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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