channel responsible for membrane potential control in lymphocytes, high affinity and selectivity toxins have been used to demonstrate therapeutic application of peptide inhibitors *in vitro* as well as *in vivo*. Margatoxin (MgTx) is often considered as a high affinity and selective inhibitor of the Kv1.3 channel. MgTx consists of 39 amino acids stabilized by 3 disulfide bridges isolated from the venom of the scorpion *Centruroides margaritatus*. This peptide is widely used in the area of ion channel research, often to verify Kv1.3 expression in the plasma membrane. However, a comprehensive study of its selectivity with electrophysiological methods has not been published yet.

We conducted electrophysiological measurements with the patch-clamp technique in voltage clamp mode to test the selectivity of MgTx. Measurements were carried out on L929 cells expressing mKv1.1 channels, human peripheral lymphocytes expressing Kv1.3 channels and transiently transfected tsA201 cells with the following ion channels: hKv1.1, hKv1.2, hKv1.3, hKv1.4-IR, hKv1.5, hKv1.6, hKv1.7, hKv2.1, Shaker-IR, hERG, hKCa1.1, hKCa3.1 and hNav1.5.

Margatoxin is indeed a high affinity inhibitor of the Kv1.3 channel (Kd = 11.7 pM) but is not selective, since it inhibited Kv1.2 channel with similarly high affinity (Kd = 6.4 pM) and Kv1.1 in the nanomolar range (Kd = 4.2 nM and 1.7 nM for human and murine Kv1.1 respectively).

Based on our comprehensive data MgTX has to be considered a non-selective Kv1.3 inhibitor, and thus, experiments aiming at elucidating the significance of Kv1.3 in *in vitro* or *in vivo* physiological responses using MgTx need to be carefully evaluated.

2792-Pos Board B484

Mechanisms of ATP-Sensitive Potassium Channel Overactivity in Cantu Mutants

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In 2012 two papers were published papers drawing a connection between genetic mutations in the ABCC9 gene and a disease called Cantu Syndrome. Patients with Cantu generally present with some or all of the following: hypertrichosis, distinct facial features, osteochndrosyplasia and cardiac defects [1] and [2]. The ABCC9 gene, that has been associated with this disease, encodes for a sulfonylurea receptor (SUR2), which couples with inwardly rectifying potassium pore forming subunits (Kir6.X) to form ATP-sensitive potassium (KATP) channels [3]. The KATP channels formed using SUR2 is expressed in two major isoforms (SUR2A and SUR2B) leading to differential expression pattern across a variety of excitable tissues including vascular smooth muscle[4], skeletal muscle and the heart[5] to serve as the link between cell metabolism and electrical activity. Harakalova et al showed some evidence for these Cantu mutations resulting in overactive KATP channels. To address the mechanism behind these potentially over-active mutations our lab has generated the equivalent of the documented human mutations in ratSUR2A, a dominant isoform of SUR2. By transiently transfecting COSm6 cells with kir6.2 along with P429L, A475V or C1039Y we have studied the effect of these mutations on channels activity. Separately we have tested both ATP-sensitivity and ADP-activation. We have found. P429L and A475V channels ATP-sensitivity remains unchanged, but the relative ADP activation for both P429L and A475V at $56.9 \pm 0.06\%$ and $59.7 \pm 0.11\%$ were significantly greater than the $26.8 \pm 0.03\%$ activation in WT. The C1039Y MgADP activation is also significantly increased $38.7 \pm 0.04\%$, but in addition C1039Y channels are 1.7 fold less sensitive to ATP. These results would suggest that while these mutations may each lead to overactive KATP channels there are a variety of mechanisms from which this over activity arises.

2793-Pos Board B485

Two-Pore-Domain TASK-1 Potassium Channels Modulate Pancreatic Islet Glucagon Secretion

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Glucose regulation of pancreatic alpha-cell Ca^{2+} entry through voltage-dependent Ca^{2+} channels is essential for normal glucagon secretion and becomes defective during the pathogenesis of diabetes mellitus. The two-pore-domain potassium (K2P) channel, TASK-1, is an important modulator of membrane voltage and Ca^{2+} entry, however, its role in alpha-cells has not been determined. Therefore, we addressed how TASK-1 channels modulate alpha-cell electrical activity, calcium entry and glucagon secretion. We find that TASK-1 channels are expressed in human and rodent alpha-cells. Furthermore, alpha-cell K2P currents are blocked by the specific and potent TASK-1 channel inhibitor A1899. Alpha-cell K2P currents are also significantly reduced following ablation of mouse alpha-cell TASK-1 channels. Inhibition of TASK-1 channels with A1899 causes membrane potential depolarization in both the human and mouse alpha-cells, which results in increased Ca^{2+} influx. While A1899 augments alpha-cell Ca^{2+} influx under low (1 mM) and high (14 mM) glucose conditions, treatment of mouse and human islets with A1899 only increases glucagon secretion under elevated (14 mM) glucose conditions. Therefore, these data suggest an important role for TASK-1 channels in limiting alpha-cell excitability and glucagon secretion during glucose stimulation. Interestingly, we also find that TASK-1 expression is reduced during the progression of type-2 diabetes mellitus in human islets. Thus, decreased TASK-1 channel activity may exacerbate the defective glucose inhibition of glucagon secretion observed in type-2 diabetes mellitus.

2794-Pos Board B486

Cloning of Equine KCNH2 and KCNQ1 as the Basis for Diagnosing Long QT Syndrome in Horses

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Long QT syndrome (LQTS) is an inherited disorder characterized by prolonged QT interval in the ECG. Human and canine patients are predisposed to syncopal episodes and sudden cardiac death (SCD). In humans mutations in 13 genes affecting ion channel function are involved in LQTS. KCNQ1 and KCNH2 represent the molecular correlate of the slow (I_{ks}) and fast (I_{kr}) repolarizing K^+ currents in the heart and mutations in these genes are responsible for about 90% of cases. Sudden death is well known in horses and unacceptable from an ethical, financial and safety point of view. Up to 68% of sudden deaths in racehorses occur of unknown reasons, but are often suspected to be exercise-induced acute cardiovascular failure. The objective of this project is to clone and characterize the equine KCNH2 and KCNQ1 channels. This may allow for the diagnosing of possible LQTS syndromes in horses.

To clone the channels and their regulatory subunits mRNA was isolated from equine ventricular myocytes and full length PCR products for KCNH2, KCNQ1, KCNE1 and KCNE2 were amplified using RACE PCR. The products were sequenced and subcloned for expression in *Xenopus* oocytes. After expression two-electrode voltage clamp was performed to characterize the electrophysiological properties of the channels.

The protein sequences for the equine channels and beta-subunits were compared with the respective human sequences and showed the following homologies, KCNH2: 99%, KCNQ: 91%, KCNE1: 80% and KCNE2: 90%. After expression in *Xenopus* oocytes, the equine KCNH2 showed electrophysiological properties resembling the human channel. The equine KCNQ1/KCNE channels await thorough electrophysiological characterization.

In conclusion, it is expected that the KCNH2 and KCNQ are responsible for repolarization of the equine heart and mutations in these proteins may lead to yet unknown equine LQTS.

2795-Pos Board B487

Isogenic Sets of Human Pluripotent Stem Cells as Model of LQT2 Syndrome

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Patient-specific induced pluripotent stem cells (iPSCs) will assist research on genetic cardiac maladies if the disease phenotype is recapitulated in vitro. However, genetic background variations may confound disease traits, especially for disorders with incomplete penetrance, such as long-QT syndromes (LQTS).

To study the LQT2-associated c.A2987T (N996I) KCNH2 mutation under genetically defined conditions, we derived iPSCs from a patient carrying this mutation and corrected it using homologous recombination. Furthermore, we introduced the same point mutation in human embryonic stem cells (hESCs), generating two genetically distinct isogenic pairs of LQTS and control lines.

Correction of the mutation normalized the current (IKr) conducted by the HERG channel and the action potential duration in iPSC-derived cardiomyocytes. Introduction of the same mutation reduced IKr and prolonged the action potential duration in hESC-derived cardiomyocytes. Further characterization of N996I-HERG pathogenesis revealed a trafficking defect associated with a ~30-40% reduction in IKr.

Our results demonstrated that the c.A2987T KCNH2 mutation is the primary cause of the LQTS phenotype. Precise genetic modification of pluripotent stem cells provided a physiologically and functionally relevant human cellular context to reveal the pathogenic mechanism underlying this specific disease phenotype.

2796-Pos Board B488

Dynamic Action Potential Clamp Investigation of Pro-Arrhythmic Risk of Drugs Binding to hERG Potassium Channels

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Many commonly used drugs can bind to and block the hERG channel and cause the potentially life threatening acquired long-QT syndrome.

Whilst obtaining an IC50 for drug block of hERG is relatively straight forward, this is a poor surrogate for risk of pro-arrhythmia. Predicting the overall consequences of hERG drug block on cardiac electrical activity is complicated by the fact that the effect of hERG channel block varies in different cells (e.g. epicardial, mid-myocardial, endocardial, Purkinje) of the heart. Furthermore it is significantly altered by the electrical remodeling that occurs in many chronic heart conditions. With the aging of our population and an increasing proportion of people with chronic heart conditions it is especially important to understand how disease states affect the consequences of hERG drug block and risk of pro-arrhythmia.

Here we describe a recently developed dynamic action potential clamp system (dAPC) to investigate the effect of hERG block on cardiomyocytes. The system consists of conventional whole cell voltage clamp study of ion channels in mammalian expression systems, coupled to a real time computer model of human cardiomyocyte action. The dAPC system integrates the current recordings from a patch clamped cell into an in-silico cell model, the output of which is then used to determine the voltage of the patch clamped cell. When used in combination with a state of the art drug perfusion system this integrative approach will permit testing of drugs on specified ion channels in a physiologically relevant environment, something that is not possible with conventional patch clamp methods.

2797-Pos Board B489

Ca^{2+} Sensitivity of L-Type Calcium Channel Inactivation Probed by Ca^{2+} Photouncaging— Window onto Calmodulinopathies

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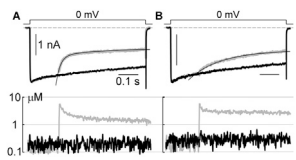
Mutations in calmodulin (CaM) cause long-QT syndrome and recurrent cardiac arrest (*Circulation* (2013) **127**: 1009). Arrhythmogenesis likely arises from impaired CaM regulation of ion channels, especially Ca^{2+}-dependent inactivation (CDI) of L-type calcium channels (LTCCs). Yet, traditional assessment of the Ca^{2+} sensitivity of CDI conflates channel gating (controlling Ca^{2+} influx), and actual Ca^{2+} responsiveness of CDI. Here, we used Ca^{2+}-photouncaging to deliver known Ca^{2+} steps to LTCCs. Li^{+} was the charge carrier to restrict the source of Ca^{2+} to that uniformly photouncaged by UV flashes. Panel A displays the CaM C-lobe component of CDI, isolated by coexpressing LTCCs with mutant CaM_{12} (Ca^{2+} binding only to C-lobe). Absent Ca^{2+} uncaging, quasi-steady currents were evoked (black). Ca^{2+} uncaging induced strong and kinetically resolved CDI (gray, with fit). Isolating the N-lobe form of CDI (panel B) yielded a slower but also strong form of CDI. Data such as these enabled full profiles of steady-state and kinetic responsiveness of CDI to Ca^{2+}. Intriguingly, disease-related CaM mutations resulted in well-resolved and specific deficits in the Ca^{2+}-to-CDI response profile, offering powerful insight into the channel mechanistic alterations that ultimately yield system-level disease.



2798-Pos Board B490

Trafficking-Defective Kir6.1 (KATP) Mutations in Sudden Infant Death Syndrome

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INTRODUCTION: KATP channels are known to provide a functional linkage between the electrical activity of cell membrane and metabolism. The KCNJ8-encoded Kir6.1 (KATP) channel is critical in the regulation of vascular tone and cardiac adaptive response to systemic metabolic stressors, including sepsis. Previously, we identified two KCNJ8 mutations (E332del and V346I) in a large sudden infant death syndrome (SIDS) cohort that exhibited a marked loss-of-function phenotype. Here we asked whether these SIDS mutations display dominant-negative effects on co-expressed wild type (WT) and further examine the mechanism underlying its abnormal channel function. METHODS AND RESULTS: A hemagglutinin (HA) epitope was introduced in an extracellular loop of Kir6.1-WT and mutations through recombinant PCR technique. Kir6.1-WT and Kir6.1 mutant (E332del or V346I) were co-expressed heterologously with SUR2A in HEK293 cells for whole cell patch clamp recordings. HA-tagged Kir6.1-WT and Kir6.1-E332del or V346I were co-expressed heterologously with SUR2A in HEK293 cells for live cell western blot with primary anti-HA antibody and the 2nd antibody labeled IRDye 800 and detected by LI-COR Odyssey infrared imaging system and for quantification of cell surface expression by flow-cytometry with FITC-conjugated anti-HA antibody. Compared with Kir6.1-WT, pinacidil-activated KATP currents for E332del and V346I were decreased 57% to 68% between −20 mV and 40 mV as reported previously in COS-1 cells. The live cell western analysis showed that the intensities of the cell surface expression of Kir6.1-E332del and V346I were 24% to 42% of Kir6.1-WT. The cell-counting studies by flow-cytometry indicated that the cell surface expression of Kir6.1-WT was suppressed 35% to 70% when co-expressed with Kir6.1-E332del or Kir6.1-V346I in a 1:1 DNA ratio. CONCLUSION: The loss-of-functional Kir6.1 KATP channel mutations found in SIDS displayed a channel trafficking defect to the plasma membrane and exerted the dominant-negative effect on Kir6.1-WT channels.

2799-Pos Board B491

Human Induced Pluripotent Stem Cell Derived Cardiomyocytes (HIPS-CM's): An Expression Model System for Investigating Cardiac Channelopathies

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Background and rationale:

Until recently the study of dysfunctional ion channels in their native environment was only possible by using transgenic animal models, which do not recapitulate human physiology. Here, we have investigated the use of hiPS-CM's as a model system for investigating cardiac channelopathies, specifically long QT Syndrome-9 (LQT9) causing Cav3 mutation (F97C-Cav3).

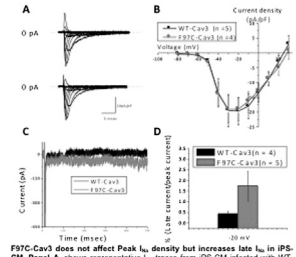
Methods:

Commercially available hiPS-CM's were obtained from Cellular Dynamic International. hiPS-CM's were infected with WT-Cav3 or F97C-Cav3 and studied with standard patch-clamp techniques.

Results:

Using HEK293 cells and in rat myocytes we previously reported that LQT9 causing mutations did not affect peak-sodium current (I_{Na-P}), but increased late-sodium current (I_{Na-L}).

In the HEK293 heterologous cell system we transfected the key components nNOS, α1-syntrophin and WT-Cav3/F97C-Cav3 to elucidate mechanism. To investigate the use of hiPS-CM's as a model, we infected hiPS-CM's with WT-Cav3/F97C-Cav3. We report that, I_{Na-p} was unchanged (A&B) but report an increase in I_{Na-L} (C&D).



F97C-Cav3 does not affect Peak I_{Na} density but increases late I_{Na} in iPS-CM. Panel A. shows representative I_{Na} traces from iPS-CM infected with WT-Cav3 (top) and F97C-Cav3 (bottom). **Panel B.** shows average INa current density from iPS-CM infected with WT-Cav3 (black) and F97C-Cav3 (red). **Panel C.** show representative traces demonstrating increase in late I_{Na} in iPS-CM infected with F97C-Cav3 as compared to WT-Cav3 at -20 mV. **Panel D.** is a bar graph showing percentage of the late I_{Na} normalized to peak density.

Conclusion:

hiPS-CM's are a relevant physiologic expression model system to study cardiac channelopathies that recapitulates the native environment of a human ventricular myocyte.

2800-Pos Board B492

Interleukin 1ß Modulates the Ventricular L-Type Calcium Current Through ROS Signalling

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Introduction & Objective: Several lines of evidence suggest that cytokines are potent mediators of cardiac remodelling including hypertrophy and generation of reactive oxidative species (ROS). Increases in serum cytokines, notably necrosis factor-alpha (TNFα) and interleukin-1β (IL-1β), have been