Regulation of coupled calcium release channels in bilayers by luminal Ca^{2+}

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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. In muscle, the RyRs are activated by ATP in the cytoplasm and by Ca^{2+} in the cytoplasm and lumen of the SR. This study investigates their mechanisms of action.

RyRs were isolated from rabbit skeletal muscle that had been removed from dead rabbits. RyRs were incorporated into artificial planar lipid bilayers separating baths corresponding to the cytoplasm and SR lumen. Single channel activity was recorded using Cs^{+} as the current carrier.

Cytoplasmic Mg^{2+} is a potent inhibitor of RyRs. Although it is recognised that Mg^{2+} binds at the cytoplasmic Ca^{2+} sites, it is not clear if Mg^{2+} is an antagonist or merely prevents Ca^{2+} from activating the channel. This distinction becomes important considering that physiological [ATP] activates skeletal RyRs in the absence of cytoplasmic Ca^{2+}. We measured the effects of Mg^{2+}-inhibition on ATP activated RyRs in the absence of Ca^{2+} and found that Mg^{2+} is indeed a RyR antagonist.

RyRs showed coupled gating when conditions favoured Ca^{2+} flow from the luminal to cytoplasmic baths (*i.e.* the rate constant for channel opening was increased by the opening of neighbouring RyRs in the bilayer). This indicates that RyRs can be in close proximity and that luminal Ca^{2+} can permeate open channels to activate neighbouring RyRs at the cytoplasmic activation sites. Curiously, we find that Ca^{2+} released by a RyR has a greater stimulatory effect on its neighbours than itself.

We describe a novel mechanism for luminal Ca^{2+} regulation of Ca^{2+} release whereby increasing luminal [Ca^{2+}] decreases the apparent affinity of the RyR for cytoplasmic Mg^{2+}. This decrease in apparent Mg^{2+} affinity was not due to competition between luminal Ca^{2+} and cytoplasmic Mg^{2+}. Rather, it appears that luminal Ca^{2+} can regulate RyRs via an allosteric mechanism independently of Ca^{2+} flow through the channel.