(8.0 ± 0.91 μM). When SR Ca leak was matched (9.0 μM) we found the SR Ca load necessary to induce that leak was significantly lower in ISO-treated myocytes (91.6 ± 1.9 μM) vs. those treated with ISO and L-NAME (129.4 ± 16.3 μM) or those left untreated (127.4 ± 2.8 μM). This evidence indicates that NOS activation, and therefore generation of nitric oxide, is necessary for ISO-dependent activation of RyR by CaMKII.

617-Pos Board B496

Discrete Proteolysis Of Neuronal Calcium Sensor 1 By μ-calpain Disrupts Calcium Binding

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Neuronal Calcium Sensor-1 (NCS-1) is a high-affinity, low-capacity calcium-binding protein abundantly expressed in many cell types. NCS-1 interacts with the inositol 1,4,5-trisphosphate receptor (InsP3R) and modulates calcium signaling by enhancing InsP3-dependent InsP3R channel activity and intracellular calcium transients. NCS-1 was also found to be a novel binding partner with the chemotherapeutic drug, paclitaxel (taxol), used to treat a variety of tumor types including ovarian, breast, lung, head, and neck cancers. The immediate response of cells to taxol is a further enhancement of the NCS-1 amplification of $InsP_3R$ dependent calcium signaling. Prolonged treatment with taxol triggers μ-calpain dependent proteolysis of NCS-1. Degradation of NCS-1 may be a critical step in the induction of peripheral neuropathy associated with the taxol treatment. To begin the process of designing a strategy that would protect NCS-1 during taxol administration, we treated NCS-1 with μ-calpain *in vitro* and identified the cleavage site by N-terminal amino acid sequencing and MALDI-mass spectroscopy. Using molecular modeling we found that μ-calpain cleavage of NCS-1 occurs within an N-terminal pseudoEF-hand domain. By sequence analysis this pseudoEF-hand domain should be unable to bind calcium. Nonetheless, our results suggest a role for this pseudoEF hand domain in forming and stabilizing the three functional EF hand domains within NCS-1. Using isothermal titration calorimetry (ITC) we found that the loss of the pseudo EF-hand domain of NCS-1 leads to a markedly decreased affinity for calcium. The inability of the μ-calpain treated NCS-1 to bind calcium may explain the reduced calcium signaling in the presence of taxol and may suggest a plausible strategy for therapeutic intervention of peripheral neuropathy in cancer patients undergoing taxol treatment.

618-Pos Board B497

Effect Of (−)-epigallocatechin Gallate (EGCG), A Green Tea Extract, On Excitation-contraction Coupling Of Murine Cardiomyocytes

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Background: Polyphenolic compounds, green tea, reportedly have protective benefit for cardiovascular disease, but the mechanism(s) are unknown. We have recently found that green tea extracts such as (-)-epigallocatechin gallate(EGCG) bind to skeletal and cardiac ryanodine receptor(RyR) Ca^{2+}-release channels. Here we examined the effect of EGCG(1μM, 100nM, 10nM) on cell shortening and Ca^{2+} kinetics in field-stimulated murine ventricular cardiomyocytes loaded with Fura-2AM and on cardiac RyR channels incorporated in lipid bilayers. **Results:** EGCG at 10 nM already maximally increased myocytes fractional shortening(%FS): ECGC 5.6 ± 0.7, N=19, *vs.* Vehicle, 2.2 ± 0.3, N=25, $p<0.01$), so 10 nM was used for all myocyte experiments. Increased contractility was caused by significantly larger Ca^{2+} transients in presence of ECGC(Fura-2 ratio: 0.64 ± 0.15 *vs.* 0.26 ± 0.03, $p<0.01$). Ca^{2+} transient decay kinetics(a measure of SERCA function) and sarcoplasmic reticulum(SR) Ca^{2+} content measured by rapid caffeine application were not significantly altered by ECGC. As a result, EGCG almost doubled the fraction of SR Ca^{2+} content released during each beat(56 ± 6 % *vs.* 32 ± 4%, $p<0.01$), even though EGCG significantly inhibited L-type Ca^{2+} current($p<0.01$). Decay of Ca^{2+} transients during caffeine application was significantly slower(-EGCG 2.31 ± 0.17 sec *vs.* Vehicle 1.72 ± 0.1 sec, $p<0.01$), suggesting that EGCG significantly inhibits Ca^{2+} extrusion via the NaCa exchanger(NCX). EGCG(500nM) enhanced RyR2 single channel activity >30-fold prolonging mean open time 15-fold without altering unitary conductance. EGCG did not alter SR Ca^{2+} loading capacity in the presence of RyR channel blocker ruthenium red. Taken together, these data suggest that EGCG enhances contractility of intact myocytes via its action on RyR channels. SR Ca^{2+} depletion is prevented by EGCG's concomitant inhibition of the NCX. **Conclusions:** EGCG potently modulates cardiac excitation contraction coupling by acting on RyR and possibly on L-type Ca^{2+}-channel and NaCa exchanger. Supported by **R01HL71670, R01HL88635, and R01AR43140**

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Properties and Functions of Store-Operated Calcium Entry in the Developing Nervous System

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Store-operated calcium channels (SOCs) open in response to depletion of calcium stores in the endoplasmic reticulum. These channels are expressed in a variety of tissues including the immune system, vasculature and hepatocytes. The most widely studied and characterized SOC is the Calcium Release-Activated Calcium (CRAC) channel in the immune system. Recent findings indicate that CRAC channels are activated by local interactions between the ER Ca^{2+} sensor, STIM1 and the CRAC channel subunit, ORAI1. Calcium influx through these channels has been shown to play an important role in transcription of inflammatory mediators such as interleukins and cytokines, mediated by the transcription factor NFAT1.

The current study aims at characterizing these channels in the developing nervous system. Calcium imaging experiments demonstrate the presence of store-operated calcium entry (SOCE) with properties similar to that mediated by the CRAC channel. Calcium influx following store-depletion is blocked by La^{3+}, a potent CRAC channel blocker. In addition, 2-APB (0.01–0.02 mM) causes a transient elevation in intracellular calcium followed by a decrease, consistent with that observed in immune cells. Functional studies done using NFAT1 tagged to GFP show translocation of this transcription factor to the nucleus upon calcium entry following store-depletion. Further, we find an increase in the levels of endogenous NFAT-dependent gene expression using Luciferase reporter assays. Altogether, these results provide evidence for the existence of store-operated calcium entry in the developing nervous system and point towards a regulatory role for this pathway in gene-transcription.

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Investigating the Architecture of the CRAC Channel Pore using SCAM

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Calcium channels are vital for numerous cellular processes in all organisms. Among the many classes of calcium channels, the calcium release-activated Ca^{2+} (CRAC) channel, a member of the Store-operated channel (SOC) family is essential for the proper development and maintenance of the immune system, mediating critical functions such as T cells proliferation, release of inflammatory mediators, and motility. This interesting channel has a biophysical fingerprint consisting of an extremely high selectivity for Ca^{2+}, a narrow pore size (3.9Å), a very small unitary conductance, and several modes of modulation. A single amino acid mutation (R91W) results in a loss of CRAC channel function also known as severe combined immunodeficiency (SCID) in human patients. Although the CRAC channel current has been well-characterized, no structural information is known about the channel or its pore.

The objective of this study is to investigate the architecture of the CRAC channel pore using the substituted cysteine accessibility method (SCAM), which has been applied to several ion channel proteins and has provided significant structural insight from topology to conformational changes. Our initial studies analyzed the effects of MTS reagents, cysteine-modifying compounds, on the TM1 and the TM1-TM2 extracellular loop region around E106, an important residue that controls Ca^{2+} selectivity and ion permeation. We find that the ability of MTS reagents to block current diminishes as the residues in TM1-TM2 become more removed from E106. These results suggest that this region of the protein may form the entrance to the channel pore, thus providing the first insight into the architecture of the CRAC channel.

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Both Membrane Depolarization And IL-6 Induce Calcium-Dependent Hsp70 Expression In Skeletal Muscle Cells

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Adaptive response of skeletal muscle to challenges imposed by contractile activity is associated to changes in specific genes expression. IL-6 and Hsp70 are proteins involved in the maintenance of skeletal muscle homeostasis during stress episodes and are markedly expressed in skeletal muscle after physiological contraction. Muscle-derived IL-6 has systemic and local effects acting in a hormone-like fashion, nevertheless the molecular bases of its functional role on skeletal muscle is poorly understood. We have demonstrated that depolarization evoked IP_3 mediated slow calcium transients, associated to cell nuclei are involved in the up-regulation of IL-6 transcriptional activity in skeletal muscle cells.