Isoform dependent properties of calsequestrin

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Skeletal and cardiac muscle contraction is dependent on Ca^{2+} release from the sarcoplasmic reticulum (SR) through the ryanodine receptor (RyR) calcium release channels. Calsequestrin (CSQ) is the major Ca^{2+} binding protein found within the SR in both skeletal and cardiac muscle, and binds Ca^{2+} with a high capacity and moderate affinity. We have shown that CSQ is a luminal Ca^{2+} sensor for skeletal RyRs (RyR1). However, CSQ1 (skeletal) and CSQ2 (cardiac) are products of different genes and share only 66-80% homology in their primary sequence and are thus likely to differ substantially in their properties and regulation of RyRs. Therefore, we have compared the Ca^{2+} binding capacity of the two isoforms and their ability to associate with and regulate their respective RyRs.

New Zealand male white rabbits were euthanized by a captive bolt and back and leg muscle used to prepare skeletal SR vesicles and CSQ1. Cardiac SR vesicles and CSQ2 were isolated from Merino sheep euthanized by overdose of 20 ml valabarb euthanasia solution (300 mg/ml) injected into the jugular vein. Ca^{2+} binding capacities of rabbit skeletal muscle CSQ1 and sheep cardiac CSQ2 were determined using a ^{45}Ca binding assay and confirmed using Stains-all staining after SDS-PAGE. The fraction of CSQ associated with the SR membrane was quantified following SDS-PAGE of SR fractions and Western blot with antibodies against CSQ1 and CSQ2. RyR channel activity was measured in lipid bilayers. Skeletal or cardiac SR vesicles were reconstituted into artificial planar lipid bilayers which separate two chambers, cytoplasmic and luminal.

We show that there are profound differences between the two isoforms in their Ca^{2+} binding capacity, their association with the RyR and their regulation of RyR activity. Despite the fact that CSQ2 has an extended acidic C-terminal tail (~30 residues longer than CSQ1), CSQ2 binds only half as much Ca^{2+} as CSQ1. To measure the fraction of CSQ associated with RyR, SR fractions (prior to 0.5% Triton X-100 solubilization - using 1 mM Ca^{2+} to retain CSQ's polymer structure), was examined as well as the supernatant and membrane fraction after centrifugation of the solubilized material. We found that 80% of CSQ1, but only 20% of CSQ2, was membrane associated. The ratio of CSQ to RyR in the SR was assessed from solubilized membrane samples using Western blot with anti-CSQ1 and anti-CSQ2 antibodies. The membrane samples contained equal amounts of RyR1 and RyR2 and the anti-CSQ antibodies were used in concentrations which had previously been determined to stain CSQ1 and CSQ2 with equal density. The CSQ/RyR ratio was ~4-fold greater in skeletal SR than in cardiac SR. These results indicate that either CSQ2 is less polymerized at 1 mM Ca^{2+} than CSQ1, or that less CSQ polymer is associated with the triadin/junctin/RyR complex in the cardiac SR.

Native RyR1 or RyR2 in lipid bilayers were treated with Ca^{2+} free luminal solution to depolymerize CSQ and to dissociate all but the residual monomer of CSQ that remains bound to the triadin/junctin/RyR. The effects of adding CSQ back to RyRs was then examined after luminal Ca^{2+} was restored to 1 mM. We found CSQ2 added to native RyR2 activates the channel, in marked contrast to the inhibition of RyR1 by CSQ1 under identical conditions . The inhibitory effect of CSQ1 and RyR1 is highly isoform specific since, at 1 mM luminal Ca^{2+}, CSQ2 activates native RyR1 and CSQ1 activates native RyR2. We have shown previously that CSQ1 regulates native RyR1, which ensures that Ca^{2+} release is decreased during transient reductions in luminal Ca^{2+} concentrations. We are currently investigating the role of CSQ2 in determining the response of native RyR2 to changes in luminal Ca^{2+}. The dissimilar properties of CSQ1 and CSQ2 suggest a differential regulation of their respective SR Ca^{2+} stores which may underlie the strong store depletion with each cardiac contraction, but retention of store Ca^{2+} load in skeletal muscle.

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