New and Notable

Cytoplasmic versus Intra-SR: the Battle of the Ca^{2+} Diffusion Coefficients in Cardiac Muscle

Godfrey Smith and
Niall MacQuaide
Biomedical and Life Sciences, Glasgow University, Glasgow, United Kingdom

Steep Ca^{2+} ion concentrations gradients within the cytosol have been recorded in many cell types. These gradients allow local (<1 μm) regulation of Ca^{2+} sensitive processes. In cardiac muscle, the ability to develop large cytosolic [Ca^{2+}] gradients is essential to the control of the force of contraction. A factor of 10× or more differences of intracellular [Ca^{2+}] can develop over a matter of ~1 μm in ~10 ms because the diffusion coefficient of Ca^{2+} is very low (~15 $\mu m^2 s^{-1}$, i.e., ~50–70× less than free solution). The poor ability of Ca^{2+} to diffuse allows the force of contraction to be modulated by varying the number of sarcoplasmic reticulum (SR) Ca^{2+} release sites active during each contraction. Despite these release sites being separated by only ~2 μm, no cross talk of Ca^{2+} between adjacent SR Ca^{2+} release units is thought to occur. In theory, the same principle should apply to the luminal side of the SR since the lumen of the SR is continuous throughout the cell. Simplistically, the Ca^{2+} diffusion coefficient within the SR must be comparable to the cytosol, otherwise the adjacent regions of the SR would be depleted by active sites, and therefore the benefit of fine digital control over Ca^{2+} released would be lost. Previously, the only estimate of luminal Ca^{2+} diffusion coefficient available was ~60 $\mu m^2 s^{-1}$ (1), a value clearly higher than estimates of cytoplasmic Ca^{2+} and therefore raising a series of interesting issues related to the relatively rapid movement of Ca^{2+} within the SR. The article in this issue by Swietach et al. (2) addresses the significant technical challenges associated with measuring intra-SR Ca^{2+} mobility with elegant experimental techniques and extensive computational analysis. They arrive at a value of 8–9 $\mu m^2 s^{-1}$ for the lumped diffusion of Ca^{2+} within the SR measured in the long-axis of the rat and guinea-pig ventricular myocytes. This value is less than current estimates of cytoplasmic diffusion coefficient and therefore supports the concept that intra-SRCa^{2+} gradients are at least as steep as cytoplasmic. This value can be used to help address a series of important quantitative questions concerning the operation of cardiac muscle under physiological and pathophysiological conditions. For example:

1. During a normal Ca^{2+} release event, how much depletion of the SR occurs? i.e., what is the minimum [Ca^{2+}] achieved on the luminal side? How much depletion from adjacent junctional SR (jSR) sites occurs?
2. How fast can the Ca^{2+} be returned from the main SR Ca^{2+} uptake sites (in the network SR; i.e., nSR) to the release sites (in the jSR)?
3. How does a Ca^{2+} wave propagate along the length of a cardiac cell?

Under certain situations, Ca^{2+} release in a discrete region of the cell has been found to propagate in a nondecrementing Ca^{2+} wave along the cell length (100–120 μm). A fire–diffuse–fire mechanism has been used to described the underlying mechanism (3) (the first term ''fire'' refers to the release of Ca^{2+} from a discrete cluster of ryanodine receptors located in the jSR; the term ''diffuse'' applies to the diffusion of cytoplasmic Ca^{2+} across the ~2 μm of the sarcomere; and the second term ''fire'' is Ca^{2+} release from the next cluster of ryanodine receptors). The triggering event is Ca^{2+}-induced Ca^{2+} release, a mechanism intrinsic to the SR Ca^{2+} release channel (RyR2). For the fire–diffuse–fire mechanism to work, the cytoplasmic Ca^{2+} should be able to diffuse between adjacent RyR2 clusters more readily than intra-SR (luminal) Ca^{2+} diffuses between adjacent jSR; otherwise, when one jSR region is depleted by a fire event, this would cause net diffusion from the lumen of the (yet-to-be activated) adjacent jSR, and the Ca^{2+} wave would ''fizzle-out''. As discussed by Swietach et al. (2), a low Ca^{2+} diffusion coefficient makes this scenario unlikely.

But there is still a lot to debate. For example:

1. Previous measurements of Ca^{2+} diffusion using a luminal Ca^{2+} indicator in rabbit cardiomyocytes arrived at a considerably higher value (1). Why the discrepancy? Swietach et al. suggest that SR Ca^{2+} leak during the period of SR depletion can explain the difference. In their study, SR Ca^{2+} leak was blocked by 0.3 mM or 2 mM tetracaine. But Wu and Bers (1) were aware of this possibility, and indicated that SR Ca^{2+} leak was not significant over the timescale of their measurements.
2. Keller et al. (4) recently published data to suggest that rapid inhibition of the SR Ca^{2+} pump slowed the propagation of the Ca^{2+} wave. Why should inhibition of a Ca^{2+} pump immediately in front of the Ca^{2+} wave slow propagation in a fire–diffuse–fire situation? The explanation suggested by Keller et al. was that Ca^{2+} wave propagation required a component of SR Ca^{2+} uptake and diffusion within the SR, i.e., a fire–SR Ca^{2+} uptake–diffusion (intra-SR)–fire mechanism. But as pointed out by Swietach et al., the low intra-SR diffusion coefficient makes this mechanism unlikely. But the experimental observation remains and requires an explanation. One resolution is simply that SR Ca^{2+} uptake is required to prime the SR Ca^{2+}

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Address reprint requests to G. L. Smith, Tel.: 00-44-14-13-30-59-63; E-mail: g.smith@bio.gla.ac.uk.

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release channel for the fire event, but the uptake occurs at the release site on the jSR (no intra-SR diffusion required). A significant amount of SERCA is thought to exist at jSR, thus the propagation process could be fire–diffuse–SR Ca^{2+} uptake–fire. As described in an abstract presented to the most recent Biophysical Society meeting by Ramay et al. (5), this latter mechanism can generate a propagated Ca^{2+} wave using very low intra-SR Ca^{2+} diffusion characteristics. Experimental evidence for this mechanism will come from high resolution measurements of intra-SR Ca^{2+}.

3. The cytoplasmic Ca^{2+} diffusion coefficient will be dependent on a number of factors including the range of $[Ca^{2+}]$ involved and physiological status of the cell. This was nicely illustrated by another recent Biophysical Society abstract, stating that factors that will contribute to the Ca^{2+} diffusion coefficient, i.e., the Ca^{2+} buffering associated with the SR Ca^{2+} pump and the myofilaments, will change during β-adrenergic stimulation (6). As discussed above, whether or not local Ca^{2+} release propagates throughout the cell may depend on the relative values of cytoplasmic and luminal Ca^{2+} diffusion coefficients. In theory, Ca^{2+} waves may be suppressed by increasing the intra-SR diffusion coefficient. Under these circumstances, the jSR region depleted by a fire event would deplete the lumen of the jSR ahead of the Ca^{2+} wavefront.
4. The intra-SR Ca^{2+} diffusion studied by Swietach et al. was along the longitudinal axis of the cardiac cell. The intra-SR Ca^{2+} diffusion coefficient may not be the same in the other two planes of the cell.

In summary, the article by Swietach et al. in this issue provides new data to inform computational models and to encourage discussion about the topic of intra-SR Ca^{2+} diffusion that hither-to had received scant attention. The implications of this data for quantitative models of Ca^{2+} fluxes in cardiac muscle are significant.

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