Store activation mechanism for cardiac ryanodine receptors

D.R. Laver, School of Biomedical Sciences, University of Newcastle, Callaghan, NSW 2308, Australia, and Hunter Medical Research Institute, Newcastle, NSW, Australia.

The Ca^{2+} load in the sarcoplasmic reticulum (SR) is an important stimulator of Ca^{2+} release which is mediated by the ryanodine receptors (RyRs) in muscle. Two quite different mechanisms have been proposed, and there is no consensus on how the Ca^{2+} load in the SR alters RyR activation. The “true luminal regulation” hypothesis attributes luminal Ca^{2+}-activation to Ca^{2+} regulatory sites on the luminal side of the RyR while the “feed-through” hypothesis proposes that luminal Ca^{2+} permeates the pore and binds to the cytoplasmic sites. This study proposes a resolution of the controversy based on measurements of luminal Ca^{2+} activation of isolated cardiac RyRs (RyR2 isoform) in artificial lipid bilayers. In the absence of Ca^{2+}_{cyt} the open probability (P_o) of RyR2 had a voltage-dependent, bell-shaped dependence on $[Ca^{2+}]_{lum}$. At -40 mV (favouring Ca^{2+} feed through) Ca^{2+}_{lum} activates with a K_a = 50 μM and inhibits with a K_i = 600 μM. K_a and K_i markedly increased with membrane depolarisation. The mechanism of $[Ca^{2+}]_{lum}$ activation appears to be luminal-triggered, Ca^{2+} feed-through. Detailed analysis of channel gating indicated that Ca^{2+} binding sites on both the luminal (K_m =50 μM) and cytosolic (K_m = 1 μM) sides of the protein mediate Ca^{2+}_{lum} activation. Ca^{2+} binding to either of these sites are sufficient to open RyR2. In the virtual absence of Ca^{2+}_{cyt} (i.e. resting $[Ca^{2+}]$) the predominant opening mechanism is the luminal site which, when bound to Ca^{2+} opens the channel briefly (P_o ~0.3%, t_o ~1 ms). Ca^{2+} feed-through from the luminal to cytoplasmic site prolongs channel openings (P_o ~10%, t_o ~10-50 ms). In this way, Ca^{2+} feed-through can produce over 90% of RyR activation yet it is completely reliant on the action of Ca^{2+} at a luminal facing site.