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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Ryanodine receptor 1 signaling in dendritic cells
Mirko Vukcevic[1], Giulio C. Spagnoli[1], Giandomenica Iezzi[1], Francesco Zorzato[2], **Susan Treves[1]**.
[1]Basel University Hospital, Basel, Switzerland, [2]University of Ferrara, Ferrara, Italy.
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.

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Understanding Interval-Force Relations in the Rat Ventricular Myocytes with a Computational Model
Ryosuke Kadoi[1], Sameer Bajikar[1], **Mohsin S. Jafri[1,2]**.
[1]George Mason University, Manassas, VA, USA, [2]University of Maryland Biotechnology Institute, Baltimore, MD, USA.
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.

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Ca++T LAB - A Computational Model for Intracellular Calcium Signaling In T Lymphocytes
Rayhan Lal, Jose L. Puglisi, Heike Wulff, Donald M. Bers.
U.C. Davis, Davis, CA, USA.
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

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Actin Branching Is Affected by Local Bending of the Mother Filament
Viviana Risca[1], Ovijit Chaudhuri[1], JiaJun Chia[2], Daniel A. Fletcher[1].
[1]University of California, Berkeley, Berkeley, CA, USA, [2]The Harker School, San Jose, CA, USA.
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
Goran Zagar, Patrick R. Onck, Erik Van der Giessen.
University of Groningen, Groningen, Netherlands.
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