**1749-Pos**

**Multiple Mechanisms Contribute to Cardiotoxicity Observed with the Antidepressant Desipramine**

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The cardiotoxicity of antidepressants is well recognized. Common electrocardiographic changes precipitated in particular by an overdose include QRS widening as well as prolongation of the QT interval and torsade de pointes tachycardia. QT prolongation by antidepressants has usually been associated with acute block of hERG/$I_{Kr}$ currents. This study has been designed to provide a more complete picture of the molecular mechanisms underlying cardiac side effects induced by the antidepressant desipramine. We have studied acute block in HEK/hERG WT cells using patch-clamp recordings and found that desipramine reduced hERG currents with an $IC_{50}$ value of 11.9μM. In HEK/hERG F656V, a mutation that reduces drug binding, hERG currents were blocked half-maximally with 48.3μM. We used Western blots to monitor the effects of desipramine on hERG trafficking. In these experiments we found that the fully-glycosylated cell surface form of hERG was reduced with an $IC_{50}$ of 5.1μM on overnight incubation. When long-term effects were studied using electrophysiological current recordings, hERG tail currents were decreased with an $IC_{50}$ of 7.5μM. Accordingly, hERG surface expression was reduced by desipramine when monitored directly using a cell-based assay ($IC_{50}$, 17.3μM) or confocal imaging. Importantly, the reduction in surface expression was not attenuated by mutation of residue F656 in the drug binding site of hERG. In guinea pig ventricular myocytes action potential duration was prolonged in a dose-dependent manner as expected on acute desipramine exposure. However, long-term exposure increased action potential duration only marginally. Finally, desipramine triggered apoptosis in cells expressing hERG channels. Taken together, desipramine exerts adverse cardiac effects by at least three different mechanisms: (1) direct hERG channel block, (2) disruption of hERG trafficking, and (3) induction of apoptosis.

**1750-Pos**

**Arrhythmogenic Activity and Channel Remodeling in Ventricles of Dilated Cardiomyopathy (DCM) Model Mice**

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**Introduction**: Dilated cardiomyopathy (DCM) is a disease characterized by weakened and dilated heart which often leads to lethal arrhythmia and sudden death. Recently a knock-in mouse model of DCM was created by mutation of cardiac troponin T (ΔK210) based on human familial DCM. Because they died suddenly at a high probability during 8-12 weeks old but rarely died before 6 weeks, we compared the properties of cardiac muscles of mutant mice between 4 and 8 weeks to explore the cause of sudden death. **Methods and Results**: Left ventricular (LV) muscles were isolated from wild type (WT) and homo mutant hearts and were loaded with di-4-ANEPPS. Membrane potential signals were determined using a laser scanning confocal microscope. Gene expression levels were quantified by real-time RT-PCR. In mutant hearts at 8 weeks, spontaneous action potentials were frequently seen and action potential duration was prolonged compared to those from WT. These features were not obvious at 4weeks. Real-time PCR analysis of mutant LV showed age dependent changes in gene expression levels of some $K^+$ channels including Kv4.2. **Conclusion**: These results suggest that the age-dependent alteration in various ion channels may contribute to both APD prolongation and abnormal automaticity, then enhance susceptibility to lethal arrhythmias in the DCM model mice.

**1751-Pos**

**New Insights into Sexual Dimorphism During Progression of Heart Failure and Rhythm Disorders**

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Mice overexpressing the human beta2-adrenergic receptors (TG4 mice) develop heart failure (HF) leading to higher mortality than WT mice. HF appears earlier in TG4 males and those animals have a more severe phenotype than TG4 females, with earlier appearance of sudden cardiac death, corroborating observations in human before menopause. We assessed the electrophysiological status of TG4 male and female mice through heart rate variability analysis (HRV), intracardiac electrophysiological exploration (IEE) and patch-clamp study in order to understand female protection. The role of gonadal hormones in HF progression was studied through gonadectomy procedure. HRV was decreased in TG4 comparing with WT, with a higher decrease in males (−48%) than in females (−35%). IEE revealed a lengthening of infrahisian conduction time (+29%) associated to a larger QRS duration (+27%) only in TG4 males. A high prevalence of spontaneous and electro-inducible premature ventricular contractions was observed only in old-TG4 males. No difference was observed in females with regards to arrhythmias. Gonadectomy improved cardiac phenotype in TG4 males whereas ovariectomy worsened it in females. TG4 left ventricular cardiomyocytes were hypertrophied only in males ($169 \pm 7$ vs. $204 \pm 11$ pF, $n = 20$) but male and female TG4 presented an increase in action potential repolarisation with no gender-related difference as compared with WT (+200%). Longer action potentials reflected a significant decrease in outward voltage-gated K+ current densities in male and female TG4 cells. Assessment of histological alterations confirmed that high mortality in TG4 males is associated with severe cardiac fibrosis while in female no difference was found between WT and TG4 mice.

In summary, the progression and severity of HF in TG4 mice are linked to sex-hormones. A link between fibrosis, conduction time, and mortality was established in relation with sex hormones.

**1752-Pos**

**Efficient Biolistic Transfection of Fresh Adult Cardiac Myocytes with a Tagged Kv1.5 Channel**

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Modulation of ion channel trafficking is a potent means by which a cardiomyocyte can regulate its excitability. Much has been learned about the roles of motifs within K+ channels that affect their trafficking to the cell surface. However, by necessity, previous studies have relied on model expression systems, because the transfection of adult cardiomyocytes has, to date, proved intractable. Rat neonatal myocytes can be transfected but the currents expressed in these cells are quite different from those of adult cardiomyocytes. Viral transduction systems are effective in adult cells but require sophisticated containment facilities and prolonged culture of the myocytes, during which time substantial dedifferentiation generally occurs.

We have developed a new method that, for the first time, allows the ready and convenient transfection of acutely isolated adult rat cardiac myocytes. Using a low pressure adaptation of a Bio-Rad Helios gene gun procedure, we have achieved efficient transfection of rat ventricular myocytes bombarded within two hours of myocyte isolation with gold particles coated with pcDNA3 constructs encoding tagged Kv1.5 constructs. Expression is rapid, robust, and detectable less than 24 hours post-transfection in myocytes retaining both current profiles and gross morphology comparable to freshly isolated cells. Using this system, we unequivocally demonstrate that tagged Kv1.5 is efficiently localized to the intercalated disk in ventricular myocytes and that it is expressed at the surface of that structure. We further demonstrate that Kv1.5 deletion mutations known to reduce the surface expression of the channel in heterologous cells similarly reduce the surface expression in transfected ventricular myocytes, although targeting to the intercalated disk per se, was generally unaffected. Thus, this new transfection method is an effective tool for the study of cardiac ion channel expression and targeting in a physiologically relevant system.

**1753-Pos**

**Generation of Sodium-Permeable Cav1.3 Channel: Insights into the Molecular Basis for the Sustained Inward Current in Cardiac Pacemaker Cells**

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The sustained inward current ($I_{st}$) is a novel pacemaker current identified in spontaneously active sinoatrial and atrioventricular node cells of rabbits, guinea-pigs, rats and mice. Because $I_{st}$ is activated and produces an inward current over the entire range of the slow diastolic depolarization, its contribution to the pacemaker activity has been suggested. However, due to the absence of specific blockers and unidentified molecular determinants, it is still difficult to directly investigate the significance of $I_{st}$ in cardiac automaticity. Although $I_{st}$ is a $Na^+$ current, its pharmacological properties are qualitatively identical with those of L-type $Ca^{2+}$ current ($I_{Ca,L}$). In the present study, we generated a $Na^+$-permeable $Ca_V1.3$ channel by a substitution of key glutamate residue

in the $Ca^{2+}$-selective filter of the pore with lysine (E1160K). The $Ca_V1.3$-E1160K channel expressed in HEK cells evoked an inward current carried by $Na^+$ even in the presence of extracellular $Ca^{2+}$ (1.8 mM). The $Na^+$ current was characterized by a slow inactivation kinetics, a low activation threshold (~−60 mV) and sensitivity to $I_{Ca,L}$ blockers such as nifedipine, diltiazem and $Cd^{2+}$. These properties of the $Ca_V1.3$-E1160K current were very similar to those of $I_{st}$, suggesting that an $I_{Ca,L}$ channel variant with altered ion selectivity may mediate $I_{st}$. Besides, application of the $Ca_V1.3$-E1160K channel to the biological pacemaker would be an intriguing approach to understand the impact of $I_{st}$ on cardiac pacemaking.

**1754-Pos**

**Evidence of a Pro-Arrhythmic Substrate in the Failing Right Ventricle of Pulmonary Hypertensive Rats**

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Arrhythmic risk is increased in patients with heart failure. We have investigated the arrhythmic state of the failing right ventricle in a model of pulmonary hypertension (PAH).

Wistar rats were injected intraperitoneally with monocrotaline (MCT, 60 mg/kg) to induce PAH and right ventricular failure within 3-4 weeks and compared to age-matched saline-injected animals (CON).

*In vivo* measurement of ECG parameters using radiotelemetry indicated modification of T wave-parameters in MCT treated animals e.g. a prolonged QT interval (CON 49.7 ± 2.0 *vs.* MCT 76.2 ± 2.5 ms, $P<0.001$) and time from the peak to the end of the T-wave (Tpe, CON 25 ± 1.8 *vs.* MCT 33.1 ± 1.7 ms, $P = 0.007$) (CON $n = 6$, MCT $n = 7$).

Animals were humanely killed upon showing clinical symptoms of HF. Monophasic action potentials (MAPs) were recorded at the right ventricular epicardial surface of isolated hearts and a S1-S2 protocol used to construct standard APD restitution curves. MAP duration was significantly prolonged in failing hearts (MAP90, 39.9 ± 1.9 ms in CON *vs.* 80.7 ± 3.5 ms in MCT, $P<0.001$) and standard restitution slopes were steeper (mean maximum slope was 0.18 ± 0.02 CON *vs.* 0.73 ± 0.28 MCT, $P<0.001$).

Optical action potentials were recorded at stimulation frequencies between 5-12 Hz using the voltage-sensitive dye di-4-ANEPPS and dynamic APD and conduction velocity restitution curves measured. The failing right ventricle exhibited steeper restitution and conduction velocity restitution curves (mean maximum slope for conduction velocity was 0.013 ± 0.004 MCT *vs.* 0.002 ± 0.001 CON, $P<0.001$). At high pacing frequencies, arrhythmias were induced in failing but not in control hearts.

T-wave modification, APD prolongation, steeper APD and conduction velocity restitution curves are typically associated with a pro-arrhythmic state. We conclude that the failing right ventricle of pulmonary hypertensive rats have an elevated risk of developing arrhythmias. The underlying mechanisms are under investigation.

**1755-Pos**

**Pregnant Mice Exhibit an Increase in the Automaticity and the Pacemaker Current $I_F$ in Sinoatrial Node Cells**

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The incidence of some types of arrhythmias is increased during pregnancy. Changes in hormonal levels, autonomic tone and hemodynamic parameters associated with pregnancy can be involved in these arrhythmias. Moreover, our preliminary findings show that resting heart rate is elevated in pregnant mice. Since increased resting heart rate is a risk factor for the development of cardiac arrhythmias it is important to understand specifically how pregnancy alters pacemaker function. Thus the purpose of the present study was to examine the effects of pregnancy on automaticity in sinoatrial cells (SANC) as well as the ion currents that underlie cardiac pacemaker function. Spontaneously beating cells were isolated from the sinoatrial node (SAN) from pregnant mice (PM) and non-pregnant mice (NPM). Current-clamp recordings revealed that the beating rate of PM-SANC (319 ± 10 bpm; $n = 17$) was elevated in comparison to SANC from NPM (282 ± 16 bpm, $n = 10$). Moreover, SANC action potential threshold ($E_{th}$) was more depolarized in PM (PM −38 ± 2, $n = 16$; NPM −43 ± 2 mV, $n = 10$; $p<0.05$) and the upstroke velocity of diastolic depolarization also was faster (PM 0.39 ± 0.05 mV/ms, $n = 14$; NPM 0.21 ± 0.05 mV/ms, $n = 10$ $p<0.05$). Next voltage-clamp experiments were used to investigate pacemaker current ($I_f$), the predominant ionic mechanism underlying cardiac automaticity. Results showed that peak $I_f$ density at −100 mV was higher in PM-SANC (−26 ± 4 pA/pF, $n = 13$) compared to NPM-SANC (−15 ± 2 pA/pF, $n = 8$; $p<0.05$). Overall, the results show that $I_f$ is increased during pregnancy and this likely contributes to the increase in beating rate in SANC. These alterations in pacemaker activity could contribute to the higher heart rate observed in pregnancy.

**1756-Pos**

**Regulation of Volume-Sensitive Chloride Current in Cardiac HL-1 Myocytes**

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HL-1 cells derived from mouse atrial myocytes retain many features of differentiated adult cardiomyocytes, continuously divide, and are emerging as a useful experimental tool. Features of several HL-1 cation channels have been described, but the characteristics of its $Cl^-$ channels are unknown. We studied regulation of volume-sensitive $Cl^-$ current, $I_{Cl,swell}$, under conditions that isolate anion currents. Modest osmotic swelling (0.85T; T, times-isosmotic) elicited robust outwardly-rectifying $Cl^-$ currents in virtually every HL-1 cell (typically, 15 - 30 pA/pF at +60 mV; $E_{Cl} = -40$ mV). As expected for $I_{Cl,swell}$, $Cl^-$ current in 0.85T was fully inhibited by DCPIB (10 μM) and was outwardly rectifying in both physiological and symmetrical $Cl^-$ gradients. Regulation of HL-1 $I_{Cl,swell}$ matched that in enzymatically dissociated adult cardiomyocytes. In 0.85T, HL-1 $I_{Cl,swell}$ was fully blocked by both the NADPH oxidase inhibitor gp91ds-tat (500 nM) and the mitochondrial ETC inhibitor rotenone (10 μM), and in isosmotic bath solution (1T), DCPIB fully suppressed $H_2O_2$-induced (100 μM) $I_{Cl,swell}$. Furthermore, as in adult cardiomyocytes, endothelin-1 (ET-1; 10 nM) activated a DCPIB-sensitive current in 1T that was outwardly-rectifying in HL-1 cells with physiological and symmetrical $Cl^-$ gradients. ET-1-induced HL-1 $I_{Cl,swell}$ was suppressed by the $ET_A$ receptor blocker BQ123 (1 μM) and by blocking ROS production with gp91ds-tat. HL-1 $I_{Cl,swell}$ also was activated by bacterial sphingomyelinase (0.03 U/mL) that produces ceramide. These findings in HL-1 cells recapitulated the biophysical and pharmacological features of $I_{Cl,swell}$ and its regulation by ROS, endothelin, and ceramides in adult myocytes. Our data indicate that HL-1 cells are a useful tool for dissecting the regulation and role of $I_{Cl,swell}$ in cardiac myocytes.

**1757-Pos**

**The LQT1 Phenotype of the KCNQ1 H258R Mutant is Unmasked by Faster Stimulation Rates**

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The long QT syndrome is a cardiac disorder caused by a delayed ventricular repolarization. LQT1 is linked to mutations in the KCNQ1 gene that codes for the six transmembrane spanning α-subunit of the channel complex that underlies $I_{Ks}$ *in vivo*. The LQT1 mutation H258R, located in the S4-S5 linker, resulted in subunits that failed to generate current in a homotetrameric condition. However, association with hKCNE1 'rescued' the mutant subunit and generated $I_{Ks}$-like currents. Compared to WT hKCNQ1/hKCNE1, H258R/hKCNE1 displayed accelerated activation kinetics, slowed channel closure and a hyperpolarizing shift of the voltage-dependence of activation, thus predicting an increased $K^+$ current. However, current density analysis combined with subcellular localization indicated that the H258R subunit exerted a dominant negative effect on channel trafficking. The co-expression hKCNQ1/H258R/hKCNE1, mimicking the heterozygous state of a patient, displayed similar properties. During repetitive stimulation the mutant yielded more current compared to WT at 1 Hz but this effect was counteracted by the trafficking defect at faster frequencies. Thus at faster stimulation rates there would be less repolarizing $K^+$ current compared to WT, explaining the disease causing effect of the mutation. In terms of H258R being 'rescued' by hKCNE1, it seems less likely that this occurs through a pure chaperone-type mechanism and based on the altered gating kinetics we suggest that hKCNE1 rescues H258R by restoring the gating machinery. It has been proposed that hKCNE1 modulates hKCNQ1 kinetics by stabilizing the interaction between the S4-S5 linker and bottom part of S6. Therefore, we speculate that the H258R mutation disrupts the contact with S6 resulting in distorted subunit folding. The association with hKCNE1 then stabilizes the electromechanic coupling and in this way compensates for the destabilization caused by the H258R mutant.

**1758-Pos**

**In Vitro Cardiac Repolarization Assays: Guinea Pig Papillary Muscles *Vs.* Canine Purkinje Fibers**

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