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