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 L-type Ca\(^{2+}\) current (I\(_{\text{Ca,L}}\)) 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\(_{\text{1/2}}\) 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\(_{\text{Ca,L}}\) in SAN pacemaker myocytes.

**1329-Pos Board B173**

**Sex Hormones And \(\beta\)-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\(_{\text{KS}}\)) in response to \(\beta\)-adrenergic stimulation. Here, we assess the role of sex hormones in slow delayed-rectifier potassium current (I\(_{\text{KS}}\)) in response to control and heart failure (HF) rabbits. I\(_{\text{KS}}\)in male control increased in response to isoproterenol (ISO, 500mM) 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 \(\beta\)-AR antagonist ICI118,551 (50mM at +50mV, 1.14 ± 0.24 pA/pF; Tail: 0.61 ± 0.06 pA/pF), but not by \(\beta\)-AR antagonist CGP-20712A, 300mM. I\(_{\text{KS}}\) in control female was significantly less (p<0.01) than control male, but did not increase with ISO (+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\(_{\text{KS}}\) in control male did not change with ISO (+50mV, Step: 0.89 ± 0.07 to 1.10 ± 0.11 pA/pF; Tail: 0.50 ± 0.03 to 0.62 ± 0.06 pA/pF, p=NS), and after ovarectomy, I\(_{\text{KS}}\) in control female now showed enhancement with ISO (+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\(_{\text{KS}}\) step comparable to the 64% increase in I\(_{\text{KS}}\) step in control male). With HF, sex differences in I\(_{\text{KS}}\) responsiveness to ISO went away. HF male exhibited reduced I\(_{\text{KS}}\) (vs control male) but I\(_{\text{KS}}\) did not enhance with ISO (+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\(_{\text{KS}}\) enhancement with ISO (+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 \(\beta\)-AR stimulation of I\(_{\text{KS}}\), that are mediated by \(\beta\)-AR, and which are modulated by sex hormones. With HF, sex differences in basal I\(_{\text{KS}}\) 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 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 electrical 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 (CPnqa, 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\(^{2+}\) ATPase (SERCA2a) gene 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, I\(_{\text{Ks}}\) is an inward rectifying potassium current, present in ventricular myocytes, which contributes to late repolarization and clamps the resting membrane potential. I\(_{\text{Ks}}\) 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 (4mM) extracellular Ca\(^{2+}\) (at 1.14 ± 0.24 pA/pF, p=NS), but not by \(\beta\)-AR antagonist CGP-20712A, 300mM, in control male. MS was achieved by automatically switching stimulus delivery among 3 electrode pairs oriented at 0, 60 and 120° in situ. 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 electrical 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 (CPnqa, CAPES, FAPESP).
non-sustained polymorphic ventricular tachycardia (VT), bi-directional VT, syncope, and mild QTc prolongation. The proband displayed dysmorphic features including micrognatia, clinodactyly 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 expressing the R260P mutant. Co-expression of WT and R260P-KCNJ2 (heterozygous expression) yielded a markedly reduced inward IK1 compared with WT alone (-3.65 ± 9.8 pA/pF vs. -143.5 ± 11.4 pA/pF, n=8, p<0.001, respectively) 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-Na1.5 and HeK-ERG Instant Cells: Study On Frozen Cells Thawed And Immediately Patched At Manual And Automated Patch Clamp Devices Ofel Shefel, Corina Ehnert, Gesa Rascher-Eggstein, Thomas Knott, Cytocentrics AG, Rostock, Germany.

Well characterized 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 IV 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 Dan Hu1, Hector Barajas Martinez2, Ryan Pfeiffer1, Alejandra Guercioffiol2, Jonathan M. Cordeiro1, Anne B. Curtis2, Guido D. Pollevick1, Yuessheng Wu1, Elena Burashnikov1, Charles Antzelevitch2, 1Masonic Medical Research Laboratory, Utica, NY, USA, 2University of South Florida, Tampa, FL, USA.

Introduction: Inherited loss of function mutations in SCN5A, the gene that encodes the α-subunit of the human cardiac sodium channel (hNa1,5), 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 CD9 DNA 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 an extrudate (300uM) 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 z1 and δ subunits of the calcium channel in TSA201 cells.

Conclusions: Our results suggest that variations leading to a loss of function in hNa1 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 Muugu V. Brahmapathi,1 Michael J. Morales2, Donald L. Campbell1, Charles Steenbergen,1 Harold C. Strauss2,1 Duke University Medical Center, Durham, NC, USA, 2UB, SUNY, School of Medicine, Buffalo, NY, USA, 3Johns Hopkins School of Medicine, Buffalo, MD, USA.

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 Ca2+ 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 distribution in atrial myocytes and a pattern 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 Said El-Hanou1, Elise Balse1, Nathalie Neyroud1, Bruno Gavillet2, Humayoun Abrishami1, Alain Coulombe1, Andreas Jeromin1, Stephane N. Hatem1, 1UMRS621, INSERM Université Pierre et Marie Curie-Paris 6, Paris, France, 2Department of pharmacology and toxicology, University of Lausanne, Lausanne, Switzerland, 3The Allen Institute for Brain Science, Seattle, WA, USA.

The Shal-type Kv channels (Kv4.x) account for a large part of the outward potassium current, Ito, 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 (1.49 ± 19/aPf vs 300 ± 52/aPf; n=11; p<0.001) without changes of current gating properties. SAP97 had no effect on Kv4.3 encoded current when channel deleted of the VSAL motif (ΔSAL-Kv4.3). Suppression of SAP97 by using shRNA inhibited Ito 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.