Phosphorylation status of calsequestrin does not alter its ability to regulate native ryanodine receptors

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Depolarisation of the sarcolemma triggers Ca^{2+} release through ryanodine receptor (RyR) calcium release channels in the sarcoplasmic reticulum (SR) of skeletal muscle. The protein responsible for calcium storage within the SR is calsequestrin (CSQ), which is located wholly within the SR lumen. CSQ is tethered to the RyR by two anchoring proteins, triadin and junctin, as well as probably forming a direct physical coupling with the channel itself.

Recent studies have shown that CSQ regulates RyRs via two mechanisms. The first (indirect) interaction is presumably mediated by triadin and junctin, resulting in RyR inhibition, whilst the second interaction is via a direct physical connection between CSQ and the RyR (Szegedi *et al.*, 1999; Herzog *et al.*, 2000; Beard *et al.*, 2000). This second interaction requires dephosphorylated CSQ to modify RyR activity. The role *in vivo* of CSQ phosphorylation is not clear, nor has a definitive phosphorylation mechanism been reported. It is unknown whether the interaction of CSQ with native RyRs (those containing RyR co-proteins, such as triadin and junctin) depends on CSQ dephosphorylation in the same manner as the direct interaction of CSQ with purified RyRs.

To study the effects of altering CSQ's phosphorylation status on its ability to regulate native RyR regulation, rabbit skeletal SR vesicles containing RyRs (isolated from back and leg muscle of New Zealand male rabbits killed by a captive bolt) were incorporated into artificial planar lipid bilayer membranes, which were formed across an aperture with a diameter of 150-200 μM in a delrin cup. The bilayer separates two chambers, *cis* (cytoplasmic) and *trans* (luminal). Solutions contained Ca^{2+} (1 mmol/l), $CsCl_2$ (20 mmol/l), caesium methane sulfonate (250/30 mmol/l; *cis/trans*) and TES (10 mmol/l). CSQ was purified according to Costello *et al.*, (1986). Phosphorylation status of CSQ was determined using ^{31}P NMR, and CSQ was dephosphorylated according to the methods of Cala & Jones (1983).

In a single channel study, RyRs were exposed to 500 mM Cs^+ to dissociate endogenous CSQ (recently shown to successfully dissociate CSQ from bilayer incorporated RyRs; Beard *et al.*, 2002). After subsequent perfusion of the *trans* chamber with 250 mM Cs^+, 20-50 μg of either phosphorylated or dephosphorylated CSQ was added to the *trans* chamber. There was no significant difference between the regulation of the RyR by phosphorylated or dephosphorylated CSQ. Both forms of CSQ significantly inhibited RyR activity.

Unlike the phosphorylation-dependant regulation of purified RyRs by CSQ, altering the phosphorylation status of exogenous CSQ did not alter CSQs ability to inhibit native skeletal RyR activity. In combination with the results of Szegedi *et al.* (1999) and Herzog *et al.* (2000), these data illustrate that CSQ imposes two very different regulatory mechanisms on RyRs, and suggest that phosphorylation-dependant changes *in vivo* do not alter the triadin/junctin mediated regulation of RyRs and SR Ca^{2+} release by CSQ.

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