change the diminishing effect of the ablation on spontaneous activity (n=3). Epicardial imaging showed that spontaneous ectopic beats were manifested as concentric epicardial breakthrough patterns, located near spatiotemporal gradients of NADH fluorescence. These data strongly suggest that in un-paced hearts from healthy rats that are perfused with Tyrode's solution, the main mechanism of spontaneous ectopic activity associated with either ischemia, low-flow or full-flow reperfusion is activation of local Purkinje fibers.

1324-Pos Board B168

Acute effects of Lipopolysaccharide on L-type Ca2+ channel currents and Transient Outward K+ channel currents in Rat Ventricular Myocytes

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Septic Shock has accounted for nearly 2 percent of all hospitalizations and has a mortality rate of 40-50%. It is a progression of sepsis, which is caused by bacterial infection in the blood and is characterized by a whole body inflammatory state known as systemic inflammatory response syndrome (SIRS). Cardiac dysfunction is one of the principal pathologies of sepsis and septic shock, along with other hemodynamic changes and dysfunction in multiple organs including the lungs and brain. While cardiac dysfunction is typically associated with the late stages of septicemia in clinical observations, animal models of septicemia have shown that cardiac dysfunction can occur well before late stages of sepsis and the induction of septic shock. Sepsis is caused by exposure to lipopolysaccharides (LPS), an endotoxin found in the outer membrane of gram-negative bacteria. LPS is a known Toll-like receptor 4 (TLR4) agonist which is associated with signaling cascades that lead to cellular inflammatory events. Studies have shown that short term exposure to LPS cause a significant increase in the amount of phosphorylated NF-κB in ARVCs. Using patch clamp single-cell electrophysiology we investigate acute effects of LPS exposure on membrane currents of adult rat ventricular cardiomyocytes (ARVC) *in vitro*. Given the necessity of L-type Ca^{2+} currents for proper myocardial function through excitation-contraction coupling (ECC) with links calcium membrane current with myocardial contraction, we investigate changes in L-type Ca^{2+} ($I_{Ca}{}^{2+}$). Due to its role in shaping the early phase of cardiac ventricular action potential we also investigate alterations of the transient outward Ca^{2+}-independent K^+ current (I_{TO}).

1325-Pos Board B169

Investigating Ion Channel Diseases With Dynamic Action Potential Clamp

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The number of ion channel mutations that are found in genetic screening of patients with cardiac arrhythmias is far outstripping our ability to functionally characterise all the mutations and assess the *in vivo* consequences of each mutant. Currently much time has to be invested in developing mathematical models of mutant ion channels in order to be able to predict their functional significance *in vivo*.

Recently the concept of a dynamic action potential clamp system was introduced that allows integration of electrophysiological recordings from mutant channels into *in silico* models of cellular systems. The current of interest is replaced in the model by current recorded from a mutant channel recorded using voltage-clamp electrophysiology, thereby bypassing the need to formulate a new mathematical model for the mutant ion channel. To simultaneously compute the action potential in the *in silico* model and record the ion current from a cell necessitates the use of a real time operating system so that the real cell can be clamped at the membrane potential of the virtual cell at all times (hence the term dynamic action potential clamp). The ionic current flow through the expressed channels would in turn contribute to changes in the membrane potential of the virtual cell model, so integrating the mutant channels into the virtual cell. We have developed a system where gating models are formulated using the graphical approach offered by Simulink / Matlab. Representation of the models in this way greatly simplifies the user interface compared to standard programming languages, making the system accessible to the less computer-savvy.

In the future we will use this system to study the effects of ion channel mutations on the cardiac function cardiac action potential.

1326-Pos Board B170

Estimating Contribution Of Individual Ionic Components To The Cardiac Pacemaker Potential

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Action potentials are generated by complicated interactions of various ionic channels and transporters through variations of membrane potential and/or the intracellular Ca^{2+} concentrations. It is still difficult to isolate the contribution of individual current from the secondary effect of modified channel activities. For example, if an ion channel is blocked, the pacemaker activity is drastically changed from the control time course. We have proposed a theoretical method to visualize the contribution of each current in the simulation study. Namely an instantaneous equilibrium potential, the lead potential (V_L), was calculated along the time course of pacemaker potential. V_L is given as,

$$V_L = (\Sigma G_X E_X - \Sigma I_{pump})/\Sigma G_X,$$

where G_X and E_X are whole cell membrane conductance and reversal potential for ion X. Contribution of a given current system is evaluated by comparing V_L obtained by fixing the gating parameter of a given current system with the control V_L. In the present study, the gating parameters are fixed during slow diastolic depolarization and action potential repolarization.

It is revealed that the initial phase of the slow diastolic depolarization is mostly attributable to deactivation of the rapid component of the delayed rectifier K^+ current (I_{Kr}). Then, the major inward currents, the hyperpolarization-activated current (I_f) and the sustained inward current (I_{st}) are activated to depolarize the membrane further to reach the threshold potential of the L-type Ca^{2+} channel current (I_{CaL}). Activation of I_{CaL} initiates generation of action potentials. Deactivation of I_{CaL} and activation of I_{Kr} and the slow component of the delayed rectifier K^+ current (I_{Ks}) occur during repolarizing phase. Contribution of I_{st} activation to the repolarization is much larger than that of I_f. Activation of I_f, I_{st}, I_{CaL} and I_{Ks} through phosphorylation during β1-adrenergic stimulation failed to modify each contribution dramatically.

1327-Pos Board B171

Extracellular Zinc Enhances Cardiomyocyte Relaxation Function in Diabetic Rats

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Diabetes mellitus (DM) leads to a cardiomyopathy in humans and rodent models. Interestingly, the chronic infusion of zinc in DM mice prevents the development of the cardiomyopathy. To elucidate the possible mechanism underlying this observation, we examined the effects of extracellular zinc ion (Zn^{2+}) on cardiomyocyte function in DM rats (n=5) compared to non-DM controls (Ctrl, n=5). Both the DM and Ctrl rats were hypothyroid, which assured similar upregulation of β-myosin heavy chain (β-MHC) in both populations. Isolated cardiomyocytes were electrically stimulated at 2, 4, 6 and 6.5 Hz, maintained at 35°C and exposed to 1.2 mM extracellular Ca^{2+}. Sarcomere shortening and relengthening dynamics were monitored using a video-based Fourier-transform technique. Without extracellular Zn^{2+} peak shortening as a fraction of diastolic sarcomere length was statistically greater ($P<0.05$) in the DM (6.56 ± 0.99 %; n = 16) compared to Ctrl (5.27 ± 1.91 %; n = 20) at 2 Hz, but not at higher frequencies. Time to 50% return to diastolic sarcomere length was not statistically different between the groups at every pacing frequency. Exposure to 12 μM extracellular Zn^{2+} significantly reduced ($P<0.001$) peak shortening in both the DM and Ctrl at all frequencies. There was a strong trend ($P = 0.070$) toward Zn^{2+} exposure significantly shortening the time to 50% return in the DM but not in the Ctrl, as revealed by repeated-measures ANOVA. Similar trends were found for time to peak shortening ($P=0.087$) and time to 10% return ($P=0.048$). These results suggest that extracellular Zn^{2+} inhibits cardiomyocyte contractile function independent of DM, perhaps by competing with intracellular Ca^{2+}, and enhances cardiomyocyte diastolic function in the DM. The enhanced sensitivity of the DM to the relaxing effects of Zn^{2+} may underlie the protective effects of Zn^{2+} against diabetic cardiomyopathy.

1328-Pos Board B172

Chronic Iron-overload Causes Sinus Bradycardia By Altering Electrical Activity In Sinoatrial Node Myocytes

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Treatment of blood disorders such as thalassemias require constant blood transfusions that cause iron-overload leading to iron-mediated cardiomyopathy, which is characterized by contractile dysfunction and electrical disturbances, especially bradyarrhythmias. In this study we explored the cellular mechanisms underlying iron-mediated bradycardia by examining the effects of chronic iron-overload (CIO; 0.6 mg/g iron-dextran 3 days/week for 4 weeks by IP injection) on heart rate and sinoatrial node (SAN) function in mice. As expected, heart rate (assessed with telemetry electrocardiograms), was lower ($p<0.001$) in CIO mice (509 ± 21 beats/min; $n = 5$) compared to controls (601 ± 12 beats/min; $n = 6$, dextrose injected). To examine intrinsic SAN function, heart rate was further studied in anesthetized mice following autonomic nervous system blockade with propranolol (10 mg/kg IP) and atropine (1 mg/kg IP), as

well as in isolated Langendorff-perfused hearts. In both cases heart rate was ~22% lower ($p<0.05$) in CIO mice suggesting iron-overload impairs SAN electrical activity. Indeed, spontaneous action potential (AP) frequency was reduced by 34% ($p<0.05$) in isolated SAN myocytes from CIO mice along with a reduction ($p<0.05$) in slope of the diastolic depolarization from 35.1 ± 3.6 V/s in controls to 18.8 ± 2.2 V/s in CIO. The maximum diastolic potential was unaltered in CIO myocytes. Voltage-clamp experiments showed that the reduction in SAN firing frequency was linked to a reduction ($p<0.05$) in L-type Ca^{2+} current ($I_{Ca,L}$) density from -4.8 ± 0.8 pA/pF to -2.6 ± 0.2 pA/pF along with a right shift ($p<0.05$) in the $V_{1/2}$ for activation from -20.2 ± 3.7 mV in control to -6.2 ± 2.6 mV in CIO SAN myocytes. In conclusion, the severe bradycardia caused by iron-overload originates from impaired intrinsic electrical activity and reduced $I_{Ca,L}$ in SAN pacemaker myocytes.

1329-Pos Board B173

Sex Hormones And β_2-adrenergic Stimulation Regulate Slow Delayed-rectifier Potassium Current In Control And Heart Failure Rabbits

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Little is known about sex differences in slow delayed-rectifier potassium current (I_{Ks}) in response to β-adrenergic stimulation.

Here, we assess the role of sex hormones on I_{Ks} in response to β_1- & β_2-AR stimulation in control and heart failure (HF) rabbits. I_{Ks} in control male increased in response to isoproterenol (ISO, 500nM) (at +50mV, Step: 1.07 ± 0.10 to 1.79 ± 0.23 pA/pF; Tail: 0.57 ± 0.04 to 0.93 ± 0.07 pA/pF, $p<0.05$), an effect blocked by β_2-AR antagonist ICI-118,551,150 nM (at +50mV, Step: 1.16 ± 0.14 pA/pF; Tail: 0.61 ± 0.06 pA/pF), but not by β_1-AR antagonist CGP-20712A, 300nM. I_{Ks} in control female was significantly less ($p<0.01$) than control male, but did not increase with ISO (at +50mV, Step: 0.62 ± 0.04 to 0.71 ± 0.04 pA/pF; Tail: 0.35 ± 0.02 to 0.41 ± 0.03 pA/pF). After castration, I_{Ks} in control male did not change with ISO (at +50mV, Step: 0.89 ± 0.07 to 1.10 ± 0.11 pA/pF; Tail: 0.50 ± 0.03 to 0.62 ± 0.05 pA/pF, p=NS), and after ovariectomy, I_{Ks} in control female now showed enhancement with ISO (at +50mV, Step: 0.74 ± 0.06 to 1.27 ± 0.09 pA/pF; Tail: 0.41 ± 0.03 to 0.72 ± 0.05 pA/pF,$p<0.01$ (a 72% increase in I_{KS} step comparable to the 64% increase in I_{Ks} step in control male)). With HF, sex differences in I_{Ks} responsiveness to ISO went away. HF male exhibited reduced I_{Ks} (vs control male) but I_{Ks} did not enhance with ISO (at +50mV, Step: 0.46 ± 0.02 to 0.50 ± 0.03 pA/pF; Tail: 0.28 ± 0.01 to 0.30 ± 0.01 pA/pF,p=NS). HF female still showed no significant I_{Ks} enhancement with ISO (at +50mV, Step: 0.61 ± 0.06 to 0.76 ± 0.11 pA/pF; Tail: 0.34 ± 0.03 to 0.42 ± 0.05 pA/pF, p=NS). Thus, there are important sex differences in β-AR stimulation of I_{Ks}, that are mediated by β_2-AR, and which are modulated by sex hormones. With HF, sex differences in basal I_{Ks} and its alterations during HF may underlie sex-based differences in arrhythmogenicity.

1330-Pos Board B174

More Effective and Safer Cardiac Electric Stimulation Using Multidirectional and Biphasic Stimuli

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Because the ability of electric fields to excite cardiac cells depends on stimulus direction, effective *in situ* cardiac stimulation requires relatively high stimulus amplitude. However, high-strength fields may cause electroporation and cell injury. In this study, we compared the effectiveness of unidirectional (US) and multidirectional stimulation (MS) in 16 populations of isolated, randomly-oriented cardiomyocytes. MS was achieved by automatically switching stimulus delivery among 3 electrode pairs oriented at 0, 60 and 120° with a reference axis. Stimuli were triplets of 5-ms voltage pulses applied 5 ms apart (total duration < refractory period). For US, single pulses were applied at only one direction at each run. Using US (monophasic pulses) for successive runs at all directions, mean threshold field (ET) was 3.8 ± 0.1 V/cm. US with 1.2xET at a single direction recruited 38 ± 1% of cells, whereas total US recruitment (the sum of recruitment at the 3 directions without intersection) was 83 ± 2%. With MS (1.2xET), recruitment reached 90 ± 2% ($p<0.05$ vs. single direction US). With biphasic pulses, ET and the stimulus amplitude required for ~90% recruitment were 20-25% lower than with monophasic stimuli ($p<0.05$). Thus the greater efficiency of MS was further enhanced by using biphasic stimuli. Experiments with high-strength pulses at a single direction showed that the field required for lethal injury in 50% of the tested cells (LE50) was 70 ± 2 (N=12) and 81 ± 1 V/cm (N=9) for monophasic and biphasic waveforms, respectively ($p<0.05$). Considering the safety index of electric stimulation as LE50/ET, we conclude that biphasic stimuli are safer (index ~26 vs. 18 for monophasic) because of both lower ET and potency of lethality (CNPq, CAPES, FAPESP).

1331-Pos Board B175

Decreased Inward-rectifier K^+ Current in Myocytes Isolated from a Mouse Model of CPVT

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Catecholamine-induced polymorphic ventricular tachycardia (CPVT) is a highly malignant inherited arrhythmia characterized by adrenergically-mediated bidirectional or polymorphic tachycardia leading to syncope and/or cardiac sudden death. Several mutations in the cardiac sarcoplasmic reticulum (SR) Ca^{2+} release channel (RyR2) with major functional consequences have been identified in human CPVT, which may cause juvenile sudden death induced by stress and exercise. Therefore CPVT showed the first demonstration that not only plasmalemmal but also SR Ca^{2+} channels are crucial in regulating cardiac excitability. The mechanism involved is still unclear and, in addition to the Na^+-Ca^{2+} exchanger, plasmalemmal ionic channels could play a role in the triggering of delayed afterdepolarizations. For example, IK1 is an inward rectifying potassium current, present in ventricular myocytes, which contributes to late repolarisation and clamps the resting membrane potential. IK1 down-regulation has been related to longer APD and both early and delayed afterdepolarizations in heart failure. In this work, we investigated the effect of the mutation R4496C of the RyR2 (mouse equivalent of the human R4497C). In freshly isolated cells, we examined IK1 in presence of low and high Ca^{2+} buffering conditions (the pipette contained 50 μM EGTA or 5 mM BAPTA, respectively) using whole cell configuration of voltage-clamp. We found that IK1 is reduced in heterozygous (R4496C +/-) myocytes dialyzed with 50 μM EGTA, as compared to WT cells. Interestingly, when 5 mM BAPTA was present in the pipette solution, IK1 was undistinguishable in R4496C +/- and WT myocytes. Theses results clearly indicate that IK1 is decreased in R4496C +/- and in the absence of fast cytosolic Ca^{2+} buffer. Accordingly, IK1 may be a target of the aberrant activity of RyR2 and may, therefore, actively contribute to the alteration of excitability of CPVT.

1332-Pos Board B176

Ventricular Sodium Currents Are Altered In CD4C/HIV Mice

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Cardiac arrhythmias have been reported in HIV patients. Studies have shown that HIV can alter ventricular potassium currents, however, little is known about the effect of HIV on ventricular sodium current (I_{Na}) even though changes in I_{Na} also can lead to rhythm disturbances. Thus, the objective of this study was to characterize the effect of HIV on ventricular I_{Na} in CD4C/HIV mice. These mice exhibit a severe AIDS-like disease. Patch-clamp techniques were used to examine I_{Na} and action potentials (AP) in ventricular myocytes isolated from HIV and wild-type (WT) mice. In HIV myocytes I_{Na} was significantly depressed between -60 and -30 mV (at -50 mV: HIV, -55.3 ± 4.3 pA/pF, n=15; WT, -79.4 ± 5.2 pA/pF, n=16). However, late I_{Na} was similar in both groups (HIV, -4.3 ± 0.4 pA/pF; WT, -4.4 ± 0.4 pA/pF n=22/group). AP amplitude was similar in HIV (90.7 ± 5.1 mV, n=11) and WT (99.8 ± 4 mV, n=15) myocytes, but the maximal velocity of the AP upstroke (V_{max}) was significantly decreased in HIV myocytes (HIV, 54.2 ± 9.6 mV/ms, n=11; WT, 99.2 ± 10.3 mV/ms, n=15). ECG telemetry recordings revealed that the QRS complex was significantly prolonged in HIV mice (HIV, 15.7 ± 0.2 ms, n=22; WT, 14.1 ± 0.5 ms, n=10). Previous studies have shown that elevated levels of cytokines can affect cardiac ion currents. In CD4C/HIV mice serum levels of TNF-alpha are elevated. The present study showed that serum levels of interleukin-1-beta also were elevated in HIV mice (HIV, 18.1 ± 3.1 pg/ml, n=3; WT, 5.1 ± 1.7 pg/ml, n=4). Overall, this study showed that I_{Na} is decreased in HIV ventricular myocytes and that this reduction is likely responsible for the observed prolongation of the QRS complex in HIV mice. These alterations could contribute to the development of cardiac rhythm disturbances.

1333-Pos Board B177

Biophysical Characterization of a Novel KCNJ2 Mutation Associated with Andersen-Tawil Syndrome and CPVT Mimicry

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Mutations in KCNJ2, the gene encoding the human inward rectifier potassium channel Kir2.1 (IK1), have been identified in Andersen-Tawil syndrome (ATS). ATS is a multisystem inherited disease exhibiting periodic paralysis, cardiac arrhythmias, and dysmorphic features at times mimicking catecholaminergic polymorphic ventricular tachycardia (CPVT). In this study, we identified a young female presenting with frequent ventricular extrasystoles and