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 ± 7 vs. 204 ± 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