(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**

619-Pos Board B498

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.

620-Pos Board B499

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.

621-Pos Board B500

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.

The aim of this work was to investigate calcium involvement in Hsp70 expression in both depolarized and IL-6 treated skeletal muscle cells.
We observed that electrical stimulation of myotubes increases Hsp70 mRNA level and protein expression. Depolarization performed in the presence of the intracellular calcium chelator BAPTA-AM resulted in a complete inhibition of Hsp70 induced expression. Inhibitors of IP_3-dependent calcium signals like 2-aminoethoxydiphenyl borate (2-APB) and LY294002, decreased Hsp70 mRNA induction and the protein expression in depolarized cells. In addition we determined that inhibitors of calcium dependent PKC abolished Hsp70 mRNA induction.
We established that IL-6 treatment of myotubes induced changes in intracellular calcium and promoted the increase of Hsp70 mRNA levels. Observed calcium transients could be associated to early events of IL-6-mediated Hsp70 expression.
Our results provide evidence for the involvement of slow calcium transients and PKC in the activation of Hsp70 expression in skeletal muscle cells and suggest that intracellular calcium signals also participate in IL-6 induced Hsp70 expression.
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622-Pos Board B501
Ryanodine receptor 1 signaling in dendritic cells
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Dendritic cells express the skeletal muscle ryanodine receptor (RyR1), yet little is known concerning its physiological role and activation mechanism. In the present report, we provide evidence that dendritic cells also express the Cav1.2 subunit of the L-type Ca2+ channel and that release of intracellular Ca2+ via RyR1 depends on the presence of extracellular Ca2+ and is sensitive to ryanodine and nifedipine. Interestingly, RyR1 activation causes a very rapid increase in expression of MHC II molecules on the surface of dendritic cells, an effect which is also observed upon incubation of mouse BM12 dendritic cells with transgenic T cells whose T cell receptor is specific for the I-Abm12 protein. Based on the present results, we suggest that activation of the RyR1 signaling cascade may be important in the early stages of infection, providing the immune system with a rapid mechanism to initiate an early response, facilitating the presentation of antigens to T cells by dendritic cells prior to their full maturation.

623-Pos Board B502
Understanding Interval-Force Relations in the Rat Ventricular Myocytes with a Computational Model
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We have developed a model of cardiac excitation-contraction (EC) coupling for the rat ventricular myocytes based upon the published experimental literature and our previous modeling work in the guinea pig ventricular myocyte. Due to the rapid rate of Ca2+ cycling in the rat (resulting from the high heart rate) there are fundamental differences from the guinea pig myocyte data which was modeled previously. This requires the reformulation of Ca2+-handling mechanisms. The rat has a shorter action potential with different morphology than the guinea pig. This is thought to be due to the properties of the potassium channels. We reformulate three potassium currents (Ito, Iss, IK1), which give strong characteristics of the cardiac AP in rat ventricular myocytes (no plateau phase and shorter APD) and the Na+ current, based on the experimental literature. This new formulation simulates the negative force-frequency relation observed in experiments on rat ventricular myocytes with the appropriate changes in action potential duration and different pacing frequencies. The SR Ca2+ load decreases with increased frequency in contrast to the guinea pig which does the opposite and has a dome shaped force-frequency relation. Furthemore, the action potential duration increases with pacing rate opposite the guinea pig. The new rat model also simulated mechanical restitution and low levels post-extrasystolic potentiation levels seen in experiments with rat.

624-Pos Board B503
Ca++T LAB - A Computational Model for Intracellular Calcium Signaling In T Lymphocytes
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Calcium signaling is crucial for T cell activation. T cell receptor activation leads to the down-stream generation of IP3 which opens IP3 receptors on the membrane of the endoplasmic reticulum (ER), resulting in an initial small rise in cytoplasmic Ca^{2+}. T cells do not have sufficiently large Ca^{2+} stores to sustain Ca^{2+} elevation and therefore require additional Ca^{2+} influx through the voltage-independent Ca^{2+} channel CRAC, which is encoded by the ORAI1 or CRACM1 gene and is activated by STIM1, which ''senses'' decrease in ER Ca^{2+} content. However, CRAC can only bring in Ca^{2+} at negative membrane potentials. This potential is generated by the potassium channels Kv1.3 and KCa3.1 which drive the membrane potential back to -60 mV and facilitate Ca^{2+} entry for the duration of T cell activation ultimately leading to cytokine production and T cell proliferation. While there have been tremendous strides acquiring biophysical data on the implicated ion channels this data has not been integrated into a dynamic model. A computer model has been developed that integrates the available data on calcium signaling in T lymphocytes and that can be utilized to illustrate and theoretically probe T cell activation. The design is implemented in LabVIEW and can be run on a PC. The program allows free reign over the cell's environment and channel characteristics. The interface provides visualization of IP3 production, membrane potential, charge flow and open probability of the aforementioned channels. We suggest this program as a tool for identifying pharmaceutical targets for intervening with immune cell activation and as a teaching aid for immunology, physiology and cell signaling.

Actin & Actin-binding Proteins

625-Pos Board B504
Actin Branching Is Affected by Local Bending of the Mother Filament
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Actin filaments serve as structural elements of the cytoskeleton subject to mechanical forces and provide binding sites for actin-binding proteins (ABPs). Structural studies have established that actin filaments can adopt several different twist structures that are stabilized by ABPs such as ADF/cofilin or scruin. We sought to investigate whether strain on the actin filament due to bending affects the binding or function of ABPs and chose the Arp2/3 complex, which is involved in the assembly of force-generating actin networks, as our initial target. The Arp2/3 complex nucleates the growth of actin branches from pre-existing filaments, making it easy to detect where on the mother filament it has bound. To test the effect of bending on branching, biotinylated and rhodamine-phalloidin-stabilized actin filaments were bound to a streptavidin-coated glass surface, immobilized in a distribution of bent conformations. These tethered mother filaments were then incubated with actin, Arp2/3, and its activator. Growth of branches was stabilized with green fluorescent phalloidin. Branches originating from highly curved sections (radius of curvature < 1.5um) of actin filaments were more likely to grow from the extended side of the filament (positive strain) than from the compressed side (negative strain), with a statistically significant ($P < 0.05$) difference. To elucidate structural distortions that may give rise to this effect, we used Monte Carlo simulations based on a coarse-grained model of the actin filament to estimate the changes in inter-monomer spacing that occur for the experimentally probed range of filament curvatures. We conclude that mother filament bend strain is sufficient to alter actin branching and may play an important role in the organization of actin networks growing under load. Similar bend-induced effects may be important for other ABPs and provide a mechanism for mechanotransduction in cells.

626-Pos Board B505
Mechanics of Biophysical Networks with Flexible Cross-links
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Various mechanical properties and functions of eucharistic cells largely originate from the cytoskeleton. The predominant cytoskeletal constituent is the biopolymer filamentous actin (F-actin). In the presence of various cross-linking proteins, F-actins can comprise two rather different structures: isotropic orthogonal networks or bundled fibers. Actin bundles are formed mostly by short and stiff cross-linking proteins (like α-actinin and scruin), while large and flexible cross-linkers, such as filamin, lead to an orthogonal network. Orthogonal networks can also be formed at lower concentrations of short cross-linking proteins, but rheological experiments of in vitro F-actin networks showed that the mechanical response of such networks is different from that of networks cross-linked with filamin. Moreover, atomic force microscope stretching experiments on filamin demonstrated the possibility of force-induced domain unfolding, characterized by a sawtooth-like pattern in the force-displacement curve. Here we present a 3D discrete model of F-actin networks that extends our previous, rigidly cross-linked network model by incorporating a flexible cross-linking model for human filamin A (hsFLNa). The implemented hsFLNa element has a highly nonlinear response to stretching, incorporating the transition to a softer response that characterizes filamin domain unfolding. Simple shear