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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Delayed or accelerated cardiac repolarization is potentially proarrhythmic. In this study, we compared a guinea pig papillary muscle APD (GPPM-APD) assay to a canine Purkinje fiber APD (CPF-APD) assay in assessing drug effects on cardiac repolarization. Papillary muscles (right ventricle) and Purkinje fibers were stimulated at 0.5 Hz, action potentials were recorded using microelectrode techniques, repolarization assessed using $APD_{30,\ 50,\ 90}$ values, and the calculated triangulation parameter, $APD_{30\text{-}90}$. Eight compounds (6 positives, 2 negatives) were tested in both preparations. Percent changes in APD_{90} values obtained with the GPPM-APD assay were less than those obtained in the CPF-APD assay (table). Repolarization parameters in the GPPM-APD assay exhibited a rank sensitivity order of $APD_{30} < APD_{50} \approx APD_{90} < APD_{30\text{-}90}$ in detecting effects of the five hERG blockers on repolarization. APD_{90} was the most sensitive parameter in detecting effects of the hERG activator (A-935142.0). These results suggest the GPPM-APD assay and the CPF-APD assay are valuable in assessing drug effects on cardiac repolarization with comparable effects in both assays.

Compounds	APD_{30}		$APD_{30\text{-}90}$
	CPF[†]	GPPM[†]	GPPM[†]
DMSO 0.1%	2 ± 1	1 ± 2	3 ± 3
Tolterodine 700 nM	55 ± 3	15 ± 5*	39 ± 10#
Moxifloxacin 450 μM	178 ± 15	81 ± 12#	140 ± 26#
E-4031 0.2 μM	146 ± 11	36 ± 5*	53 ± 6*
Cisapride 10 μM	34 ± 4	22 ± 4*	63 ± 11#
Haloperidol 2.66 μM	3 ± 4	12 ± 3*	32 ± 5*
A-935142.0 60 μM	-24 ± 2	-18 ± 2*	-13 ± 5*

[†]Values are expressed as a mean percent change from baseline ± SEM. n = 4-6
*p<0.05 vs. vehicle (unpaired t-test); #p<0.05 vs. vehicle (Mann-Whitney)

1759-Pos

Increased Cardiac Risk in Concomitant Methadone and Diazepam Treatment: Pharmacodynamic Interactions in Cardiac Ion Channels

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Methadone, a synthetic opioid used in the treatment of chronic pain and in maintenance of withdrawal from opioid dependence, has been linked to QT prolongation, potentially fatal torsades de pointes, and sudden cardiac death. Concomitant use of benzodiazepines, such as diazepam, in methadone maintenance treatment appears to increase the risk of sudden death. Our objective was to determine the effects of methadone and diazepam singly and in combination on the major cardiac ion channels, responsible for the cardiac repolarization, stably expressed in mammalian cells. Using automated patch-clamp technique (PatchXpress®) for ion channel current recording, we found that methadone produced concentration-dependent block of hERG (IC_{50} = 1.7 μM), $hNa_v1.5$ (11.2 μM tonic block; 5.5 μM phasic block), $hCa_v1.2$ (26.7 μM tonic; 7.7 μM phasic) and hK_vLQT1/hminK (53.3 μM). Diazepam demonstrated much less potent block to block of these ion channels: the IC_{50} values were 53.1, >100 tonic and 47.7 phasic, 89.0 tonic and 82.1 phasic, and 86.4 μM for hERG, $hNa_v1.5$, $hCa_v1.2$ and hK_vLQT1/hminK, respectively. Co-administration of 1μM diazepam with methadone had no significant effects on methadone-induced block of hERG, $hCa_v1.2$ and hK_vLQT1/hminK channels, but caused a 4-fold attenuation of $hNa_v1.5$ block (44.2 μM tonic and 26.6 μM phasic). Thus, although diazepam alone does not prolong the QT interval, the relief of the methadone-induced Na^+ channel block may leave hERG K^+ channel block uncompensated, thereby creating a potentially greater cardiac risk.

1760-Pos

Optimization of a Cav1.2 Cell Line for Use on QPatch and PatchXpress

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Ion channel currents comprise the cardiac action potential and are important in cardiac safety liability assessment of potential drug candidates. The gold standard for assessing ion channel activity is the voltage clamp technique, but this technique is a very low throughput process. Planar patch technology (QPatch and PatchXpress) allows for moderate throughput by providing automated, simultaneous whole cell voltage clamp recordings from cells heterologously expressing the channel of interest. Ion channels routinely screened for cardiovascular safety are hERG (Kv11.1), Nav1.5, Kir2.1 and KvLQT/minK using either PatchXpress or QPatch instruments. In this study, we highlight the validation of Cav1.2 (L-type calcium channel) on our automated electrophysiology systems for cardiovascular safety screening. The L-type calcium channel is expressed in the cardiovascular system both in smooth and cardiac muscle. Potent L-type calcium channel antagonists can lower blood pressure, reduce cardiac contractility, and potentially increase the P-R interval on the electrocardiogram (ECG). Cav1.2 was expressed in CHO cells using a tetracycline inducible vector. Because of this we needed to optimize expression level by varying the induction variables. Addition of an L-type antagonist (verapamil) also provided benefit by keeping well-expressing cells viable after induction. In order to optimize flexibility in performing experiments, we also prepared the cells as a cryo-preserved subtrate. Cav1.2 channel kinetics for both activation and inactivation were investigated, and potencies of 8 reference compounds (weak and strong antagonists) were assessed on both platforms. In conclusion, we have optimized tissue culture conditions, cell preparation and voltage clamp protocols on two automated electrophysiology platforms to provide cardiac safety evaluation of drug candidates using an inducible Cav1.2 cell line.

1761-Pos

Pacemaker Cells of the Atrioventricular Node are $Ca_V1.3$ Dependent Oscillators

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The atrioventricular node (AVN) can generate pacemaker activity in case of failure of the sino-atrial node (SAN). However, the mechanisms underlying pacemaking in AVN cells (AVNCs) are poorly understood. Voltage-dependent ion channels such as hyperpolarization-activated HCN channels, L-type $Ca_V1.3$ and T-type $Ca_v3.1$ channels are known to play a role in pacemaking of sino-atrial node cells (SANCs). Here, we investigate the role of these channels in AVNCs pacemaker activity using genetically modified mouse strains and show that they differentially impact pacemaking of AVNCs than of SANCs. Indeed, contrary to SANCs, $Ca_v1.3$ channels are necessary for pacemaking of AVNCs and accounted for the predominant fraction of $I_{Ca,L}$. Inactivation of $Ca_v3.1$ channels impaired automaticity in AVNCs by promoting sporadic block of automaticity and spontaneous cellular arrhythmia.
Abolition of the cAMP sensitivity of HCN channels shifted the I_f activation to voltages negative to that spanning the diastolic depolarization and prevented AVNCs automaticity in basal conditions. However pacemaker activity could be restored to control levels by adrenergic receptor stimulation.
Inactivation of both $Ca_v1.3$ and $Ca_v3.1$ results in abolishment of pacemaking. Inhibition of TTX-resistant (I_{Nar}) Na+ current showed that this is a key contributor of the action potential (AP) threshold and upstroke velocity.
Conclusion: 1. Spontaneous firing rate in AVNCs is strongly dependent from $Ca_v1.3$-mediated L type calcium current and from TTX resistant sodium current ($I_{Na,r}$). 2. In AVNCs the $Ca_v1.3$ isoform seems to be predominant compared to $Ca_v1.2$ isoform. 3. The fact that hyperpolarization of $Ca_v1.3^{-/-}$AVNCs pacemaking can be observed suggests that the absence of pacemaker activity is not due to the impossibility to generate the upstroke phase of the AP but probably due to an imbalance between outward and inward currents during the diastolic depolarization.

1762-Pos

Development of Novel System for the Functional Analysis of the Cardiomyocytes Network Model Using *On-Chip* Cellomics Technology

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Spatial and temporal regulation of cellular orientation is one of the key to resolve the mechanism of organs and tissues that are complexly intertwined with epigenetic factors, such as cellular network size and orientation of cellular-type. To study the dynamics of synchronous beating rhythm in the cardiac myocytes, we tried to develop the agarose micro-chamber (AMC) system on the multi-electrode array (MEA) chip, and extra-cellular signals of cardiomyocytes in geometrically patterning chambers were recorded with *On-Chip* MEA system. The chip set consists with the type of MEA chip, pattern of AMC and cellular-type, for example, primary *mouse* embryonic, ES and iPS derived cardiomyocyts. By using the cell handling by micropipette and additional fabrication to the AMC during the cultivation, we are able to construct the normal and disordered model. For example, we made the loop structure as reentry model having length of the circuit above the millimeter. Under this system, it is possible to obtain a multiple of information about individual cell (as constitutional unit) and entire network (as organ model) by field potential recordings (FPs) and optical imaging. Pseudo-ECG, which sum the FPs obtained from each electrode, means whole network signal and duration time of pseudo-ECG corresponds to QT interval. From analysis of individual FPs, direction of the excitement propagation and conduction velocity is resolved. Waveform analysis of FPs give us the relative intensity of Na^+, Ca^{2+} and K^+ currents and field potential duration (FPD) corresponding to action potential duration (APD). *On-Chip* geometric re-constitutive approaches are powerful tools for stepwise cell network construction and long-term measurement of it, and also will make possible the development of the novel system for the toxicity studies of drugs.