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

Katja E. Odening[1], Xuwen Peng[2], Bum-Rak Choi[1], Michael Brunner[3], Leonard Chaves[1], Lorraine Schofield[1], Manfred Zehender[3], Gideon Koren[1].
[1]Cardiovascular Research Center, Division of Cardiology, Rhode Island Hospital, Warren Alpert Medical School of Brown University, Providence, RI, USA, [2]Department of Comparative Medicine, Pennsylvania State University College of Medicine, Hershey, PA, USA, [3]Innere Medizin III, Kardiologie, University of Freiburg, Freiburg, Germany.

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

Barbara Rosati[1], Min Dong[2], Lan Cheng[3], Qinghong Yan[1], Shian-Ren Liou[1], Michael Sanguinetti[3], Hong-Sheng Wang[2], **David McKinnon**[1].
[1]Stony Brook University, Stony Brook, NY, USA, [2]University of Cincinnati, Cincinnati, OH, USA, [3]University of Utah, Salt Lake City, UT, USA.

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

Jakob Gierten[1], Eckhard Ficker[2], Ramona Bloehs[1], Kathrin Schlömer[1], Sven Kathöfer[1], Eberhard Scholz[1], Edgar Zitron[1], Hugo Katus[1], Christoph Karle[1], **Dierk Thomas**[1].
[1]Department of Cardiology, University of Heidelberg, Heidelberg, Germany, [2]MetroHealth Campus, Case Western Reserve University, Cleveland, OH, USA.

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

Philippe Comtois.
Montreal Heart Institute, Montreal, QC, Canada.

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