pattern can be enhanced by exposure to agonist concentrations (1-5 μM) of ryanodine in OT terminals only. RyR antagonists, 8-Br-cADPR or higher concentrations (>10 μM) of ryanodine had the opposite effect; significantly reducing the amount of OT associated with the membrane area.
Additionally, Ca^{2+}-evoked NP release from permeabilized terminals was increased by agonist concentrations of ryanodine and conversely, decreased by antagonist concentrations of this drug. Agonist concentrations of ryanodine were also able to increase the asynchronous phase of low frequency electrically stimulated capacitance increases from isolated NH terminals. Thus, the ryanodine-sensitive mobilization of secretory granules seems to have a functional role in modulating secretion of neuropeptides from NH terminals. [Supported by UMass Grant P60037094900000 (SOM) and NIH Grant NS29470 (JRL)]

1583-Pos
Reactive Cysteines of Ryanodine Receptor Type 1 Influence Function and Response to Oxidative Stress
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Redox modulation of the skeletal muscle ryanodine receptor1 (RyR1) plays a key role in determining the responsiveness of the Ca^{2+} release channel to physiological modulation. The sensitivity of RyR1 to redox stress may be conferred by seven previously identified hyper-reactive cysteines (1040, 1303, 2426, 2606, 2611, 2625 and 3635). Wild type RyR1 ($_{wt}$RyR1), and seven hyper-reactive cysteine mutations of RyR1 were stably expressed in HEK-293 cells and their contribution to RyR1 function evaluated. Addition of RyR1 activator 4-chloro-*m*-cresol (4-CMC) elicited an increase in $[Ca^{2+}]_i$ in the $_{wt}$RyR1cells but failed to produce a Ca^{2+} response in the C1303S, C2606S, C2436S and C7S (expressing all seven cysteine mutations) expressing cells, while the C1040S and C2611S mutations significantly attenuated 4-CMC mediated Ca^{2+} response. Microsomal fractions isolated from C1040S, C2611S, C2436S and C3635S bound to ^{3}[H]Ry while C7S and C1303S showed significantly lower, levels of RyR-binding, although significantly above preparations from RyR-null HEK 293. The sensitivity of RyR1 to 1, 4-napthoquinone (NQ) appears to depend on the expression of RyR1 and the presence of reactive cysteines. Sensitivity to NQ-induced cytotoxicity was determined by the multiTox fluorescence assay. NQ decreased cell viability in a dose-dependent manner, but the $_{wt}$RyR1 cells were less sensitive than C2606, C1040S, C2611S and the C7S mutants. These data indicate the role of hyper-reactive cysteines in regulating RyR1 function and its response to oxidative stress. Supported by NIH AR43140.

1584-Pos
Triclosan Uncouples Excitation-Contraction Coupling in Skeletal Myotubes Without Blocking RyR1
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The chlorinated diphenylethers are a class of broad-spectrum antimicrobial agents. One of the most potent and widely used member of this group is triclosan (TCS; 2,4,4′-trichloro-2′-hydroxydiphenylether). We studied the effects of TCS in primary myotube cultures using Ca2+ imaging with Fluo 4 and whole-cell voltage clamp. Acute perfusion with 10 mM TCS resulted in a significant but transient elevation in cytosolic Ca2+ in unstimulated (resting) myotubes, an effect not seen in RyR null (dyspedic) cells. TCS caused a rapid decline in the amplitude of electrically evoked Ca2+ transients culminating in complete loss of Ca2+ transients. Upon failure of excitation-contraction (EC) coupling, RyR1 remained responsive to application of caffeine (20mM). Caffeine-induced release of SR Ca2+ in the presence of TCS was comparable to, or greater than, that measured in the control period indicating that the release channels remained functional and the SR stores were replete with prolonged TCS exposure. Acute submicromolar TCS (0.5 μM) enhanced Ca2+ transient amplitude at 0.1 Hz stimulus, whereas pre-incubation of myotubes with TCS for 24 hr was sufficient to alter the relationship between stimulus frequency and Ca2+ transient amplitude across the entire stimulation frequency range. TCS (10 μM) also completely inhibited depolarization-triggered extracellular Ca2+ entry and suppressed DHPR mediated Ca2+ current to that observed in dyspedic cells. These ucoupling effects were observed without any influence on the magnitude of store-operated Ca2+ entry (SOCE) in myotubes. These results are the first to identify that TCS (and possibly related structures) impairs EC coupling by uncoupling orthograde and retrograde signaling between RyR1 and DHPR in skeletal muscle. Supported by NIH AR055104 (K.G.B.), AR43140 and ES011269 (I.N.P.), and MDA4319 (K.G.B.) and MDA4155 to (R.A.B.).

Motions of the Cell Surface Molecules

1585-Pos
Method for In-Vitro Studies of Cellular Interactions at the Interface of Two Tissues
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We describe a simple and reliable experimental technique that enables one to create a high fidelity linear interface between two opposing cell layers. The method employs a custom designed lid that fits a standard 3cm cell culture dish. During cell plating, the dish is divided by a 200 micron thick separator that is part of the lid. The separator is covered in a thin layer of parafilm that forms a hermetic seal with the underlying coverslip and creates a temporary gap between the two cell plating environments. After cells attach, the custom lid is replaced with a standard lid and cells are allowed to grow under standard cell culture conditions. When expanding cell layers fill the gap, a linear interface is formed between the two opposing fields. Paracrinal factors released from an approaching cell front as well as direct physical and molecular interactions between two cell types affect intercellular orientation, individual cell morphology, and the degree of cells invasion into the opposing layer. The local interface appearance thus depends on a specific cell pair and may vary dramatically. We describe several types of such interfaces for different cell pairs, including cardiomyocytes, fibroblasts, melanocytes, endothelial cells and colon carcinoma cell lines. The method serves as a practical in vitro tool to study cell growth and invasion that occur on the interface of two neighboring tissues.

1586-Pos
A Zoo of Dynamic Pattern Formation by Bacterial Cell Division Proteins
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Min proteins of the *Escherichia coli* cell division system oscillate between the cell poles *in vivo. In vitro* on a solid-surface supported lipid bi-layer these proteins exhibit a number of interconverting modes of collective ATP-driven dynamic pattern formation including not only the previously described propagating waves, but also near uniform in space surface concentration oscillation, propagating filament like structures with a leading head and decaying tail, and moving and dividing amoeba-like structures with sharp edges. We demonstrate that the last behavior most closely resembles *in vivo* system behavior. The simple reaction-diffusion models previously proposed for the Min system fail to explain the results of *in vitro* self-organization experiments. We propose hypotheses that initiation of MinD binding to the surface is controlled by counteraction of initiation and dissociation complexes; the binding of MinD is stimulated by MinE and involves polymerization-depolymerization dynamics; polymerization of MinE over MinD oligomers triggers dynamic instability leading to detachment from membrane.

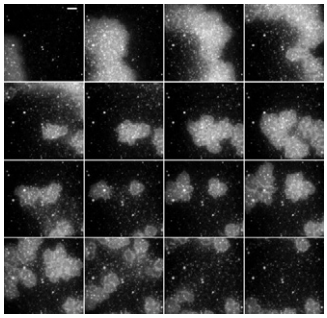

Fig. 1. MinD (green) and MinE (red) wave transforms into running and dividing amoebas. Frames were taken every 40 s, the scale bar is 5 μm.

1587-Pos
Active Re-Modelling of Cortical Actin Regulates Spatiotemporal Organization of Molecules on a Living Cell Surface
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Cell surface proteins such as lipid-tethered GPI-anchored proteins, Ras-proteins and several glycoproteins, are distributed as monomers and nanoclusters on the surface of living cells. The spatial distribution and dynamics of formation and breakup of these nanoclusters is unusual and controlled by the active remodeling dynamics of the underlying cortical actin (CA). To explain these results, we propose a novel mechanism of nanoclustering, based on the active hydrodynamics of the CA and its coupling to local membrane composition. In addition, our theory makes a falsifiable prediction – GPI-APs must exhibit anomalous concentration fluctuations resembling those at criticality; we confirm this using a fluorescence-based assay. Our work addresses a central issue