Regulation of ryanodine receptors from skeletal and cardiac muscle by components of the cytoplasm and lumen

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Contraction in skeletal and cardiac muscle occurs when Ca^{2+} is released from the sarcoplasmic reticulum (SR) through ryanodine receptor (RyR) Ca^{2+} release channels. Ca^{2+}, Mg^{2+} and ATP are key regulators of RyRs. Skeletal (RyR-1) and cardiac (RyR-2) RyRs are modulated differently by these ligands and these differences may underlie the different characteristics of excitation-contraction (EC) coupling in skeletal and cardiac muscle. RyRs are regulated by two Ca^{2+}/Mg^{2+}-dependent mechanisms. They are activated at ~1 μmol/l [Ca^{2+}] and inhibited at mmol/l [Ca^{2+}] in the cytoplasm. Mg^{2+} can inhibit RyRs by binding at the Ca^{2+} activation and inhibition sites. ATP strongly activates RyR-1 in the virtual absence of cytoplasmic Ca^{2+} while in RyR-2, ATP primarily enhances Ca^{2+} activation.

The Ca^{2+} load of the SR is an important stimulator of Ca^{2+} release in skeletal and cardiac muscle. It is known that luminal Ca^{2+} stimulates RyRs but the mechanisms for this are not understood. In cardiac muscle, the release of Ca^{2+} from the SR strongly reinforces RyR activation, a process called Ca^{2+}-induced Ca^{2+} release (CICR). Although CICR should provide an explosive, positive feedback in Ca^{2+} release, the quantity of Ca^{2+} released from the SR has a graded, stable dependence on the magnitude of the Ca^{2+} inflow through the DHPRs.

In order to understand the mechanisms controlling Ca^{2+} release in skeletal and cardiac muscle, single RyRs and RyR arrays were incorporated into artificial lipid bilayers. SR vesicles were prepared from the back and leg muscles of New Zealand rabbits and 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 30-230 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.

Several proteins influence the way RyRs are regulated by luminal Ca^{2+}. The luminal proteins, calsequestrin (CSQ), triadin and junctin are associated with RyRs. By dissociating CSQ from RyR-1 it was shown that CSQ inhibits RyRs and can enhance the activating effect of luminal Ca^{2+}. In addition, CSQ dissociates from RyRs when luminal Ca^{2+} exceeds 4 mmol/l. These observations reveal several possible mechanisms by which CSQ can act as a sensor for luminal [Ca^{2+}].

The action of luminal Ca^{2+} on RyR-1 and RyR-2 was strongest in the absence of cytosolic Ca^{2+} and the potency of the luminal Ca^{2+} was enhanced by membrane potentials favouring Ca^{2+} flow from lumen to cytoplasm. At these voltages, RyR-1 activity rose ~5-fold by raising luminal [Ca^{2+}] from zero to ~100 μmol/l while a further increase to mmol/l levels caused ~30% fall from peak activity. RyR-2 had a more exaggerated Ca^{2+} dependence than RyR-1. RyR-2 activity increased ~100 fold between zero and 100 Ca^{2+} and decreased by 90% from peak activity at 1 mmol/l Ca^{2+}. Luminal Mg^{2+} inhibited RyRs by competing with luminal Ca^{2+} for both activating and inhibiting luminal sites. Thus Ca^{2+} and Mg^{2+} regulated RyR activity in a very similar way from both the luminal and cytoplasmic sides.

RyRs showed coupled gating when conditions favoured Ca^{2+} flow from the luminal to cytoplasmic baths. The rate constant for channel opening was increased by the opening of other RyRs in the bilayer. This indicates that the close packed RyR arrays seen in muscle are retained during isolation and bilayer incorporation. In these arrays, luminal Ca^{2+} can permeate an open channel to activate neighbouring RyRs. Coupled openings were followed by a rapid and complete closure of all the channels that occurred within 10 ms. This may be the first inactivation phenomena demonstrated *in vitro* that could possibly explain the rapid termination of Ca^{2+} sparks and the graded control of Ca^{2+} release in cardiac EC coupling.