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Ryanodine Receptor Pore Structure and Function
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Ryanodine Receptors (RyRs) are ion channels that regulate muscle contraction by releasing calcium ions from intracellular stores into the cytoplasm. Mutations in skeletal muscle RyR (RyR1) give rise to congenital diseases such as the Central Core Disease. The absence of high-resolution structures of RyR1 has limited our understanding of channel function and disease mechanisms at the molecular level. Here, we report a structural model of the pore-forming region of RyR1 and electrophysiological studies on a Central Core Disease mutant RyR1-G4898R. Molecular dynamics simulations on the structural model show preferential localization of Ca2+ over K+ in the selectivity filter. We observe high ion binding to the residues D4899, E4900, D4938 and D4945 along the pore, which are experimentally known to be critical for channel function and selectivity. Furthermore, simulations on the mutant RyR1-D4899Q show a loss of preference to Ca2+ in the selectivity filter as seen experimentally. Electrophysiological experiments on RyR1-G4898R show constitutively open channels that conduct K+ but not Ca2+. Our simulations with G4898R likewise show a decrease in preference of Ca2+ over K+ in the selectivity filter. Together, the computational and experimental results shed light on the functioning of the RyR1 pore at an atomistic level.

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Characterization of Conformation-Specific Monoclonal Antibodies Against Skeletal Ryanodine Receptor
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Conformation-specific monoclonal antibodies are powerful tools for studies of proteins structure and function. Monoclonal andibodies suitable for structure studies of membrane proteins are those that bind the target molecule at a conformation-sensitive epitope, typically one that comprises a three dimensional structure. Monoclonal antibody Fab fragments have been shown to help crystallization of membrane proteins both by stabilizing a conformation and by increasing hydrophilic surface for crystal ''packaging''.
We have immunized mice with full-length rabbit skeletal muscle ryanodine receptor, isolated clonal hybridoma cell lines, cloned the Fab fragments, and tested the purified antibodies for specificity, affinity and conformation sensitivity. Two monoclonal antibodies showed conformation specificity. These antibodies detect the native receptor in an immunoprecipitation assay but not the denatured receptor in a Western blot analysis. Furthermore, these antibodies show sensitivity to calcium and affect ryanodine binding activity suggesting specificity to the closed state of the receptor rather then the open state.

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Homer-RyR1 Associations are Physiological Regulators of Intracellular [Ca2+]
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Homer proteins belong to family of multifaceted proteins that enhance signaling by linking transmembrane proteins with signaling pathways and may be involved in intracellular Ca^{2+} regulation. We have measured resting intracellular Ca^{2+} ($[Ca^{2+}]_{rest}$) by means of Ca^{2+} selective microelectrodes, and Ca^{2+} transients using Fluo-4 in primary Wt and pan Homer KO myotubes at 23°C under different experimental conditions. $[Ca^{2+}]_{rest}$ in Wt myotubes was 122 ± 8 nM ($n = 23$) while in Homer KO it was 313 ± 30 nM ($n = 20$). Incubation with either Cd/La, 30 μM dantrolene or 25 μM ryanodine (overnight) and subsequently in 20 μM bastadin-5 (B5) for 5 min prior to $[Ca^{2+}]_{rest}$ measurements induced significant reductions in $[Ca^{2+}]_{rest}$ in both groups of cells, but the reduction was greater in Homer KO than *Wt* cells. In ryanodine+B5 treated cells both 0.5 mM Cd^{2+}/ 0.1 mM La^{3+} or 30 μM dantrolene produced a further reduction of $[Ca^{2+}]_{rest}$ which again was more evident in Homer KO. The amplitude of Ca^{2+} transients elicited by electrical trains of square pulses (0.5–1ms duration) at 60 Hz were always smaller in Homer KO than those produced by Wt myotubes, confirming that Homer regulates RyR1 gain. The present findings reveal that the absence of Homer in myotubes dysregulates several aspects of Ca^{2+} homeostasis. High $[Ca^{2+}]_{rest}$ appears to be mediated by both increased sarcolemmal Ca^{2+} entry and enhanced leak from RyR1 channels. These data demonstrate that Homer-RyR1 associations are significant physiological regulators of basal and evoked intracellular $[Ca^{2+}]$. Supported by **AR17605 and AR43140 (PDA, INP)**

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Mitsugumin 29 as a TRPC3-interacting Protein
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Canonical-type transient receptor potential cation channel type3 (TRPC3) allows the entry of Ca^{2+} into various cells. In skeletal myotubes, functional interaction between TRPC3 and RyR1 (ryanodine receptor1) regulates the gain of excitation-contraction coupling. Mitsugumin 29 (MG29) is a TRPC3-interacting protein in skeletal myotubes. To identify critical region(s) of MG29 that participate in binding to TRPC3, N-terminus, three intervenient loops among four transmembrane regions, and C-terminus of MG29 were expressed in E. coil as N-terminal GST-fused forms. The five GST-fused MG29 portions were subjected to co-immunoprecipitation assay with intact TRPC3. Cytoplasmic N-terminus and a loop between first and second transmembrane regions of MG29 effectively bound to TRPC3.

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Cross-Reactivity of Ryanodine Receptors with Putative Modulators of Ion Channels in the Plasma Membrane
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The role of specific ion channels for cell function is often investigated using ''pharmacological agents'' (PA). PA specificity is typically determined using the whole-cell voltage clamp technique, which allows for the contrasting of many plasma membrane ion channels but not intracellular calcium release channels such as the ryanodine receptors (RyRs), which are capable of triggering numerous cellular processes. We determined that an UV-visible cuvette assay can be utilized for the screening of RyR cross-reactivity with PA, allowing for rapid determination of modulators that affect the rate of Ca^{2+} leak from skeletal muscle sacroplasmic reticulum microsomes. Agents that tested positive (both agonists and antagonists) were also tested on skeletal RyRs reconstituted into planar lipid bilayers. One of our main focus was TRP modulators, as early evidence suggested that these agents could target RyRs. At doses normally used to modulate TRP channels, menthol activated and anandamide inhibited RyRs. On the contrary, pseudocapsaicin and capsaicin were without effect. We have hypothesized that the cytosolic vestibular regions in the RyRs conduction pathway could have structural homology with that of voltage-gated Na^+ and K^+ channels. We then tested agents thought to act on the cytosolic vestibular region of those channels and determined that lamotrigine isethionate (Na^+ channel blockers) and UCL1964 (K^+ channel blocker) also block RyRs. Thus, our results suggest that RyR channels are targets for various ion channel modulators and could mediate some of their therapeutic actions. Exploring RyRs cross-reactivity has the potential for identifying pharmacological tools to better understand RyRs gating and RyR-mediated Ca^{2+} signaling in cells. (Supported by NIH R01 GM078665 to JAC)

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The Na^+/Ca^{2+} exchanger Inhibitor KB-R7943 is also a potent inhibitor of EC coupling and ryanodine receptors
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Background: Na^+/Ca^{2+} exchanger (NCX) is a plasma membrane transporter that moves Ca^{2+} in or out of the cell depending on membrane potential and transmembrane ion gradients. NCX is the main pathway for Ca^{2+} extrusion from ventricular myocytes and has been extensively studied in this tissue. NCX inhibitors can ameliorate cardiac ischemia-reperfusion injury and this result has been attributed to inhibition of the Ca^{2+} inward mode of NCX. Here we tested two NCX inhibitors, KB-R7943 and SN-6 on adult dissociated skeletal muscle fibers loaded with Fluo-4 and [^{3}H]ryanodine binding analysis. **Results:** KB-R7943 (5 and 10 μM) reversibly inhibits Ca^{2+} transients elicited by electrical stimulation. At the same concentrations SN-6 has no effect on the properties of transients. In order to evaluate a possible direct effect of KB-R7943 and SN-6 on RyR activity we performed [^{3}H]-ryanodine binding assays because ryanodine binds to the channel in the open state and is used to evaluate channel conformation. Using a skeletal muscle SR fraction we found that 5 or 10 μM KB-R7943 inhibits high affinity binding of [^{3}H]-ryanodine in a dose-dependent manner whereas SN-6 has negligible activity. **Conclusion:** Our results suggest that KB-R7943 is a potent and reversible blocker of RyR channels and EC coupling in adult fibers. The effects of these compounds on cardiac ischemia-reperfusion injury may be partially explained by their effect on RyR activity. Support: **R01AR43140 & P01 AR52354**