The results suggest that aromatic residues do not increase the binding affinity of bupivacaine to Kv channels. Rather, the affinity decreases. The Kd value for the wildtype channel is 300 μM, for the V473F channel 550 μM and for the P474F channel 740 μM. Thus, aromatic residues seem not to be necessary for high-affinity local anaesthetic binding to voltage-gated ion channels. The specific role of aromatic residues in Nav and hERG channels seems thus related to specific structural constraints in these channels.

2890-Plat
A Regulator for Eag Family Channels
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Ether-a-go-go (Eag) family channels, which include hErg1, are voltage-gated K^+ channels that are important in cardiac and neural function. From the genetic sequence of this family of channels, we identify two probable ligand-binding sites based on their similarities with well-characterized ligand binding domains. The first putative ligand-binding domain is in the carboxy-terminal region and shares sequence similarity with the cyclic nucleotide-binding domain of cyclic nucleotide-gated (CNG) channels. Yet, this binding domain of Eag family channels lack a critical arginine required for cyclic nucleotide binding, and channel gating was not altered by cAMP or cGMP. The second potential ligand-binding site is a Per-Arnt-Sim (PAS) domain in the amino-terminal region. High conservation of these putative binding domains amongst all Eag family channels indicates their functional importance. We therefore categorize these channels as orphan receptors. We reasoned that a chemical screen of cellular metabolites will lead us to physiologically relevant channel-regulators. Using a novel, high through-put screen of the ''Fragments of Life'' chemical library of metabolites and metabolite-like compounds (deCODE Biostructures) and inside-out patch-clamp recording, we have identified regulators of Eag family channels. We identified six regulators that cluster into four chemical families. One indole, and one indole-like compound were increased Eag channel opening at hyperpolarizing potentials. Indoles are particularly interesting because of their structural similarity to purines, the core of the cyclic nucleotides that bind to and regulate CNG channels. In contrast to indoles, compounds from the flavonoid family strongly inhibited Eag current. These results indicate that metabolites regulate Eag family channels and may lead us to physiologic channel regulators.

Platform BC: Cardiac Electrophysiology

2891-Plat
The Vena Cava Is Pacing The Embryonic Heart
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The mechanism and location of the pacemaker in the embryonic heart is highly controversial due to the lack of physiological *in vivo* recordings. Here we report fluorescent macroscopic *in vivo* recordings of embryonic hearts (E12.5-E14.5) from mice with cardiac expression (α-MHC or Cx40 promotor) of the Ca^{2+} sensor GCaMP2.
Initial observations from the ventral surface showed regular uniform Ca^{2+} transients in the atrium, ventricle and a structure below/behind the right atrium that preceded every atrial activation. To better understand the origin of these Ca^{2+} transients we established a dorsal preparation leaving the heart and veins intact. Ca^{2+} transients activated in the region of the putative sinus node, propagated bidirectionally along the superior right and in a u-shaped pattern into the coronary sinus and left superior vena cava, and conducted faster (24 ± 4 mm/sec) than the atria (15 ± 2 mm/sec; n=5; $p<0.02$); we have termed this the ''streak''. In most hearts the streak fired before every atrial activation with a delay of 79 ± 10 ms (n=12); variations in this delay was not dependent on the heart rate (104 ± 15 bpm, n=12). Some hearts showed 2:1 coupling and in others only the streak fired at 178 ± 39 bpm (n=5). The streak contracted, could be electrically paced and spontaneous local field potentials were recorded as sharp spikes at the onset of Ca^{2+} transients. In some experiments we observed a diastolic Ca^{2+} increment just prior the field potential, in line with the proposed role of Ca^{2+} oscillations for the initiation of pacemaking in embryonic heart cells. The spontaneous activity of the streak prior to atrial activation, higher intrinsic frequency of this region, and diastolic Ca^{2+} release establish the pacemaker function of the streak. Thus, cardiomyocytes within/on the vena cava walls are involved in murine embryonic heart pacemaker activity. Values: mean ± SEM.

2892-Plat
Increased Vulnerability To Atrial Fibrillation Under Vagal Hyperinnervation Associated With Vasoactive Intestinal Polypeptide'S Release In Dog'S Atrium
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Increased vagal tone promotes atrial fibrillation (AF). Vasoactive intestine polypeptide (VIP, a neural transmitter co-released with acetylcholine) was shown to shorten atrial refractory periods. AF was reportedly associated with VIPoma, a rare tumor secreting excessive VIP. Yet, the effects of VIP on atrial electrophysiology remain unclear. **Methods:** Canine left atria were isolated with intact coronary perfusion. Programmed stimulation (300 ms drive cycle length followed by up to 4 extrastimuli) was used to assess AF induction. Action potential duration (APD) at 500 ms pacing was recorded with optical mapping system. Potassium currents (I_{Ks}) were recorded with patch-clamp techniques. Immunohistochemical staining for VIP receptors was performed on atrial tissue/myocytes. **Results:** AF was induced in 1 of 6 atria at baseline but 5 of 6 during 1 μM VIP perfusion ($p=0.021$) and 2 of 6 after 15 min washout. VIP shortened APD and increased inhomogeneity in a dose-dependent manner at 0, 0.1, 1.0, 10 μM and washout (n=13): APD_{75} was 134.61 ± 5.36, 116.31 ± 6.80, 117.50 ± 7.86, 100.71 ± 8.73 and 124.50 ± 4.84 ms; standard deviation of APD_{75} was 15.83 ± 1.55, 15.59 ± 2.33, 21.58 ± 3.57, 25.76 ± 1.16 and 18.67 ± 1.62 ms, respectively ($p<0.05$). VIP (1 μM) increased I_{Ks} current density (14.5 ± 9.5%, n = 9, $p<0.01$). Staining on isolated atrial myocytes revealed the expression of VIP receptor 1 and 2 was highly variable among cells and tissue staining showed spatial heterogeneity. **Conclusions:** VIP shortens APD and increases APD spatial inhomogeneity that could lead to increased AF vulnerability. Enhanced I_{Ks} and heterogeneity of receptor expression may contribute to these effects.

2893-Plat
Ablation of Protein Kinase A or Calmodulin Kinase II Phosphorylation Sites on Phospholamban Confer Arrhythmia Resistance in Sinoatrial Nodal Pacemaker Cells
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Phospholamban (PLN) is a negative regulator of the sarcoplasmic reticulum Ca^{2+} ATPase (SERCa). PLN is phosphorylated by Protein Kinase A (Ser16) or Calmodulin Kinase II (Thr17). These phosphorylations reduce the inhibitory effects of PLN on SERCa. Phosphorylation at PLN Ser16 and Thr17 are important for catecholamine effects on excitation-contraction coupling in ventricular myocytes, but their role in sinoatrial nodal (SAN) cells is unknown. We isolated SAN cells from wild type (WT) controls and from transgenic mice where Ser16Ala or Thr17Ala PLN mutants are expressed in lieu of WT PLN. We recorded spontaneous action potentials by perforated patch clamp at 35 ± 1°C. SAN cell automaticity rates ('beats'/min) were not significantly different between Ser16Ala, Thr17Ala or WT at baseline: WT (n=18) 269 ± 12; T17A (n=9) 264 ± 23; S16A (n=16) 256 ± 17. No early (EAD) or delayed (DAD) afterdepolarizations were observed at baseline. EADs and DADs were induced by isoproterenol (1-5 μM) in WT SAN cells (cells with afterdepolarizations/total cells tested: 10/13) but not in Ser16Ala (0/6) and Thr17Ala (0/5) SAN cells ($p<0.01$ in both cases compared to WT). These results suggest that catecholamine induced EADs and DADs require phosphorylation of PLN Ser16 or Thr17 and highlight the importance of the intracellular Ca^{2+} cycling machinery for determining SAN cell vulnerability to adverse effects of catecholamine stimulation.

2894-Plat
Arrhythmogenic potential of activated fibroblasts
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Many cardiovascular disorders including ischemic heart disease and heart failure are associated with extensive fibrosis. A critical event in the development of cardiac fibrosis is the transformation of fibroblasts into an active fibroblast phenotype or myofibroblast. Fibroblasts isolated from healthy hearts and grown under standard tissue culture conditions start expressing the myofibroblast marker α-SMA 24-48 hours after isolation. These cells have been referred to as myofibroblasts. However, there is evidence indicating *in vitro* phenotypic changes due to culture conditions do not fully replicate the *in vivo* activation process.

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