Consequently, we investigate the role of kinases/phosphatases and cytoskeleton modulators (colchicine and cytochalasin D) on RyR-mediated Ca^{2+} leak from SR microsomes as well as on coupled RyR1. We also investigated role of FKBP12 in coupled RyR1 gating by adding FKBP12 to partially coupled RyR1 or rapamycin to coupled RyR1 in planar lipid bilayers. Our results suggest the RyR1-RyR1 interactions that are essential for coupled gating were not significantly affected by addition of kinases/phosphatases, cytoskeleton modulators or the addition/removal of FKBP12 by rapamycin. Yet, some of the agents affected the overall activity of the RyR1. (Supported by NIH R01 GM078665).

2251-Pos Board B237

CGP37157 is an Inhibitor of the Sarcoplasmic Reticulum Calcium ATPase and an Activator of Ryanodine Receptors in Striated Muscle

Jake T. Neumann, Paula L. Diaz-Sylvester, Sidney Fleischer, Julio A. Copello.

CGP-37157 (CGP), a benzothiazepine derivative of clonazepam, is commonly utilized as a blocker of the mitochondrial Na^+/Ca^{2+} exchanger. Yet, evidence suggests that CGP could also affect other targets, such as L-type Ca^{2+} channels and plasmalemma Na^+/Ca^{2+} exchanger. Here, we tested the possibility of a direct modulation of ryanodine receptors (RyR) and/or sarcoplasmic reticulum (SR) Ca^{2+} stimulated ATPase (SERCA) by CGP. SERCA activity was measured in SR microsomes with a ATP-ase activity assay or Ca^{2+} loading in presence of ruthenium red (RyR blocker). The effects of CGP on RyR activity were performed in SR microsomes with a Ca^{2+} leak assay or after reconstitution of the channels into planar lipid bilayers. CGP inhibited SERCA-mediated Ca^{2+} uptake of cardiac and skeletal SR microsomes (IC_{50}'s = 6.6 and 9.9 μM, respectively). The CGP effects on SERCA activity correlated with a decreased V_{max} of ATPase activity of SERCA-enriched skeletal SR fractions without an apparent change in K_m. CGP ($\geq$ 5 μM) also increased RyR-mediated Ca^{2+} leak from skeletal SR microsomes. Planar bilayer studies confirmed that both cardiac and skeletal RyR are directly activated by CGP (EC_{50}'s = 9.4 and 12.0 μM, respectively). In summary, we found that CPG inhibits SERCA and activates RyR channels. Hence, the action of CGP on cellular Ca^{2+} homeostasis reported in the literature of cardiac, skeletal muscle as well as other non muscle systems requires further analysis to take into account the contribution of all CGP-sensitive Ca^{2+} transporters. (Supported by NIH R01 GM078665).

2252-Pos Board B238

Tetracaine is a Potent Inhibitor of SR Ca Leak in Ventricular Cardiac Myocytes

Stephen Shonts.

Tetracaine is commonly used to inhibit RyR-dependent SR Ca leak, however ability to do so in intact cardiac myocytes has not been fully characterized. In single RyR channel bilayer a significant portion of leak would not be inhibited even by low millimolar concentrations of tetracaine. In this study we investigated the effectiveness of tetracaine on RYR-dependent SR Ca leak in ventricular myocytes. Experiments were performed in isolated rabbit myocytes loaded with fluo-4. Myocytes were perfused with 2mM Ca NT with 200μM caffeine in order to increase basal SR Ca leak in the presence of varying concentrations of tetracaine (1, 10, 30, 100μM). Myocytes were then rapidly perfused with 0 Na, 0 Ca NT with 1mM tetracaine following washout with same solution but with varying amounts of tetracaine. SR Ca leak was quantified as the shift of Ca from the cytosol to the SR upon blockage of the RyR with high tetracaine. The EC_{50} for tetracaine is significantly reduced in intact myocytes compared to single RyR channel. Among the potential explanations for this effect is the possibility that Ca flux regulation makes the RyR clusters in the SR junction more sensitive to blockage of release.

2253-Pos Board B239

Tetracaine Inhibits RyRs by Three Mechanisms

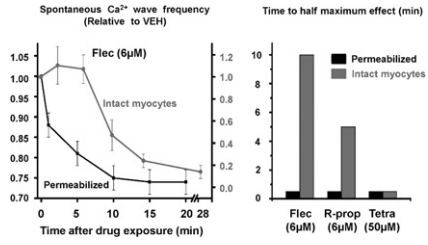
Derek R. Laver, Dirk F. vanHelden.

Tetracaine is a tertiary amine local anaesthetic (pKa = 8.5) which induces closures in RyRs. Tetracaine has been extensively used to study the role of the SR Ca^{2+} fluxes in Ca^{2+} handling in muscle cells. We use single channel recording of rabbit skeletal and sheep cardiac RyRs in lipid bilayers to identify three mechanisms for tetracaine inhibition. First, luminal and cytoplasmic tetracaine induce closed events (~100 ms- slow mechanism). Cytoplasmic/luminal competition indicates a common site for cytoplasmic and luminal action. The binding rate decreases with increasing RyR open probability, consistent with a closed state mechanism with no detectable binding to open channels. At pH 7.4, its voltage-dependence points to a cation binding site within the bilayer, near its luminal interface. At pH 9.5, the slow mechanism is independent of voltage, indicating that it also responds to neutral tetracaine molecules. Second, cytoplasmic and luminal tetracaine induce brief closures (~1 ms- fast mechanism). It is insensitive to the open state of the RyR, independent of voltage and markedly diminished at pH 9.5 indicating that it is caused exclusively by tetracaine cations binding outside the transmembrane electric field. The action of trans-membrane pH gradients on the fast mechanism points to a cytoplasmic location for the binding site. Finally, cytoplasmic tetracaine reduces the ionic conductance of the channel at mM concentrations. We predict that under diastolic conditions, the slow mechanism (IC_{50} of ~200 μM) is a more potent form of block than the fast mechanism or conduction block (IC_{50} ~ 2mM) and these mechanisms can explain the differences in the tetracaine inhibition seen at low and high concentrations.

2254-Pos Board B240

Slow Diffusion Across Cell Membrane Delays Onset of RyR2-Channel Block and Ca Wave Suppression by Flecainide in Intact Myocytes

Hyun Seok Hwang, Eleonora S. Galimberti, Bjorn C. Knollmann.



RyR2 channel inhibitors such as flecainide suppress Ca waves in myocytes and effectively prevent ventricular arrhythmias in vivo. Since the RyR2 channel is located in an intracellular organelle, the sarcoplasmic reticulum, differences in diffusion rates across the cell membrane and gaining access to the RyR2 may contribute to variability of drug action in intact myocytes and in vivo. To test this hypothesis, we compared three RyR2 channel inhibitors to suppress Ca waves in permeabilized and intact myocytes: flecainide (Flec:6μM), R-propafenone (R-prop:6μM) and tetracaine (Tetra:50μM). All three drugs exhibited rapid onset of action in permeabilized myocytes (**Figure**). In intact myocytes, only Tetra exhibited rapid onset of action, whereas Flec and R-prop exhibited significant lag times of 10 min and 5 min, respectively (**Figure**). Flec's slow onset of action in intact myocytes occurred even though its potency was higher intact (IC_{50}=2μM) than permeabilized myocytes (IC_{50}=12μM).

Conclusion: Slow diffusion of RyR2 channel blockers such as flecainide across intact membranes importantly determines the onset of Ca wave suppression, which has to be taken into account when evaluating drug efficacy in intact myoyctes and in vivo.

2255-Pos Board B241

Inhibition of Cardiac Ca^{2+} Release Channels by Class I Anti Arrhythmic Drugs as Therapy for Arrhythmia

Divya R. Mehra, Dirk F. vanHelden, Bjorn C. Knollmann, Derek R. Laver.

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an arrhythmia resulting from mutations in either the cardiac Ca^{2+} release channel (RyR2) or calsequestrin. Flecainide is a class-Ic compound that is highly effective in suppressing arrhythmia in CPVT patients and a calsequestrin-null mouse model of CPVT. Its efficacy is a combination of reduced membrane excitability via its known Na^+ channel block and stabilisation of SR Ca^{2+} release by a recently discovered RyR2 block. Here we report RyR2 blocking kinetics of other class I anti arrhythmic drugs.

RyR2 was isolated from sheep and human hearts, incorporated in lipid bilayers and investigated by single-channel recordings in presence of diastolic Ca^{2+} (100 nM cytoplasmic, 0.1 mM luminal). The class 1c drugs, propafenone, flecainide and encainide decreased RyR open times (IC_{50} = 17.3 ± 1.6, 16.7 ± 4 and 22.5 ± 1.2 ìM respectively) whereas classes Ia and Ib had no significant effect up to 50 μM.

Blocking kinetics was also measured in fully activated RyR2 (1 mM Ca^{2+}, 2 mM ATP). All class I drugs tested caused voltage-dependent induction of subconductance states with similar actions from luminal and cytoplasmic sides. However, class Ic drugs produced subconductances with longer duration and lower conductance than classes 1a and 1b. These differences may explain the different efficacy of these drugs to curb Ca^{2+} release in diastole and prevent arrhythmia.

Our results suggest that the efficacy of class I drugs in preventing CPVT depends on a reduction in RyR2 open time with a short blocking duration; kinetics typical of low affinity binding. This may lead to a paradigm shift in drug development process by directing strategies away from discovering high affinity compounds.

2256-Pos Board B242

Designing New Ca2+ Release Channel Inhibitors Based on Enhanced Electron Donor Characteristics

Yanping Ye, Daniel Yaeger, Laura J. Owen, Jorge O. Escobedo, Jialu Wang, Robert M. Strongin, **Jonathan J. Abramson**.

New drugs with enhanced electron donor properties that target the Ca^{2+} release channel (CRC) from sarcoplasmic reticulum are potent inhibitors of single channel activity. In this study we synthesize derivatives of the channel activator 4-chloro-3 methyl phenol (4-CmC) and the 1,4-benzothiazepine channel inhibitor K201 (JTV519) with enhanced electron donor properties. Instead of activating channel activity (~100 uM), 4-CmC's 4-methoxy analog (4-methoxy-3-methyl phenol) inhibits channel activity at sub-micromolar concentrations (IC_{50} = 0.34 ± 0.08 uM). Increasing the electron donor characteristics of K201, by synthesizing its dioxole congener, results in a new compound which is 16 times more potent an inhibitor of single channel activity (0.24 ± 0.05 uM) than K201(3.98 ± 0.79 uM). These alterations to chemical structure do not affect Ca^{2+} dependent ATPase activity of SERCA1. Both K201 and its dioxole derivate show a similar potency toward inhibiting ATPase activity (IC_{50} ~50 uM), while 4-methoxy-3-methyl phenol does not inhibit ATPase activity at concentrations up to 1 mM. Moreover, the FKBP12 protein, which stabilizes RyR1 in a closed configuration, is shown to be a strong electron donor. It appears as if FKBP12, K201, its dioxole derivative, and 4-methoxy-3-methyl phenol inhibit the skeletal muscle SR CRC channel activity by virtue of their electron donor characteristics. We also show that the inhibitory action of K201 is independent of FKBP12 binding to RyR1. These results embody strong evidence that designing new drugs that target the CRC with enhanced electron donor characteristics results in more potent channel inhibitors. This represents a novel approach toward designing new more potent drugs aimed at functionally modifying the CRC from sarcoplasmic reticulum. Supported by PSU Faculty Development Award, University Venture Development Fund, ONAMI, and NIH (R01 AR48911) to JJA.

2257-Pos Board B243

Altered Ca2+ Sensitivity and Gating Properties of Skeletal Muscle Ryanodine Receptors in Aged Mice

Albano C. Meli, Steve Reiken, Ran Zalk, Daniel C. Andersson, Matthew J. Betzenhauser, Andrew R. Marks.

Calcium (Ca2+) release channel/ryanodine receptor 1 (RyR1) plays a fundamental role in the transient increase of cytosolic free Ca2+ upon depolarization of transverse tubules in skeletal fast-switch muscles.

Sarcopenia can be defined as the age-related loss of muscle mass, strength and function. Using an animal model of sarcopenia, we found that fast-switch muscle RyR1 from aged mice (24 months) compared to younger mice (3-6 months) was oxidized, cysteine-nitrosylated, and depleted of the channel stabilizing subunit FK506 binding protein (FKBP12 or calstabin1). Such modifications of the RyR1 macromolecular complex are known to impair RyR1 channel activity.

Here, we looked at the single-channel properties of RyR1 in young and aged mice using RyR1 agonists (calcium, ATP, caffeine). Age-related changes in RyR1 Ca2+ sensitivity were manifested by an increased open probability (Po), particularly at low activating calcium concentration (e.g., 150 nM). Increased Po was associated with an increased opening frequency (Fo) while the mean open-time (To) was unchanged. The concentration-response curve for calcium dependent activation for RyR1 from aged muscle was left shifted. These data suggest that RyR1 may be ''leaky'' in skeletal muscle from aged mice.

Membrane Receptors & Signal Transduction II

2258-Pos Board B244

Investigating the Functional Dynamics of Bacterial Chemoreceptors Using Hydrogen Exchange Mass Spectrometry

Seena S. Koshy, Stephen J. Eyles, Robert M. Weis, Lynmarie K. Thompson.

Bacterial chemotaxis is an ideal system to study the underlying mechanisms involved in transmembrane signaling and signal processing. Bacteria such as E. coli sense chemicals through chemoreceptors, and transmit information from the periplasmic space to the cytosol, to ultimately control the swimming direction of the cell. Chemoreceptors function as large multimeric complexes that also contain a histidine kinase (CheA) and an adaptor protein (CheW). Previously, we and others have shown that the cytoplasmic domain of the receptor is highly dynamic, and small changes in a few amino acids can dramatically stabilize this domain. To investigate whether modulation of cytoplasmic domain dynamics plays a role in the signaling mechanism, we have developed a mass spectrometry method to measure hydrogen exchange of the cytoplasmic domain in active, membrane-bound complexes with CheA and CheW. Our global dynamics data clearly shows that cytoplasmic domain dynamics are significantly reduced in active complexes relative to the non-functional solution state. Current efforts to optimize pepsin digest conditions will enable us to determine whether local dynamics change with signaling state, to provide insight into the role of dynamics in the transmembrane signaling mechanism.

This research supported by GM 47601, GM085288, and a Fellowship to Seena Koshy from the University of Massachusetts as part of the Chemistry-Biology Interface Training Program (NRSA T32 GM08515).

2259-Pos Board B245

Conformational Changes during Kinase On-Off Switching in the Chemosensory Signaling Array of the Bacterial Cell Membrane: Detection by One-Sample FRET

Annette H. Erbse, Adam J. Berlinberg, **Joseph J. Falke**.

Fluorescence resonance energy transfer (FRET) is a powerful tool to study macromolecular assemblies *in vitro* under near physiological conditions. One Sample FRET (OS-FRET) employs a novel, non-fluorescent methanethiosulfonate-linked acceptor that can be reversibly coupled to a target protein Cys residue (Erbse *et al*, submitted). The design of OS-FRET provides distinct advantages over existing methods for quantitative FRET measurements in virtually any fluorimeter or detection device. We previously demonstrated the utility of the method by applying it to a soluble complex formed by the CheA and CheW proteins of the bacterial chemosensory pathway. Here, OS-FRET is applied to the functional, membrane-bound bacterial chemosensory signaling array. OS-FRET reveals that attractant binding to transmembrane chemoreceptors triggers large, relative domain motions in the CheA kinase proteins of the signaling array. These findings provide the first molecular view of the structural changes underlying receptor-mediated kinase on-off switching in bacterial chemosensing.

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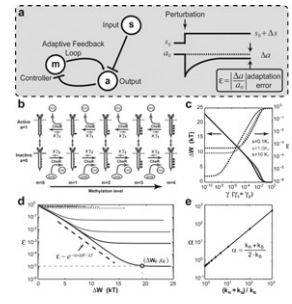
2260-Pos Board B246

The Energy Cost of Sensory Adaptation

Ganhui Lan, Pablo Sartori, Yuhai Tu.

Biological sensory systems adapt to prolonged stimuli in order to maintain high sensitivity in different environments. Sensory adaptations are carried out by various molecular feedback mechanisms. Here, we show that all adaptation dynamics are dissipative and feedback control consumes energy to achieve high adaptation accuracy against intrinsic fluctuations in the underlying molecular signaling pathways. A universal relation among energy dissipation rate, adaptation time, and the optimum adaptation accuracy is established in a general continuum model and for the specific case of adaptation in E. coli chemotaxis. Our study finds that sensory adaptations are fueled by high-energy biomolecules (e.g., ATP), which provide the energy necessary in stabilizing the adapted state. For E. coli chemotaxis, hydrolysis of S-adenosylmethionine (SAM) drives the chemo-receptor adaptation, and the high energy content in SAM is crucial in maintaining the near perfect adaptation of the system. Finally, we point out that the energy-accuracy relation found here has deep connections with the energy dissipation required for molecular level error-correction and information processing.



2261-Pos Board B247

Increases in Amplitude and Frequency of Ciliary Beating by a β2-Agonist, Procaterol, in Small Airways of Mice

Takashi Nakahari.

The beat frequency (CBF) and the beat angle (CBA) of mice small airway cilia were measured using a light microscope equipped with a high-speed camera (500 Hz). This study demonstrated that a β2-agonist, procaterol, increases not only CBF but also CBA in small airways and that an increase in CBA enhances the rate of mucociliary transport. A low concentration of procaterol (1-10 pM) increased CBA by ~30%, but not CBF, whereas a high concentration (10 nM) increased both CBA and CBF by ~100%. Procaterol actions were mimicked by forskolin (FK) and isobutyl-methylxanthine (IBMX) and