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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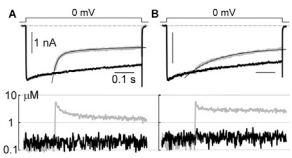
Mutations in calmodulin (CaM) cause long-QT syndrome and recurrent cardiac arrest (*Circulation* (2013) **127**: 1009). Arrhythmogenesis likely arises from impaired CaM regulation of ion channels, especially Ca^{2+}-dependent inactivation (CDI) of L-type calcium channels (LTCCs). Yet, traditional assessment 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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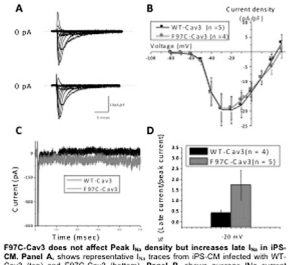
Background and rationale:
Until recently the study of dysfunctional ion channels in their native environment was only possible by using transgenic animal models, which do not recapitulate human physiology. Here, we have investigated the use of hiPS-CM's as a model system for investigating cardiac channelopathies, 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