of interest, we demonstrated a straightforward transfer to Rap1B. As understanding the precise functions of closely related family members is a current frontier in Ras research, this specific control over the activity of a given member is of particular interest. More importantly, successful sensitization of a different G protein to the compounds controlling the activity of the previously engineered H-Ras demonstrates the potential breadth of application of this approach.

2120-Symp
Protein Structure Prediction by Golbal Optimization and its Applications
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One of the fundamental goals of modern sciences is to understand the nature of life, and deciphering the protein structure and its working mechanism lies at the very heart of this agenda. Due to the tremendous success of many genome projects, the number of available protein sequences reached over 5.3 million as of 2007, but less than 1% of these protein structures are known. Reliable and accurate protein structure prediction using only the sequence information is greatly in demand, but it remains as an unsolved problem even after many years of efforts. We intend to establish a successful protein modeling method that is solely based on direct application of principles excluding human interference in modeling steps. This should be contrasted to the common conception in the field that human expertise accumulated by many years of protein modeling is the most important asset for accurate protein structure prediction. In this talk we will discuss recent progresses of our efforts in protein structure prediction. It appears that our newly proposed method, which is based on the direct and rigorous optimization of relevant score functions, can provide significantly improvement for 3D modeling of proteins in the category of High-Accuracy Template-Based Modeling. Applications of highly accurate proteins 3D models to various biological systems will be discussed.

2121-Symp
Finding Small Molecule Ligands for Protein-Protein Interactions and Other "undruggable" Targets
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The central tenant of chemical biology and small-molecule drug discovery is that biology can be manipulated using small, organic compounds. Nevertheless, the known drugs act on only ~1% of the proteome, and the realm of undrugged targets is vast. Protein complexes occupy much of this realm, yet are widely considered ''undruggable'' or, at best, ''challenging.'' Thus, there is an opportunity to greatly expand the range of chemical tools and drugs if we can identify which protein-protein interactions are most amenable to small-molecule interference, and what small-molecule discovery approaches are most likely to yield potent and selective modulators. This presentation will describe some of the outstanding issues and promising advances in tackling protein-protein interactions. For example, we note that many protein interfaces are structurally adaptive, and therefore could have low-energy conformations that are amenable to binding small ligands. Additionally, many enzymes are allosterically regulated by protein complexation, and these protein-protein interfaces are also targets for unconventional enzyme inhibitors.

Platform AC: Cardiac Electrophysiology

2122-Plat
Diverse Effects of a Familial Atrial Fibrillation (FAF)-Related KCNE2 Mutation, R27C, on Cardiac Voltage-Gated Potassium (Kv) Channels
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Background: KCNE2 (E2) is expressed in human heart and can potentially associate with all major cardiac Kv channels to modulate their current amplitude and/or gating kinetics. An E2-R27C mutation was identified in fAF patients, and shown to have a gain-of-function effect on E2/(KCN)Q1 channel complex. However, it is not clear whether/how E2-R27C affects E2 modulation of other cardiac Kv channels, and the biophysical nature of its gain-of-function phenotype when associated with Q1. **Methods**: We express E2-WT or E2-R27C with partner Kv channel α-subunits (E2:α-subunit = 3:1), and record currents using TEVC. **Results**: Coexpressing E2-WT with Kv4.3 (pore-forming subunit of I_{to} channels) reduces peak current amplitude and induces a depolarizing shift in $V_{0.5}$ of inactivation (from −45+5 to −37+5 mV). Relative to E2-WT/Kv4.3, E2-R27C reduces the current-suppressing effect and shifts $V_{0.5}$ of inactivation in the hyperpolarizing direction (to −41+5 mV). Relative to E2-WT/hERG (pore-forming subunit of I_{Kr} channels), E2-R27C induces a modest current suppressing effect along with a hyperpolarizing shift in $V_{0.5}$ of activation (from 10+4 to −8+1 mV). Relative to E2-WT/Q1 (pore-forming subunit of I_{Ks} channels), E2-R27C markedly increases the estimated fully-available current amplitude and induces a hyperpolarizing shift in $V_{0.5}$ of activation (from −7+2 to to −41+5 mV). **Conclusion**: E2-R27C affects how E2 modulates the current amplitude and voltage-dependence of gating of Kv4.3 and hERG channels. The net results can be gain-of-function or loss-of-function, depending on the resting membrane potential (RMP, depolarizing RMP exacerbates Kv4.3 inactivation by E2-R27C) and action potential plateau voltage (APPV, loss of APPV favors currents through E2-R27C/hERG channels). E2-R27C exerts a strong gain-of-function effect on E2/Q1 channels by 2 mechanisms: increasing the fully-available current amplitude and shifting the voltage range of activation in the hyperpolarizing direction.

2123-Plat
Simulation of the Impact of Elevated Cytosolic Na+ on Ca2+ Handling, Mitochondrial Energetics and Cellular Electrophysiology in Guinea Pig Myocytes
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Chronic heart failure is one of the leading causes of morbidity and mortality in the United States. One of the classical strategies for treating heart failure is to inhibit sarcolemmal Na+/K+-ATPase (NKA). Blocking NKA can result in dramatic elevation of [Na+]i, increasing the sarcoplasmic reticulum (SR) Ca2+ load by acting on the plasmalemmal Na+/Ca2+ exchanger (NCX). Whether and how change of [Na+]i affects mitochondrial Ca2+ dynamics and energetics is still under investigation. Since intracellular Na+ is regulated by a complex system involving multiple ions, channels, exchangers and membrane potentials, unraveling its effect on cell physiology and function requires an integrative view of cardiomyocyte physiology. In the present study we developed a mathematical model of cardiomyocyte that incorporates mitochondrial energetics, ion channels and exchangers, and E-C coupling. Using this model, we simulated the effect of elevated cytosolic Na+ on Ca2+ handling, mitochondrial energetics and reactive oxygen species (ROS) generation. Model simulations show that inhibition of NKA (50%) dramatically increased [Na+] in both the cytosol and mitochondria, which consequently caused Ca2+ overload in the cytoplasm during increased workload. Elevated Na+ also decreased ATP concentration and increased mitochondrial ROS production. Concomitant inhibition of mitochondrial Na+/Ca2+ exchanger (mNCE) ameliorated these effects by attenuating cellular Ca2+ overload and increasing [Ca2+]m. Furthermore, inhibiting mNCE also prevented the [ATP]i drop and decreased ROS production. The findings indicate that increasing cytosolic Na+ has an adverse effect on mitochondrial energetics that can be attenuated by simultaneous inhibition of mNCE.

2124-Plat
Biexcitability and Early Afterdepolarization-Mediated Cardiac Arrhythmias
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Under normal conditions in ventricular tissue, both planar wave propagation and spiral wave reentry are mediated by Na current (I_{Na})-mediated depolarization. Under diseased conditions in which repolarization reserve is reduced, however, secondary depolarizations can occur in the plateau or repolarizing phase of the action potential (AP) due to reactivation of the L-type calcium current ($I_{Ca,L}$), known as early afterdepolarizations (EADs). Under these conditions, we observed a novel behavior in which both I_{Na}-mediated spiral wave reentry and $I_{Ca,L}$-mediated spiral wave reentry coexisted in the same homogeneous tissue. I_{Na}-mediated spiral waves were similar to those observed under normal condition, with high rotation frequency (~10 Hz) and nearly full repolarization between beats. $I_{Ca,L}$-mediated spiral waves, however, rotated much slower (2-3 Hz) with membrane voltage remaining above −40 mV, at which I_{Na} is inactivated. We call this novel property of an excitable medium ***biexcitability***. In heterogeneous tissue with transmural AP gradients, pause-induced EADs initiated $I_{Ca,L}$-mediated rotors from the M-cell region. The resulting arrhythmia was characterized by co-existing $I_{Ca,L}$- and I_{Na}-mediated wavefronts, with a frequency and electrocardiographic appearance resembling Torsades de Pointes. The arrhythmia either terminated spontaneously or degenerated to ventricular fibrillation. We propose biexcitability as a novel mechanism of Torsades de Pointes in long QT syndromes.

2125-Plat
B-Type Natriuretic Peptide (BNP) Prolongs Action Potential Duration through Suppressing Transient Outward Potassium Current in Rat Hearts
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Background: Nesiritide (B-type natriuretic peptide) improves hemodynamic function and heart failure status for patients with decompensated congestive heart failure. However, studies associated the use of nesiritide with an increased risk of sudden death, to date, little is known of the underlying mechanisms. In this study, the effect of BNP on action potential duration and underlying electrophysiologic mechanisms were investigated in rat hearts.
Method: Wistar rats were anesthetized with sodium pentobarbital (40mg/kg) and injected with BNP-32 (12μg/kg) from abdominal vena cava. EKG was recorded using electrodes inserted into the subcutaneous layer of the paws. Action potentials from 8 rat left ventricles were recorded by a high-resolution optical mapping with voltage-sensitive dye RH237 in Langendoff perfusion system. Transient outward potassium current of rat ventricular myocytes was recorded by the whole cell configuration of patch clamp technique.
Results: BNP-32, at a clinically relevant concentration, prolonged corrected QT interval (QTc = QT / $\sqrt{}$ RR) obviously in adult rats, whereas heart rate was comparable during and after BNP-32 treatment. BNP-32 (0.1μM) increased action potential duration at 50% (APD_{50}) repolarization (45.62 ± 4.45ms, $P<0.01$) and this effect persisted after 15 min of washout (57.71 ± 12.62ms, $P<0.05$) compared to baseline (BL: 41.22 ± 2.88ms). In control group of 6 rat hearts, APD_{50} had no obvious changes over the same time period without BNP perfusion. The peak transient outward potassium current at +60mV was significantly reduced (7.11 ± 4.97 to 2.85 ± 3.30 pA/pF, n=6; $P<0.05$) by 0.01μM BNP-32, and then partially recovered to 5.85 ± 4.12 pA/pF (n=6, $P<0.05$) after washout of BNP-32.
Conclusion: BNP prolongs action potential duration and reduces transient outward potassium current in rat hearts, which might contribute to BNP-induced increase of death risk in decompensated heart failure patients.

2126-Plat
The Mitochondrial Bioenergetic Phenotype for Protection from Ischemia in Sur2-Mutant Mice
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ATP-sensitive potassium channels (K_{ATP}) in mitochondria are postulated to play a key role to protect mitochondria and myocytes from cardiac ischemic insult. The sulfonylurea receptor-2 (SUR2) is a subunit of K_{ATP} in sarcolemma, although its role in mitochondrial physiology is unclear. Mice where the SUR2 gene was disrupted (SUR2 mutant) have been shown to be constitutively protected from ischemic injury.
We characterized the bioenergetic phenotype of mitochondria in SUR2 mutant mice to gain insight into mechanisms of protection from ischemia.
Membrane potential ($\Delta\Psi_m$), Ca^{2+} uptake, and reactive oxygen species (ROS) generation were studied in the isolated mitochondria by fluorescence based assays and K^+-influx was studied by volume measurements. Mitochondrial respiration was studied in normoxia and after hypoxia-reoxygenation. Myocyte protection against metabolic inhibition was also investigated. $\Delta\Psi_m$ was depolarized (53.37 ± 1.5 vs 48.4 ± 1.8 %), tolerance to Ca^{2+} loading was increased (163 ± 26 vs 116 ± 23 μM), and ROS generation was increased (9.3 ± 0.4 vs 7.4 ± 0.6 FU/sec) in the SUR2 mutant mitochondria compared to wild type (Wt). SUR2 mutant mitochondria had greater swelling (30.2 ± 3.1%) compared with Wt (14.5 ± 0.6%) indicating greater K^+ influx. SUR2 mutant mitochondria recovered better from post hypoxia-reoxygenation than Wt as measured by the respiration control index (RCI). Finally, the SUR2 mutant myocytes viability was better protected against metabolic inhibition.
We concluded that SUR2 plays a key role in mitochondrial mechanisms of protection from ischemia by altering a potassium conductance consistent with a mitochondrial K_{ATP} and causing a protected mitochondrial phenotype.

2127-Plat
Multiple Mechanisms of hERG Liability: K^+ Current Inhibition, Disruption of Protein Trafficking, and Apoptosis Induced by Amoxapine
Sabrina Obers[1], Ingo Staudacher[1], Ramona Bloehs[1], Eckhard Ficker[2], Adrienne Dennis[2], Jana Kisselbach[1], Patrick Schweizer[1], Ioana Baldea[1], Hugo Katus[1], **Dierk Thomas**[1].
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The antidepressant amoxapine has been linked to QT prolongation, acute heart failure, and sudden death. Drug binding to cardiac hERG (Kv11.1) potassium channels causes prolonged repolarization and induces apoptosis. This study was designed to investigate amoxapine effects on hERG currents, hERG protein trafficking, and hERG-associated apoptosis in order to elucidate molecular mechanisms underlying cardiac side effects of the drug. hERG channels were expressed in *Xenopuslaevis* oocytes and HEK 293 cells, and potassium currents were recorded using patch clamp and two-electrode voltage clamp electrophysiology. Protein trafficking was evaluated in HEK 293 cells by Western blot analysis, and cell viability was assessed by immunocytochemistry and colorimetric MTT assay. Amoxapine caused acute hERG blockade in oocytes (IC_{50} = 21.6 μM) and in HEK 293 cells (IC_{50} = 5.1 μM). Mutation of residues Y652 and F656 attenuated hERG blockade, suggesting drug binding to a receptor inside the channel pore. Channels were mainly blocked in open and inactivated states, and voltage-dependence was observed with reduced inhibition at positive potentials. Amoxapine block was reverse frequency-dependent and resulted in accelerated and leftward-shifted inactivation. Furthermore, amoxapine caused chronic reduction of hERG trafficking into the cell surface membrane (IC_{50} = 15.3 μM). Finally, the antidepressant drug triggered apoptosis in cells expressing hERG channels. Triple mechanisms of hERG liability associated with a single compound, are revealed. Amoxapine causes direct hERG current inhibition and disruption of hERG protein trafficking. Furthermore, the drug induces apoptosis of cells expressing hERG potassium channels.

2128-Plat
Cardiac Glycoside Chronotropic and Arrhythmogenic Effects in Sinoatrial Nodal Pacemaker Cells (SANC) Occur Along a Continuum of Electrochemical Gradients of Na^+ (E_{Na}) and Ca^{2+} (E_{Ca})
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Cardiac glycosides reduce E_{Na} and E_{Ca} (due to an increase of Na^+_i via Na-K pump inhibition, and of Ca^{2+}_i, due to a secondary reduction in Ca efflux for Na influx via NaCa exchange). Here we show that exposure of single rabbit SANC to the cardiac glycoside, digoxigenin (10-20μM) results in a continuum of time-dependent effects. Within 30s to 1 min, the rate of rhythmic spontaneous action potentials (AP) increases by 20% (n=3) and this is associated with an earlier occurrence (reduced period) of local sub-membrane Ca^{2+} releases (LCR's) during diastolic depolarization, detected by confocal Ca^{2+} imaging. Approximately 1-3 minutes following AP rate acceleration, LCR period lengthens by 40%, accompanied by a similar reduction in the rhythmic AP rate. The changes in LCR period during the biphsic changes in rhythmic AP firing rate increase are highly correlated with the changes in AP cycle length (R^2=0.98). A progressive increase in the steady level of diastolic Ca^{2+} beneath the surface membrane then ensues usually within 4 to 6 additional minutes, LCR's became undetectable, and dysrhythmic and chaotic AP firing occurs. Numerical model simulations (Maltsev-Lakatta model, AJP 2009) in which Na_i was increased progressively 5-15mM during glycoside exposure reproduced the experimental results. That rate and rhythm regulation of SANC AP firing during cardiac glycoside exposure occurs along a continuum of E_{Na}/E_{Ca} is in agreement with repeated observations over the last decade, showing that the SANC spontaneous AP firing rate is critically dependent on the timing of acute changes in sub-membrane E_{Ca} during DD caused by LCR occurrence (LCR period) that accelerates DD by activation of an inward Na/Ca exchange current.

2129-Plat
Calcium Currents in Chronically Dysfunctional Pig Myocardium
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Chronic reduction in coronary artery blood flow due to stenosis results in reduced myocardial contractile function, termed "hibernating" myocardium. This change involves electrical remodeling and a propensity for sudden cardiac death in the absence of infarction. We used a pig model of chronic left anterior descending artery stenosis to study calcium currents associated with the hibernating myocardium. We developed a cell isolation technique from punch biopsies of the left ventricle. This yields calcium-tolerant isolated myocytes with brick shaped morphology and clear striation patterns. Myocyte length from hibernating myocardium averaged 145 ± 7.7 (n=15) vs. 124 ± 4.8 μm (n=42) for control, suggesting cellular hypertrophy. The cell shortening of hibernating cells was also reduced compared to the remote region from 6.4 ± 1% (n=21) to 4.45 ± 1% (n=11) suggesting that the reduced contractility seen in the hibernating region was preserved in the isolated myocytes. Furthermore, cells isolated from hibernating myocardium had significantly higher numbers of premature contractions 4/15 for hibernating vs. 0/19 for control suggesting a cellular propensity for arrhythmias. The L-type calcium channel current ($I_{Ca,L}$) in myocytes from hibernating myocardium (1.35 ± 0.08 pA/pF, n=22) was reduced compared to normal myocardium (1.96 ± 0.07 pA/pF, n=33; $P<0.01$). The voltage dependence of steady state activation and inactivation were nearly