The purpose of this study was to investigate the potential of activated fibroblasts to alter conduction velocity (CV) and contribute to an arrhythmogenic substrate. Cardiac fibroblasts isolated from ventricles of healthy (Fb) and infarcted (MI-Fb) hearts 7 days after LAD ligation were plated on top of confluent neonatal rat myocyte monolayers and optically mapped 16-20 hours later. Homocellular myocyte monolayers (Myo) were used as controls. Fb significantly decreased (17.0 ± 0.5 cm/s; $p=0.01$) and MI-Fb increased (22.0 ± 0.6 cm/s; $p=0.02$) average CV compared to Myo (19.7 ± 0.7 cm/s). In addition, CV was significantly faster in MI-Fb compared to Fb (p=2.0E-8). Action potential duration (APD50) was significantly reduced in MI-Fb (85.7 ± 3.2 ms) compared to Myo (109.2 ± 4.6 ms; p=2.0E-4) and Fb (97.7 ± 3.7 ms; $p=0.02$). Proliferation assays confirmed these changes were not due to differences in the rate of cellular division between Fb and MI-Fb. Cx40 and Cx43 mRNA detected by qRT-PCR were significantly upregulated in MI-Fb compared to Fb. Cx45 mRNA levels were not different between the groups. These data demonstrate significant electrophysiological differences between fibroblasts isolated from healthy and infarcted hearts that could contribute to the greater incidence of arrhythmias observed in fibrotic hearts. These findings may lead to the development of new anti-arrhythmic therapeutic approaches targeting the fibroblast activation process.

2895-Plat

Estradiol and Progesterone Exert Opposite Effects on Cardiac Repolarization and Arrhythmogenesis in Transgenic Long QT Syndrome 2 Rabbits

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Introduction: Adult women with LQT2 are at higher risk for sudden cardiac death (SCD) than men with an increased risk during the postpartum. We have created transgenic rabbits over-expressing a pore mutant of the human ERG channel in the heart (LQT2) and showing the human long QT phenotype (Brunner et al. JCI, 2008). 4/4 LQT2 females used for breeding died of SCD during the postpartum. We hypothesize that sex hormones modulate cardiac repolarization and arrhythmogenesis in LQT2 females.

Methods: Prepubertal ovariectomized LQT2 females were implanted with 90day release-pellets of estradiol (EST), progesterone (Prog), dihydrotestosterone (DHT), or placebo (OVX) (n=6 each). All groups underwent telemetric ECG monitoring and in vivo electrophysiological studies (EPS) after 8 weeks and first optical mapping experiments were performed in OVX, EST- and DHT-treated rabbits.

Results: EST treatment steepened the QT/RR slope of prepubertal rabbits ($p<0.05$), whereas DHT or OVX decreased the QT/RR slope steepness ($p<0.05$). Prog did not alter the QT/RR slope. In vivo EPS revealed a longer ventricular refractory period (VERP) in EST- than in DHT- or Prog-treated rabbits (DHT: $p<0.05$, Prog: $p<0.01$). Within 8 weeks of hormone-treatment, 4 of 6 EST-treated rabbits died of polymorphic VT, while no SCD occurred in 6 DHT- and 6 Prog-treated LQT2 females ($p<0.05$). Preliminary optical mapping experiments revealed heterogeneous APD dispersion with islands of prolonged refractoriness in EST- rabbits contrasting with smoother APD dispersion in OVX and DHT-treated rabbits.

Conclusions: EST increases the QT/RR steepness and prolongs cardiac refractoriness, whereas DHT and Prog shorten cardiac refractoriness. EST predisposes prepubertal LQT2 rabbits to polymorphic VT. Heterogeneous ADP dispersion might underlie this proarrhythmic effect of EST. We are currently using high-throughput molecular approaches to elucidate the underlying mechanisms.

2896-Plat

Evolution of Ventricular Myocyte Electrophysiology

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The relative importance of regulatory versus structural evolution for the evolution of different biological systems is a subject of controversy. The primacy of regulatory evolution in the diversification of morphological traits has been promoted by many evolutionary developmental biologists. For physiological traits, however, the role of regulatory evolution has received less attention or has been considered to be relatively unimportant. To address this issue for electrophysiological systems, the importance of regulatory and structural evolution in the evolution of the electrophysiological function of cardiac myocytes was examined in mammals. In particular, two related phenomena were studied: the change in action potential morphology in small mammals and the scaling of action potential duration across mammalian phylogeny. In general, the functional properties of the ion channels involved in ventricular action potential repolarization were found to be relatively invariant. In contrast, there were large changes in the expression levels of multiple ion channel and transporter genes. For the Kv2.1 and Kv4.2 potassium channel genes, which are primary determinants of the action potential morphology in small mammals, the functional properties of the proximal promoter regions were found to vary in concordance with species dependent differences in mRNA expression, suggesting that evolution of cis-regulatory elements is the primary determinant of this trait. Scaling of action potential duration was found to be a complex phenomena, involving changes in the expression of a large number of channels and transporters. In this case, it is concluded that regulatory evolution is the predominant mechanism by which the scaling is achieved.

2897-Plat

Inhibition of $hK_{2P}3.1$ (TASK-1) Potassium Channels by the Tyrosine Kinase Inhibitor Genistein

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Two-pore-domain (K_{2P}) channels mediate potassium background currents, controlling excitability by stabilizing membrane potential below firing threshold and expediting repolarization. Inhibition of K_{2P} currents permits membrane potential depolarization and excitation. Signaling via protein tyrosine kinases has been implicated in ion channel modulation. The objective of this study was to investigate tyrosine kinase regulation of $K_{2P}3.1$ channels. The two-electrode voltage clamp technique was used to record K_{2P} currents in *Xenopus* oocytes, and $K_{2P}3.1$ channels were studied in CHO cells using the whole cell patch clamp technique. Human $K_{2P}3.1$ (TASK-1) was blocked by the tyrosine kinase inhibitor, genistein, in *Xenopus* oocytes ($IC_{50} = 10.7$ μM) and in Chinese hamster ovary cells ($IC_{50} = 12.3$ μM). The channel was not affected by genistin, an inactive analogue of genistein. Peroorthovanadate, an inhibitor of tyrosine phosphatase activity, slightly attenuated the inhibitory effect of genistein. Current reduction was voltage-independent and did not require channel protonation at position H98. Genistein-associated blockade occurred independently of channel phosphorylation at the single tyrosine kinase phosphorylation site, Y323, suggesting that tyrosine kinase activity does not directly affect $K_{2P}3.1$ channel function. In addition to $K_{2P}3.1$, genistein also reduced $K_{2P}6.1$ (TWIK-2), $K_{2P}9.1$ (TASK-3), and $K_{2P}13.1$ (THIK-1) currents, respectively. Modulation of K_{2P} channels by genistein is revealed to be a novel mechanism to alter background K^+ channel function.

2898-Plat

Acetylcholine-dependent Prolongation Of Atrial Action Potentials By Acute Stretch

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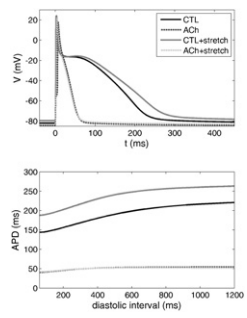
Mechanical stretch of cardiomyocytes modulates the action potential (AP) via stretch-activated channels. The electrical activity is also modulated by the parasympathetic nervous system via the acetylcholine (ACh)-dependent potassium current. The ACh effect is however heterogeneous throughout the atria thus facilitating arrhythmic events. Simultaneous activation of both systems could occur and may facilitate atrial arrhythmias. Simulations of a canine atrial ionic model in an isolated cell and linear tissue strand were computed with varying stretch and ACh levels. Pacing at 1Hz, AP duration (APD) is increased (see APs in panel A) by ~47 ms with 20% stretch compared to control (CTL). However, stretch did not increase APD in presence of 25 nmol/L of ACh (ACh vs. ACh+stretch in panel A). Restitution curves calculated with the S1-S2 protocol (S1=1 Hz) are plotted in panel B. Stretch (20%) results in an upshift of ~47 ms of the restitution curve compared to CTL. ACh almost eliminate atrial restitution compared to CTL with no important changes with stretch (ACh vs. ACh+stretch curves). Preliminary results obtained in a cable with



heterogeneous ACh distribution showed an increased interval for block of electrical propagation with tissue stretch.

Symposium 20: Membrane Trafficking

2899-Symp
The interplay between lipid and protein trafficking
Frederick Maxfield.
Weill Cornell Medical College, New York, NY, USA.

2900-Symp
Watching t-SNAREs And Their Interaction With Secretory Granules In Live Cells
Wolfhard Almers[1], Michelle Knowles[2], Sebastian Barg[3], Lei Wan[1].
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The SNARE proteins Syntaxin and SNAP25 inhabit the plasma membrane, and VAMP/synaptobrevin the membrane of secretory vesicles. When all three combine in a 1:1:1 complex they are thought to fuse the secretory vesicle to the plasma membrane. To explore the interaction of Syntaxin and SNAP25 with secretory vesicles, we have imaged live cells using TIRF microscopy in two colors. Cells co-expressed a fluorescent granule marker as well as EGFP-tagged t-SNAREs at low copy number. Fluorescence was calibrated by single molecule measurements. Granules formed nanodomains beneath them, each with room for 100 syntaxin molecules. The nanodomains repeatedly and spontaneously emptied of syntaxin and then re-filled. They exchanged their syntaxin with plasma membrane with a half time of a few seconds, and when a granule performed exocytosis its nanodomain disassembled. SNAP25 was concentrated beneath granules but with 10 fold lower affinity than Syntaxin. Most Syntaxin and nearly all SNAP25 molecules were seen to move freely in the plasma membrane, but a minor proportion of each t-SNARE was almost immobile. Single Syntaxin molecules could be observed as they were captured and released from granule sites. We have tracked the recruitment and release of SNAREs at exocytic sites in a time-resolved manner and with single molecule sensitivity.

2901-Symp
Molding The Plasma Membrane At Sites Of Endocytosis
Pietro De Camilli.
Yale University School of Medicine, New Haven, CT, USA.
An important goal of our laboratory is to elucidate mechanisms in the biogenesis and traffic of synaptic vesicles at neuronal synapses, with emphasis on the processes that mediate their reformation by endocytic recycling after each cycle of secretion. We use a variety of complementary approaches that include reconstitution experiments with purified endocytic proteins and lipid membranes, broken cell preparations, intact cells, model synapses and genetically modified mice. With these studies we hope not only to improve knowledge of synaptic transmission but also to advance the understanding of fundamental mechanisms in endocytosis. In my talk I will discuss the role of the GTPase dynamin, a protein implicated in the fission reaction of endocytosis, and the impact of the lack of dynamin on cell structure and physiology. We have generated KO mice for each of the three dynamin isoforms. These mice, as well as cells derived from them, allow us to study the fundamental function of dynamin as well as isoforms specific functions. Surprisingly, cells without any dynamin live, although they fail to proliferate and they display major alterations in the structure of the cell surface. I will also discuss the function of dynamin binding partners with curvature generating and curvature sensing properties (proteins with BAR and F-BAR domains), and the mechanisms through which these proteins deform membranes (Roux et al. Nature 441: 528-531; Ferguson et al. Science 316: 570-574; Frost et al. Cell 132:807-817).

2902-Symp
Phosphoinositides in Ca2+ Signaling and Plasma Membrane Biogenesis: Roles for Electrostatic Interactions
Barbara Baird, Lavanya Vasudevan, Nathaniel Calloway,
Alice Wagenknecht-Wiesner, Kirsten Elzer, David Holowka.
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Phosphoinositides are implicated in a wide range of cellular pathways, both at the plasma membrane and at other organelles. During IgE receptor activation in mast cells, phosphatidylinositol 4,5-bisphosphate (PIP_2) synthesized by PIP5-kinase Igamma at the plasma membrane is hydrolyzed by phospholipase Cgamma to produce inositol 1,4,5- trisphosphate, which initiates store-operated Ca^{2+} influx (SOCE). In contrast, PIP_2 synthesized by another isoform, PIP5-kinase Ialpha, regulates SOCE in these cells in an apparently bimodal manner: It promotes the interaction between the endoplasmic reticulum (ER) Ca^{2+} sensor STIM1 and the Ca^{2+} channel protein Orai1/CRACM1, yet it plays a net negative role in SOCE, possibly by inhibiting Orai1/CRACM1 gating. Functional coupling between STIM1 and Orai1/CRACM1 involves electrostatic interactions: Coupling is blocked by positively charged sphingosine derivatives at the inner leaflet of the plasma membrane, and also by mutation of six acidic amino acid residues in the coiled-coil C-terminus of Orai1/CRACM1. We hypothesize that PIP_2 participates in this electrostatic coupling.
Phosphoinositol 4-phosphate participates in ER-to-plasma membrane biogenic trafficking, and it is synthesized from phosphatidylinositol (PI) at the cytoplasmic face of the ER by PI4-kinase IIIalpha. We find that expression of the polybasic MARCKS effector domain in the lumen of the ER reduces PI4P content in the Golgi complex and inhibits ER-to-plasma membrane protein trafficking in parallel with this inhibition. We hypothesize that ER-targeted MARCKS effector domain traps PI at the luminal face by an electrostatic interaction to inhibit PI4P synthesis and thereby ER-to-plasma membrane trafficking. These results highlight the importance of negatively charged phosphoinositides in multiple cellular pathways and point to the roles of electrostatic interactions in regulating these processes.

Symposium 21: Receptor-mediated Channel Activation

2903-Symp
Conformation Changes Before Opening And The Activation Mechanism In Glycine And Nicotinic Receptors
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Channels in the nicotinic superfamily are pentameric membrane proteins that respond to the binding of transmitter molecules to their extracellular domain by opening their integral membrane pore. One of the best ways to obtain information on the chain of events that follows transmitter binding is by single channel analysis. Mechanisms of receptor activation can be fitted to sets of experimental recordings for the purpose of validating a particular model and quantifying the rate at which each step occurs. We use HJCfit, a program developed by David Colquhoun (available from http://www.ucl.ac.uk/pharmacology/dc.html) to obtain maximum-likelihood, global mechanism fits with full missed event correction to steady-state recordings obtained at different agonist concentrations and idealised by time-course fitting. By the use of this technique on wild-type glycine receptors, we were able for the first time to detect an intermediate conformational change that follows agonist binding but precedes channel opening. The short-lived, partially-activated intermediate shut state (which we termed ''flip'') has a higher affinity for the agonist than the resting state, which suggests that this conformational change involves some degree of domain closure in the extracellular domain. Activation models that include this flipped state can also accurately describe the properties of ACh nicotinic receptors and of startle disease mutants of the glycine channel. Analysis of the activation of nicotinic channels and glycine channels by partial agonists showed that the difference between partial and full agonists resides in the first conformational change (flipping) rather than in the open-shut reaction as has always been supposed previously. Partial agonists are poor at eliciting the change from resting to flipped, but once in the flipped state the opening and shutting of the channel is much the same for all agonists.

2904-Symp
Probing Structure on Well-defined Functional States of the Nicotinic Receptor Using Systematically-engineered Ionizable Residues and Proton-transfer Events
Gisela D. Cymes, **Claudio Grosman**.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.
The conformational changes that underlie the closed-open transition in members of the nicotinic-receptor superfamily remain elusive and controversial. To gain insight into the structural properties of the pore-domain of the muscle-nicotinic acetylcholine-receptor channel (AChR) in the open state, while retaining the advantages of studies on intact cells and in real time, we engineered basic residues along the M1, M2, and M3 transmembrane segments of all four types of subunit and recorded the individual proton-transfer events using single-channel patch-clamp electrophysiology. Proton binding-unbinding reactions to and from individual side chains were manifest as blocking-unblocking events of the passing cation current. Two observables, namely, the extent to which the current is attenuated upon side-chain protonation, and the pKa-shifts of the engineered ionizable groups relative to bulk water, were analyzed to reveal the electrostatic properties of the local microenvironment around the transmembrane segments in the open-channel conformation. In turn, these data were interpreted in terms of secondary and tertiary structure, and compared with existing structural models of the closed state in order to elucidate the change in conformation that opens the AChR. Our open-channel data suggests that the