The response of the ryanodine receptor to reduced luminal Ca^{2+} concentrations is depressed by calsequestrin

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The ryanodine receptor (RyR) Ca^{2+} release channel in the sarcoplasmic reticulum (SR) of skeletal and cardiac muscle is essential for excitation-contraction (EC) coupling. Calsequestrin (CSQ) is the major Ca^{2+} binding protein in the SR and also regulates RyRs. Since Ca^{2+} release from SR is determined by the Ca^{2+} load, CSQ is possibly a luminal Ca^{2+} sensor for the RyR. There are contradictory reports about the effect of changing luminal free Ca^{2+} concentration ($[Ca^{2+}]_{free}$) on RyR activity, which are unexplained but may depend on the channels association with CSQ.

To investigate the responses of RyRs to altering luminal $[Ca^{2+}]_{free}$ in the presence and absence of CSQ, rabbit skeletal SR vesicles (from freshly euthanased rabbits) were reconstituted in artificial lipid bilayers, which separates two chambers, denoted cytoplasmic and luminal respectively. Luminal $[Ca^{2+}]_{free}$ was adjusted between 1 mM to 100 nM by adding BAPTA or EGTA, and channel activities were tested in both CSQ-associated and CSQ-dissociated RyRs at both sub-activating (100 nM) and activating (50 m M) cytoplasmic Ca^{2+}.

Lowering luminal $[Ca^{2+}]_{free}$ from 1 mM to 100 nM resulted in immediate activation of RyRs in CSQ-dissociated RyRs. In contrast, either less increase or in fact decreased activity was observed in CSQ-associated RyRs when luminal Ca^{2+} was decreased. The changes were independent of initial channel activity and the type of Ca^{2+} chelator.

The data show that the RyR response to changing luminal $[Ca^{2+}]_{free}$ depends on CSQ association. The activation by a fall in luminal $[Ca^{2+}]_{free}$ was depressed in the presence of CSQ. This suggests that CSQ acts as a luminal Ca^{2+} sensor for the RyR at lower than physiological $[Ca^{2+}]$s and could effectively reduce excess Ca^{2+} release from the SR during stress or fatigue and thus act to conserve the store load.