

change in heart failure ($n = 5$, $P < 0.05$) but a $230 \pm 92\%$ increase with isoprenaline treatment ($n = 6$, $P < 0.05$). Basal levels of phosphorylation are thus relatively low at RyR Ser2030, phospholamban Ser16, and troponin I Ser23/24, and are not significantly increased in heart failure, but are substantially increased by isoprenaline treatment. In contrast, the phosphorylation level at Ser 2809 is already high and can be increased only moderately by isoprenaline.

1578-Pos

Inhibition of RyR2-S2814 Phosphorylation Prevents Heart Failure by Reducing SR Ca Leak

Ralph J. van Oort, Na Li, Jonathan L. Respress, Angela DeAlmeida, Xander H. Wehrens.

Baylor College of Medicine, Houston, TX, USA.

Abnormal regulation of RyR2 by Ca^{2+} /calmodulin-dependent protein kinase 2 (CaMKII) has been suggested as a cause of sarcoplasmic reticulum (SR) Ca^{2+} leakage and contractile dysfunction in heart failure. We hypothesized that CaMKII phosphorylation of RyR2 is crucial for heart failure development. We generated RyR2 knockin mice in which CaMKII phosphorylation site S2814 was mutated to alanine (S2814A) to prevent phosphorylation. Cardiac function and dimensions monitored by echocardiography were similar in S2814A and WT mice up to 12 months of age. WT ($n=13$) and S2814A mice ($n=9$) were subjected to transverse aortic constriction (TAC) to induce pressure overload. At 8 weeks after TAC, S2814A mice displayed a similar hypertrophic response and decrease in ejection fraction (EF) as WT mice. At 16 weeks after TAC, however, EF was significantly lower in WT ($32.5 \pm 3.4\%$) compared to S2814A mice ($43.0 \pm 2.9\%$) suggesting inhibition of heart failure development in the latter. This rescue effect was further verified by a lower lung-weight-to-tibia-length ratio and a decrease in expression levels of the cardiac stress genes ANF and BNP in S2814A mice compared to WT mice at 16 weeks after TAC. Ca^{2+} imaging in cardiomyocytes, isolated from S2814A and WT mice at 16 weeks after sham or TAC surgery, demonstrated an decreased incidence of spontaneous SR Ca^{2+} release (SCR) events in S2814A (29.7% of myocytes) compared to WT (61.3% of myocytes; $P < 0.01$). Whereas CaMKII inhibitor KN93 reduced the incidence of SCR events in WT to 36.1% ($P < 0.01$ vs. WT TAC), KN93 did not have an effect of SCR in S2814A myocytes (30.0%; $P = \text{NS}$ vs. S2814A TAC). Together, our results demonstrate that blocking CaMKII phosphorylation of RyR2 prevents SR Ca^{2+} leak, and inhibits or delays the progression to congestive heart failure in S2814A mice.

1579-Pos

Luminal Regulation of Single RyR2 Channels by Cardiac Calsequestrin

Marcia Cortes-Gutierrez¹, Heather R. Orrell¹, Simon Williams², Patricia Velez¹, Ariel L. Escobar¹.

¹UC Merced, Merced, CA, USA, ²TTUHSC, Lubbock, TX, USA.

We examined the hypothesis that calsequestrin (CSQ2) can regulate the RyR2 activity using a combination of single channel electrophysiology and lanthanide resonance energy transfer (LRET). Under steady-state, open probability (P_o) of RyR2 of cardiac SR fractions from dog is modulated by luminal $[\text{Ca}^{2+}]$. P_o increased when luminal $[\text{Ca}^{2+}]$ was increased from 6 μM to 2 mM at a fixed cytosolic $[\text{Ca}^{2+}]$ of 2 μM . This effect on RyR2 appears to be mediated by luminal sites. Interestingly, RyR2 P_o also increases when 2 mM Mg^{2+} was added to the luminal side. To gain mechanistic insights on the Casq2-mediated luminal regulation, we used the binding of Casq2 to Tb+3 as a functional assay. Luminescence produced by LRET between the tryptophan's of purified dog Casq2 and the lanthanide increased as function of the $[\text{Tb}+3]$. This fluorescence was reduced as $[\text{Ca}^{2+}]$ increased suggesting that Ca^{2+} binds to purified Casq2 by displacing tightly bound Tb+3 from a common binding site. To further explore the specificity of this regulation, we expressed recombinant dog Casq2 in *E. coli*. The specificity of this interaction was assessed by LRET lifetime measurements. Interestingly, we found that the displacement of Tb3+ by Ca^{2+} was not significantly different than the displacement of Tb3+ by Mg^{2+} (~ 2.5 mM) suggesting that Ca^{2+} and Mg^{2+} share a common binding site. Finally, we explore the hypothesis that Tb3+ bound to Casq2 was able to modulate the RR2 activity. At fixed cytosolic $[\text{Ca}^{2+}]$ of 2 μM , P_o of single RyR2 increased as a function of luminal $[\text{Tb}+3]$ ($\text{KD} \sim 500$ nM, Hill coeff. ~ 4). These results are consistent with the idea that a multimeric form of Casq2 acts as luminal divalent cation sensor and translates it into changes in RyR2 gating. Supported by NIH R01-HL-084487 to AE.

1580-Pos

Ca SR Leak is Modulated by CaMKII Phosphorylation on RyR2-S2814

Yi Yang¹, Laetitia Pereira¹, Ralph J. van Oort², Xander H.T. Wehrens², Donald M. Bers¹.

¹UC Davis, Davis, CA, USA, ²Baylor College of Medicine, Houston, TX, USA.

CaMKII has been shown to increase cardiac SR Ca leak through RyRs. RyR2-S2814 has been suggested as the phosphorylation site responsible for SR Ca leak triggering cardiac arrhythmias in heart failure. Here we test the requirement of S2814 for these effects, in knock-in mice expressing only RyR2-S2814A or -S2814D. Ca spark frequency (CaSpF) and SR load were studied in intact and permeabilized cardiomyocytes using confocal microscopy. At baseline CaSpF is higher in S2814D vs. WT (8.7 ± 0.4 ($n=9$) vs 6.44 ± 0.3 , $n=8$, $p < 0.01$) without altered SR Ca load. Activation of endogenous CaMKII (1.2 μM Ca -calmodulin) in WT increases CaSpF as described by Guo et al (Circ. Res. 2006), but only to the level seen in S2814D at baseline. Moreover, CaMKII did not further increase CaSpF in S2814D myocytes. In RyR2-S2814A CaMKII activation produces a very small CaSpF increase vs WT (19 vs 60%), and that response in S2814A is secondary to increased SR Ca load (by 15%, $n=10$, $p < 0.01$). In intact myocytes (as above), basal CaSpF was highest in S2814D vs. WT and S2814A (which were similar). Baseline Ca transients were not different among the groups. The cAMP activated GTP exchange factor Epac may activate CaMKII to induce SR Ca leak (Pereira et al, J Physiol, 2007). In WT mice the Epac activator 8-CPT enhanced CaSpF and decreased both SR Ca load and Ca transient amplitude (consistent with a primary effect on SR Ca leak). However, 8-CPT had no effect on any of these parameters in either S2814A or S2814D myocytes. These data indicate that RyR2-S2814 is the critical RyR2 site responsible for CaMKII-dependent enhancement of SR Ca leak and potential arrhythmogenesis in heart failure, and confirm that Epac signaling can work through this same pathway.

1581-Pos

RyR2 NH2-terminal Mutations Associated with Cardiomyopathies Reduce the Threshold for Ca^{2+} Release Termination

Yijun Tang.

Department of Physiology and Pharmacology, University of Calgary, CALGARY, AB, Canada.

Naturally occurring mutations in the cardiac Ca^{2+} release channel/ryanodine receptor (RyR2) have been linked not only to cardiac arrhythmias and sudden death, but also to cardiomyopathies. The causal mechanisms underlying RyR2-associated cardiomyopathies are unknown. We have previously shown that RyR2 mutations linked to catecholaminergic polymorphic ventricular tachycardia (CPVT) reduce the threshold for store overload induced Ca^{2+} release (SOICR), also known as spontaneous Ca^{2+} release during Ca^{2+} overload. To determine the impact of RyR2 mutations associated with cardiomyopathies, we generated stable, inducible HEK293 cells expressing the RyR2 wt and the exon-3 deletion, R420W, and L433P mutants, and monitored the luminal Ca^{2+} dynamics in these wt and mutant cells using the fluorescence resonance energy transfer (FRET)-based luminal Ca^{2+} sensing protein, D1ER. Consistent with other CPVT mutations, the exon-3 deletion, R420W, and L433P mutations reduce the SOICR threshold. Interestingly, we found that these mutations also lower the critical luminal Ca^{2+} level at which Ca^{2+} release is terminated (the termination threshold). To further assess the role of the NH2-terminal region of RyR2 in Ca^{2+} release termination, we deleted the first NH2-terminal 305 amino acid residues and found that this NH2-terminal deletion also lowers the termination threshold for Ca^{2+} release. Our data demonstrate that the NH2-terminal region of RyR2 plays an important role in Ca^{2+} release termination, and suggest that alterations in the termination of Ca^{2+} release via RyR2 may cause cardiomyopathies (Supported by NIH and CIHR).

1582-Pos

Modulation of Neurosecretory Granule Mobilization and Neuropeptide Release by Ryanodine Receptors in Neurohypophysial Terminals

Jose R. Lemos, Edward Custer, James McNally, Sonia Ortiz-Miranda.

Univ. of Massachusetts Medical School, Worcester, MA, USA.

The neuropeptides oxytocin (OT) and vasopressin (AVP) are contained in large dense core vesicles (LDCV) and are released at the neurohypophysis (NH). The mobilization and translocation of vesicles to exocytotic release sites is modulated by cytosolic Ca^{2+} . Intracellular structures and organelles able to store and release Ca^{2+} are significant contributors of cytosolic Ca^{2+} . The presence of ryanodine receptors (RyR) in LDCV of NH terminals, coupled with the demonstration that pharmacological activation of these receptors can induce spontaneous focal Ca^{2+} transients, make them ideal modulators of cytosolic levels of Ca^{2+} , and therefore, vesicle mobilization and subsequent neuropeptide (NP) release.

To test this hypothesis, the association of LDCV in an area within 0.45 μm of the plasma membrane was assessed using immunolabeling of Neurophysin I (OT) and II (AVP) along with high stringency deconvolution techniques in isolated NH terminals. We found that the total amount of membrane associated NP-immunoreactivity varies significantly between terminal type; significantly higher in OT than in AVP terminals. This membrane associated distribution

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

Diptiman D. Bose¹, Genaro C. Barrientos¹, Benjamin T. Yuen¹, Stevie Maxwell¹, Claudio F. Perez², Paul D. Allen², Isaac N. Pessah¹.

¹University of California, Davis, Davis, CA, USA, ²Brigham and Women's Hospital, Boston, MA, USA.

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 (w_t 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 $[\text{Ca}^{2+}]_i$ in the w_t RyR1 cells 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 ^3H 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-naphthoquinone (NQ) appears to depend on the expression of RyR1 and the presence of reactive cysteines. Sensitivity to NQ-induced cytotoxicity was determined by the multi-Tox fluorescence assay. NQ decreased cell viability in a dose-dependent manner, but the w_t 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

Gennady Cherednichenko¹, Roger A. Bannister², Kurt G. Beam², Isaac N. Pessah¹.

¹Department of Molecular Biosciences, University of California, Davis, CA, USA, ²Department of Physiology and Biophysics, University of Colorado, Denver, CO, USA.

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 Ca^{2+} imaging with Fluo 4 and whole-cell voltage clamp. Acute perfusion with 10 mM TCS resulted in a significant but transient elevation in cytosolic Ca^{2+} in unstimulated (resting) myotubes, an effect not seen in RyR null (dyspedic) cells. TCS caused a rapid decline in the amplitude of electrically evoked Ca^{2+} transients culminating in complete loss of Ca^{2+} transients. Upon failure of excitation-contraction (EC) coupling, RyR1 remained responsive to application of caffeine (20mM). Caffeine-induced release of SR Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} transient amplitude across the entire stimulation frequency range. TCS (10 μM) also completely inhibited depolarization-triggered extracellular Ca^{2+} entry and suppressed DHPR mediated Ca^{2+} current to that observed in dyspedic cells. These uncoupling effects were observed without any influence on the magnitude of store-operated Ca^{2+} 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

Ara Arutunyan, Zaruhi Karabekian, Nikki Gillum-Posnack, **Narine Sarvazyan**.

The George Washington University, Washington, DC, USA.

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. Paracrine 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

Vassili Ivanov, Kiyoshi Mizuuchi.

NIDDK, NIH, Bethesda, MD, USA.

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.

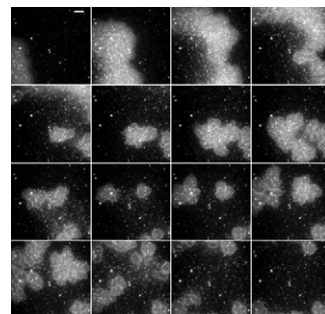


Fig. 1. MinD (green) and MinE (red) wave transforms into running and dividing amoebas. Frames were taken every 40 s, the scale bar is 5 μm .

1587-Pos

Active Re-Modelling of Cortical Actin Regulates Spatiotemporal Organization of Molecules on a Living Cell Surface

Kripa Gowrishankar.

Raman Research Institute, Bangalore, India.

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