Regulation of ryanodine receptors from cardiac muscle by luminal Ca^{2+} and Mg^{2+}

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Muscle contraction occurs when Ca^{2+} is released from the sarcoplasmic reticulum (SR) through ryanodine receptor Ca^{2+} release channels (RyRs). In heart, uptake and release of Ca^{2+} from the SR causes the free $[Ca^{2+}]$ within the lumen ($[Ca^{2+}]_L$) to cycle between ~0.3 to 1.0 mmol/l during the normal heart beat (Ginsburg *et al.*, 1998). $[Ca^{2+}]_L$ is known to regulate the Ca^{2+} releasing excitability of this store by stimulating the RyRs in its membrane. The resulting negative feedback between store depletion and Ca^{2+} release is believed to drive pacemaking and rythmicity cardiac muscle (Vinogradova *et al.*, 2005) as well as smooth muscle (Van Helden, 1993) and neurons (Verkhratsky, 2005). Luminal stimulation of RyRs involves three Ca^{2+} sensing mechanisms on both the luminal and cytoplasmic side of the RyR (Laver, 2007); namely the luminal Ca^{2+}-activation site (*L*-site, 60 μmol/l affinity), the cytoplasmic activation site (*A*-site, 0.9 μmol/l affinity) and the high affinity cytoplasmic Ca^{2+}-inactivation site (I_2-site, 1.2 μmol/l affinity). Cardiac RyR (RyR2 isoform) activation by luminal Ca^{2+} occurs by a multi-step process dubbed "luminal-triggered Ca^{2+} feed-through". Ca^{2+} binding to the *L*-site initiates channel openings where upon luminal Ca^{2+} can flow through to the *A*-site (producing prolongation of openings) and to the I_2-site (causing inactivation at high levels of Ca^{2+} feed-through). Cytoplasmic Mg^{2+} inhibits RyRs by displacing Ca^{2+} from the *A*-site (Laver *et al.*, 1997) and plays an important role in regulating Ca^{2+} release. However, the possibility that similar processes occur at the *L*- and I_2-sites has not been explored.

To explore this possibility, single RyRs and RyR arrays were incorporated into artificial lipid bilayers. SR vesicles were prepared from sheep hearts. Animals were killed by barbiturate overdose prior to muscle removal. SR vesicles containing RyRs were incorporated into artificial planar lipid bilayers which separated baths corresponding to the cytoplasm and SR lumen. The baths contained 230 mmol/l $CsCH_3O_3S$, 20 mmol/l CsCl, 10 mmol/l TES (pH 7.4) plus various amounts of Ca^{2+}, Mg^{2+} and ATP. Channel activity was recorded using Cs^+ as the current carrier. A novel, high affinity inhibition of RyR2 by luminal Mg^{2+} was observed, pointing to an important physiological role for luminal Mg^{2+} in cardiac muscle. At diastolic cytoplasmic $[Ca^{2+}]$ ($[Ca^{2+}]_C$ = 100 nmol/l) luminal Mg^{2+} inhibition was voltage-independent and was alleviated by increasing luminal $[Ca^{2+}]$. The K_i for Mg^{2+} inhibition increased from 90 μmol/l at $[Ca^{2+}]_L$ = 0.3 mmol/l to 1 mmol/l at $[Ca^{2+}]_L$ = 1 mmol/l. At systolic $[Ca^{2+}]_C$ (1- 10 μmol/l), Mg^{2+} inhibition was substantially reduced and its properties were consistent with luminal Mg^{2+} flowing through the channel and binding to the cytoplasmic *A*-site. Under these conditions K_i was voltage-dependent; 13 mmol/l at -40 mV and >100 mmol/l at +40 mV. The data could be accurately fitted by a model in which Mg^{2+} and Ca^{2+} compete at both the *L*- and *A*-sites and where the *L*-site has similar affinities for both ions. The model predicts that under physiological divalent ion concentrations (1 mmol/l free Mg^{2+} in the cytoplasm and lumen) and membrane potential (0 mV), $[Ca^{2+}]_L$ activation of Ca^{2+} release is primarily due to displacement of Mg^{2+} from the *L*-site and that luminal Mg^{2+} is an essential cofactor for the phenomenon. Therefore competition between luminal Ca^{2+} and Mg^{2+} may play an essential role in store-load dependent Ca^{2+} release.

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