was a dose dependent shift in the open time constants towards the faster components. The binding assays revealed a [Fe^{2+}] dependent, co-operative reduction in [^{3}H]ryanodine binding to HSR vesicles. Preliminary data of [^{3}H]ryanodine binding in increasing [Ca^{2+}] showed a rightward shift in the presence of Fe^{2+}.
The results presented here show for the first time that Fe^{2+} is a potent inhibitor of RyR2. The mechanism of this inhibition may be due to competition with Ca^{2+} for RyR2 activation sites. Suppression of RyR2 activity by Fe^{2+} may therefore be one of the mechanisms involved in iron-induced cardiomyopathies.
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Increased Expression of Ryanodine Receptors and the Iron Transporter DMT1 in Hippocampal Neurons by Brain Derived Neurotrophic Factor (BDNF), NMDA or Spatial Memory Training
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Ryanodine receptors (RyR) mediate skeletal and cardiac muscle contraction and amplify via CICR postsynaptic calcium signals generated by activity-dependent calcium influx through NMDA receptors (NMDAR) in hippocampal glutamatergic synapses. We have recently shown that reactive oxygen species (ROS) and iron, which as shown here promotes ROS generation in neurons, stimulate RyR-mediated calcium release when added to primary hippocampal neurons. Here, we report that 5 min incubation of hippocampal cells in primary culture with NMDA (50 μM), induced RyR-mediated calcium signals that were inhibited by pre-incubation with the iron chelator desferroxamine. Incubation with NMDA also enhanced >2-fold the expression (measured 24 h later) of the iron transporter DMT1 (IRE form), while incubation with BDNF (50 ng/ml) increased >5-fold RyR expression. Additionally, we investigated if spatial memory training of male rats in a Morris water maze affected RyR and DMT1 expression. The hippocampus was dissected 6 h after the last behavioral task (5d, 2d rest, 1d platform free) and samples from tissue were prepared for Western blot and RT-PCR experiments. We found that spatial memory training increased the mRNA and protein expression of DMT1, RyR2 and RyR3. Our results confirm enhanced RyR2 expression following spatial memory training and correlate for the first time enhanced in vivo expression of the iron transporter DMT1 and RyR3 with spatial memory acquisition/consolidation. We propose that iron-induced ROS production stimulates the emergence of RyR-mediated intracellular calcium signals that promote RyR and DMT1 expression during the spatial memory task.
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Increased Levels Of Type 2 Ryanodine Receptor (RyR2) In Rat Heart Mitochondria During Diabetes
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Diabetes reduces myocardial contractility. Studies attribute this defect in part to a dysfunction of cardiac mitochondria. However, molecular mechanisms responsible for mitochondrial dysfunction during diabetes remain incompletely defined. The present study was designed to determine whether diabetes alter expression and activity of cardiac mitochondrial ryanodine receptor (mRyR). Type 1 diabetes was induced in male Sprague-Dawley rats using streptozotocin (STZ). Two and eight weeks after STZ injection, diabetic rats were sacrificed, hearts harvested, and cardiac mitochondria were purified using differential and Percoll gradient centrifugations. In Western blots, bottom and middle Percoll fractions from control hearts immuno-reacted with VDAC and COX IV, but not with SERCA2 antibodies. These fractions also contained a protein of $M_w \approx 500$kDa that immuno-reacted with RyR2 but not with RyR1 antibodies. Trypsin digestion followed by mass spectroscopic analysis revealed this high molecular weight protein to be RyR2 (mRyR2). After two weeks of diabetes, mRyR2 protein level in bottom fraction increased 1.8 fold, as was total [^{3}H]ryanodine bound (11.9 ± 4.8 fmol [^{3}H]ryanodine bound/mg protein for control *vs* 17.5 ± 2.0 fmol/mg protein for diabetic at 900μM Ca^{2+}). After 8 weeks of diabetes, mRyR2 protein level remained elevated. Interestingly, the activity of mRyR2 as assessed from [^{3}H]ryanodine bound increased 5-fold (from 46.9 ± 8.1 fmol/mg protein in control to 262.7 ± 40.1 fmol/mg protein at 200μM Ca^{2+}). Two weeks of insulin-treatment initiated after 6 weeks of diabetes, normalized expression and activity of mRyR2 to near control values. These data are the first to show mRyR2 expression increases in heart during diabetes. This increase in expression of mRyR2 during diabetes could perturb mitochondrial Ca^{2+} homeostasis resulting in disrupting of ATP production and a reduction in myocyte function. (Supported in part by NIH grants to S-SS and KRB)

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Intracellular Calcium Release Channels Mediate Their Own Countercurrent: The Ryanodine Receptor Case Study
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The ryanodine receptor (RyR) and inositol trisphosphate receptor (IP3R) calcium release channels mediate large calcium release events lasting >5 ms from intracellular calcium storage organelles. For these channels to mediate such a long-lasting calcium efflux, a countercurrent of other ions is necessary to prevent the membrane potential from rapidly (<1 ms) reaching the calcium Nernst potential. A recent model of ion permeation through a single, open RyR channel is used here to show that the vast majority of this countercurrent is likely conducted by the release channel itself. Consequently, changes in membrane potential are minimized locally and instantly, assuring maintenance of a calcium driving force. This auto-countercurrent is possible because of the poor calcium selectivity and high conductance for both monovalent and divalent cations of the calcium release channels. For example, the RyR model suggests that in normal cellular ionic conditions this auto-countercurrent clamps the membrane potential near 0 mV within ~150 micros. Consistent with experiment, this model demonstrates how RyR calcium current is defined by luminal calcium concentration, surrounding permeable ion composition, pore selectivity and conductance. Since the RyR and IP3R channels have homologous pores and permeation characteristics, we predict this will also be true for IP3R-mediated calcium release as well. If so, then auto-countercurrent may be essential to nearly any RyR or IP3R mediated calcium release event observed in cells.

Calcium Signaling Proteins

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Essential Roles for Coiled-coil Domains in STIM1 Oligomerization and CRAC Channel Activation
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The calcium release-activated calcium (CRAC) channel is activated by depletion of Ca2+ from the ER. Store depletion causes the ER Ca2+ sensor, STIM1, to translocate to sites of close ER-plasma membrane apposition, where it interacts with Orai1, the pore-forming subunit of the CRAC channel and activates Ca2+ entry. STIM1 self-associates in resting cells (Baba et al., PNAS 103:16704, 2006) and further oligomerizes after store depletion (Liou et al., PNAS 104:9301, 2007), an event that triggers the self-assembly and activation of STIM1-Orai1 clusters at ER-PM junctions (Luik et al., Nature 454:538, 2008). STIM1 has several protein interaction domains, including a lumenal sterile alpha motif (SAM) and two putative cytosolic coiled-coil regions. The isolated lumenal EF hand - SAM region is known to oligomerize upon Ca2+ removal in vitro (Stathopulos et al., JBC 281:35855, 2006), but the roles of the coiled-coil domains in the functions of STIM1 in situ are not as well understood. Using fluorescence recovery after photobleaching, co-immunoprecipitation, and blue native PAGE analysis on truncated mutants of STIM1 we show that the two coiled-coil domains of STIM1 affect STIM1 oligomerization in different ways. The ER-proximal coiled-coil is sufficient for the self-association of STIM1 in resting cells but does not by itself support oligomerization in response to store depletion. The distal coiled-coil is required for depletion-induced oligomerization. Mutation of specific residues within the predicted hydrophobic interface of the distal coiled-coil prevents the formation of STIM1 puncta and the activation of CRAC channels. These results reveal an essential role for the distal coiled-coil of STIM1 in the oligomerization step that controls store-operated Ca2+ entry.

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Atomic Force Microscopy of Copine I and Annexin A1 on Supported Phospholipid Bilayers: Structure and Synergism
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