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

non-sustained polymorphic ventricular tachycardia (VT), bi-directional VT, syncope, and mild QTc prolongation. The proband displayed dysmorphic features including micrognatia, clinodactylia and syndactyly. The patient's symptoms continued following administration of propranolol, but subsided after treatment with flecainide. Molecular genetic screening revealed a novel heterozygous mutation (c.779G>C/p R260P) in KCNJ2. Whole-cell patch-clamp studies conducted in TSA201 cells transfected with wild type human KCNJ2 cDNA (WT-KCNJ2) yielded robust IK1, but no measurable current in cells expressing the R260P mutant. Co-expression of WT and R260P-KCNJ2 (heterozygous expression) yielded a markedly reduced inward IK1 compared with WT alone (-36.5 ± 9.8 pA/pF vs. -143.5 ± 11.4 pA/pF, n=8, p>0.001, respectively at -90 mV) indicating a strong dominant negative effect of the mutant. The outward component of IK1 measured at -50 mV was also markedly reduced with the heterozygous expression vs. WT (0.52 ± 5.5 pA/pF vs. 23.4 ± 6.7 pA/pF, n=8, p>0.001, respectively). Conclusion: We report a novel KCNJ2 mutation associated with classical phenotypic features of Andersen-Tawil syndrome and CPVT mimicry. The R260P mutation produced a strong dominant negative effect leading to marked suppression of the inward rectifier potassium current.

1334-Pos Board B178

Ready-to-use CHO-NaV1.5 And Hek-hERG Instant Cells: Study On Frozen Cells Thawed And Immediately Patched At Manual And Automatic Patch Clamp Devices

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Well characterised cell lines and constant high cell quality are prerequisites for reliable data in electrophysiological studies. We developed the cell culture system ''Instant Cells'' that enables quality control of frozen cell batches and guarantees a constant cell quality. To show that the Instant Cells are suited for pharmacological studies, we have adapted CHO-K1 cells stably expressing hNaV1.5 and HEK 293 cells stably expressing hERG to the Instant Cells system.

The frozen Instant Cells were thawed, spun down and resuspended in a physiological buffer. Afterwards, the cell suspension was kept for four hours in the Cell Reservoir, a bench-top cell storage device. During this time span the cells were taken from the Cell Reservoir to be evaluated on a conventional and on an automated patch clamp device, the CytoPatchTM instrument.

We show that both types of Instant Cells have the same characteristics in terms of electrophysiological and pharmacological properties compared to permanently cultured cells:

- Trypan-blue tests showed 95 % vital cells after preparation.
- The mean peak current of the NaV1.5 Instant Cells was 12.5 nA, the mean tail current of the hERG Instant Cells was 1.2 nA.
- More than 80 % of the cells sealed (above 1 GOhm), more than 60 % were stable for 15-25 min (Rm was above 500 MOhm).
- The isochronal I/V relationship of the hERG activation and tail current and the NaV1.5 activation current were similar to freshly prepared cultured cells.
- No difference was observed in the dose-response relationship for blocking compounds between the Instant cells and the running culture of both cell types.

This proves that the Instant Cells are well suited to investigate ion channel pharmacology.

1335-Pos Board B179

Dual Variations in SCN5A and CACNB2b Underlie Cardiac Conduction Disease without Brugada Syndrome

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Introduction: Inherited loss of function mutations in *SCN5A*, the gene that encodes the α-subunit of the human cardiac sodium channel ($hNa_v1.5$), have been linked to overlapping syndromes including cardiac conduction disease (CCD) and Brugada syndrome (BrS). The mechanisms responsible for the development of one without the other are poorly understood.

Methods: Direct sequencing analysis was performed in a family with CCD. Wild type (WT) and variant channels were co-expressed with CD_8 cDNA in TSA201 cells for electrophysiological study. Green fluorescent protein (GFP)-fused WT or mutant *SCN5A* genes were used for confocal microscopy to assess channel trafficking.

Results: A novel *SCN5A* missense mutation, P1008S, was identified in all family members displaying 1st degree AV block, but not in unaffected family members nor in 430 reference alleles. Peak P1008S current was 11.77% of WT ($p<0.001$). Confocal microscopy showed that WT channels tagged with GFP were localized on the cell surface, whereas GFP-tagged P1008S channels remained trapped in intracellular organelles. P1008S current and trafficking could be rescued by incubation at room temperature, but not by incubation with mexiletine (300μM) at 37°C. We also identified a novel polymorphism (D601E) in CACNB2b. The variation in the β subunit of the calcium channel caused a slowing of inactivation of the L-type calcium channel current (ICa), significantly increasing total charge, when co-expressed with the $\alpha 1$ and $\alpha_2\delta$ subunits of the calcium channel in TSA201 cells.

Conclusions: Our results suggest that variations leading to a loss of function in INa coupled with a gain of function in ICa may underlie the development of cardiac conduction disease without Brugada syndrome.

1336-Pos Board B180

Expression and Distribution of Voltage Gated Ion Channels in Ferret SA Node

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Spontaneous diastolic depolarization in the sinoatrial (SA) node enables it to serve as pacemaker of the heart. The combination of variation of cellular morphology within the SA node and heterogeneity of ion channel expression in the atrium predict that ion channel expression would be different and more heterogeneous than in the atrium. To evaluate ion channel heterogeneity within the SA node, we used fluorescent in-situ hybridization to examine ion channel transcript expression in the ferret SA nodal region and atrial appendage. We analyzed transcripts for 24 voltage-gated K^+ channel alpha subunits, 4 hyperpolarization-activated cation channels, 3 voltage-gated Ca^{2+} channels and 6 voltage-gated Na^+ channels and 3 ancillary subunits. Immunofluorescence was used to verify localization patterns of voltage-dependent K^+ channels. Co-localizations were performed to observe any preferential patterns. Neuronal antibodies were used in association with K^+ channel transcripts and antibodies to segregate the associated patterns in cardiac tissue. There were some overlapping and non-overlapping binding patterns observed. As positive controls, oligonucleotide probes from Troponin I slow and Troponin I cardiac sequences were used. Measurement of different K^+ channel transcripts showed heterogeneous expression with many different patterns of expression, attesting to the complexity of electrical activity in the SA node. This study enabled us for the first time to analyze the microscopic distribution of different transcripts in contiguous images and in a continuous manner over a cross-section of the SA nodal region. Such information provides a better understanding of the role that ion channel heterogeneity might play a role in SA node pacemaker activity.

1337-Pos Board B181

The Anchoring Protein SAP97 Is Crucial For The Surface Expression Of *Shal* Kv Potassium Channels And Their Regulation By CaMKII In Cardiac Myocytes

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The *Shal*-type Kv channels (Kv4.x) account for a large part of the outward potassium current, I_{to}, in heart. Membrane-associated guanylate kinase proteins are major determinants of the organization of several ion channels however, few are known on the interaction between Kv4.x channels and cardiac MAGUK, SAP97 in the heart. Here using pulldown assays we found a direct interaction via the VSAL amino acid motif between the Kv4.x C-terminus and the SAP97 in rat and human myocardia. In Kv4.3-KChIP stable CHO cell line and using the whole cell patch clamp technique, SAP97 increased the Kv4.3 encoded current by a factor 2 (145 ± 19pA/pF *vs* 300 ± 52 pA/pF; n=11; $p<0.001$) without changes of current gating properties. SAP97 had no effect on Kv4.3 encoded current when channel were deleted of the VSAL motif (ΔSAL-Kv4.3). Suppression of SAP97 by using shRNA inhibited I_{to} in cardiac myocytes. In CHO cells ΔSAL-Kv4.3 channel-encoded current showed a marked acceleration of its time-dependent inactivation and was insensitive to CaMKII inhibition achieved by intracellular application of the CaMKII inhibitor KN93, or of inhibitory peptide. In HEK293 cells, SAP97 silencing reproduced the effects of CaMKII inhibition on the current kinetic and suppressed the interaction between Kv4.x C-terminus and CaMKII studied by pull down assay. Conclusion: The anchoring protein SAP97 enhances the functional expression of Kv4.x channels and facilitates its regulation by the CaMKII in cardiac myocytes.