Background: Nesiritide (B-type natriuretic peptide) improves hemodynamic function and heart failure status for patients with decompensated congestive heart failure. However, studies associated the use of nesiritide with an increased risk of sudden death, to date, little is known of the underlying mechanisms. In this study, the effect of BNP on action potential duration and underlying electrophysiologic mechanisms were investigated in rat hearts.
Method: Wistar rats were anesthetized with sodium pentobarbital (40mg/kg) and injected with BNP-32 (12μg/kg) from abdominal vena cava. EKG was recorded using electrodes inserted into the subcutaneous layer of the paws. Action potentials from 8 rat left ventricles were recorded by a high-resolution optical mapping with voltage-sensitive dye RH237 in Langendoff perfusion system. Transient outward potassium current of rat ventricular myocytes was recorded by the whole cell configuration of patch clamp technique.
Results: BNP-32, at a clinically relevant concentration, prolonged corrected QT interval (QTc = QT / $\sqrt{}$ RR) obviously in adult rats, whereas heart rate was comparable during and after BNP-32 treatment. BNP-32 (0.1μM) increased action potential duration at 50% (APD_{50}) repolarization (45.62 ± 4.45ms, $P<0.01$) and this effect persisted after 15 min of washout (57.71 ± 12.62ms, $P<0.05$) compared to baseline (BL: 41.22 ± 2.88ms). In control group of 6 rat hearts, APD_{50} had no obvious changes over the same time period without BNP perfusion. The peak transient outward potassium current at +60mV was significantly reduced (7.11 ± 4.97 to 2.85 ± 3.30 pA/pF, n=6; $P<0.05$) by 0.01μM BNP-32, and then partially recovered to 5.85 ± 4.12 pA/pF (n=6, $P<0.05$) after washout of BNP-32.
Conclusion: BNP prolongs action potential duration and reduces transient outward potassium current in rat hearts, which might contribute to BNP-induced increase of death risk in decompensated heart failure patients.

2126-Plat
The Mitochondrial Bioenergetic Phenotype for Protection from Ischemia in Sur2-Mutant Mice
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ATP-sensitive potassium channels (K_{ATP}) in mitochondria are postulated to play a key role to protect mitochondria and myocytes from cardiac ischemic insult. The sulfonylurea receptor-2 (SUR2) is a subunit of K_{ATP} in sarcolemma, although its role in mitochondrial physiology is unclear. Mice where the SUR2 gene was disrupted (SUR2 mutant) have been shown to be constitutively protected from ischemic injury.
We characterized the bioenergetic phenotype of mitochondria in SUR2 mutant mice to gain insight into mechanisms of protection from ischemia.
Membrane potential ($\Delta\Psi_m$), Ca^{2+} uptake, and reactive oxygen species (ROS) generation were studied in the isolated mitochondria by fluorescence based assays and K^+-influx was studied by volume measurements. Mitochondrial respiration was studied in normoxia and after hypoxia-reoxygenation. Myocyte protection against metabolic inhibition was also investigated. $\Delta\Psi_m$ was depolarized (53.37 ± 1.5 vs 48.4 ± 1.8 %), tolerance to Ca^{2+} loading was increased (163 ± 26 vs 116 ± 23 μM), and ROS generation was increased (9.3 ± 0.4 vs 7.4 ± 0.6 FU/sec) in the SUR2 mutant mitochondria compared to wild type (Wt). SUR2 mutant mitochondria had greater swelling (30.2 ± 3.1%) compared with Wt (14.5 ± 0.6%) indicating greater K^+ influx. SUR2 mutant mitochondria recovered better from post hypoxia-reoxygenation than Wt as measured by the respiration control index (RCI). Finally, the SUR2 mutant myocytes viability was better protected against metabolic inhibition.
We concluded that SUR2 plays a key role in mitochondrial mechanisms of protection from ischemia by altering a potassium conductance consistent with a mitochondrial K_{ATP} and causing a protected mitochondrial phenotype.

2127-Plat
Multiple Mechanisms of hERG Liability: K^+ Current Inhibition, Disruption of Protein Trafficking, and Apoptosis Induced by Amoxapine
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The antidepressant amoxapine has been linked to QT prolongation, acute heart failure, and sudden death. Drug binding to cardiac hERG (Kv11.1) potassium channels causes prolonged repolarization and induces apoptosis. This study was designed to investigate amoxapine effects on hERG currents, hERG protein trafficking, and hERG-associated apoptosis in order to elucidate molecular mechanisms underlying cardiac side effects of the drug. hERG channels were expressed in *Xenopuslaevis* oocytes and HEK 293 cells, and potassium currents were recorded using patch clamp and two-electrode voltage clamp electrophysiology. Protein trafficking was evaluated in HEK 293 cells by Western blot analysis, and cell viability was assessed by immunocytochemistry and colorimetric MTT assay. Amoxapine caused acute hERG blockade in oocytes (IC_{50} = 21.6 μM) and in HEK 293 cells (IC_{50} = 5.1 μM). Mutation of residues Y652 and F656 attenuated hERG blockade, suggesting drug binding to a receptor inside the channel pore. Channels were mainly blocked in open and inactivated states, and voltage-dependence was observed with reduced inhibition at positive potentials. Amoxapine block was reverse frequency-dependent and resulted in accelerated and leftward-shifted inactivation. Furthermore, amoxapine caused chronic reduction of hERG trafficking into the cell surface membrane (IC_{50} = 15.3 μM). Finally, the antidepressant drug triggered apoptosis in cells expressing hERG channels. Triple mechanisms of hERG liability associated with a single compound, are revealed. Amoxapine causes direct hERG current inhibition and disruption of hERG protein trafficking. Furthermore, the drug induces apoptosis of cells expressing hERG potassium channels.

2128-Plat
Cardiac Glycoside Chronotropic and Arrhythmogenic Effects in Sinoatrial Nodal Pacemaker Cells (SANC) Occur Along a Continuum of Electrochemical Gradients of Na^+ (E_{Na}) and Ca^{2+} (E_{Ca})
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Cardiac glycosides reduce E_{Na} and E_{Ca} (due to an increase of Na^+_i via Na-K pump inhibition, and of Ca^{2+}_i, due to a secondary reduction in Ca efflux for Na influx via NaCa exchange). Here we show that exposure of single rabbit SANC to the cardiac glycoside, digoxigenin (10-20μM) results in a continuum of time-dependent effects. Within 30s to 1 min, the rate of rhythmic spontaneous action potentials (AP) increases by 20% (n=3) and this is associated with an earlier occurrence (reduced period) of local sub-membrane Ca^{2+} releases (LCR's) during diastolic depolarization, detected by confocal Ca^{2+} imaging. Approximately 1-3 minutes following AP rate acceleration, LCR period lengthens by 40%, accompanied by a similar reduction in the rhythmic AP rate. The changes in LCR period during the biphsic changes in rhythmic AP firing rate increase are highly correlated with the changes in AP cycle length (R^2=0.98). A progressive increase in the steady level of diastolic Ca^{2+} beneath the surface membrane then ensues usually within 4 to 6 additional minutes, LCR's became undetectable, and dysrhythmic and chaotic AP firing occurs. Numerical model simulations (Maltsev-Lakatta model, AJP 2009) in which Na_i was increased progressively 5-15mM during glycoside exposure reproduced the experimental results. That rate and rhythm regulation of SANC AP firing during cardiac glycoside exposure occurs along a continuum of E_{Na}/E_{Ca} is in agreement with repeated observations over the last decade, showing that the SANC spontaneous AP firing rate is critically dependent on the timing of acute changes in sub-membrane E_{Ca} during DD caused by LCR occurrence (LCR period) that accelerates DD by activation of an inward Na/Ca exchange current.

2129-Plat
Calcium Currents in Chronically Dysfunctional Pig Myocardium
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Chronic reduction in coronary artery blood flow due to stenosis results in reduced myocardial contractile function, termed "hibernating" myocardium. This change involves electrical remodeling and a propensity for sudden cardiac death in the absence of infarction. We used a pig model of chronic left anterior descending artery stenosis to study calcium currents associated with the hibernating myocardium. We developed a cell isolation technique from punch biopsies of the left ventricle. This yields calcium-tolerant isolated myocytes with brick shaped morphology and clear striation patterns. Myocyte length from hibernating myocardium averaged 145 ± 7.7 (n=15) vs. 124 ± 4.8 μm (n=42) for control, suggesting cellular hypertrophy. The cell shortening of hibernating cells was also reduced compared to the remote region from 6.4 ± 1% (n=21) to 4.45 ± 1% (n=11) suggesting that the reduced contractility seen in the hibernating region was preserved in the isolated myocytes. Furthermore, cells isolated from hibernating myocardium had significantly higher numbers of premature contractions 4/15 for hibernating vs. 0/19 for control suggesting a cellular propensity for arrhythmias. The L-type calcium channel current ($I_{Ca,L}$) in myocytes from hibernating myocardium (1.35 ± 0.08 pA/pF, n=22) was reduced compared to normal myocardium (1.96 ± 0.07 pA/pF, n=33; $P<0.01$). The voltage dependence of steady state activation and inactivation were nearly