1524-Pos  Board B416
Human ES- and Induced Pluripotent Stem-Derived Cardiomyocytes. A Comparative Electrophysiological Study
Fernando López-Redondo, Junko Kurokawa, Fumimasa Nomura, Tomoyuki Kaneko, Tomoyo Hamada, Kenji Yasuda, Tetsushi Furukawa.
Tokyo Medical Dental University, Tokyo, Japan.
Special attention is directed to the potential application of human induced pluripotent stem (iPS) cell-derived cardiomyocytes for cardiac safety pharma-
ocology and toxicology with fewer legal and ethical issues. Supply of commer-
cially available products enables many researchers to test the utility in their own systems. Although variations of electrophysiological properties have been reported among pluripotent cell lines by classifying cardiac subtypes into nodal, atrial and ventricular cells, most cells are spontaneously contracting, which makes difficult to be implicated in adult human hearts. Thus, we sought objective description on action potential (AP) parameters recorded from perfused patch-clamped iCell cardiomyocytes (iCell-CMs, AJ/CDI) by system-
tically comparing with those from human ES-derived cardiomyocytes (hES-CMs, Cellartis). Mean +/- SE values of APD90 in iCell-CMs (382 +/- 38 ms, n=36) were significantly longer than those in hES-CMs (278 +/- 28 ms n=64), while APD90 in iCell-CMs (1210 +/- 346 ms, n=36) tended to be longer than those in hES-CMs (776 +/- 136 ms n=64). As for APD50, despite of the large sample size, there was no statistical significance between iPS-CM and hES-CMs. Detailed analysis of the variation by Gaussian fitting revealed that marked differences in shapes of probability distribution between iPS-CMs and hES-CMs. Although further studies are necessary to know if the variation of AP parameters affect drug responses, our data provide important information for cardiac safety assessments.

1525-Pos  Board B417
Examining the Causes and Consequences of Calcium Overload in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes
Masonic Medical Research Laboratory, Utica, NY, USA.
Intracellular calcium overload has been linked to arrhythmias in conditions such as ischemia reperfusion, heart failure, catecholaminergic polymorphic ventricular tachycardia and digitalis intoxication. While this link has been extensively studied at the cellular level using animal models, there is a paucity of information on the causes and consequences of Ca\(^{2+}\) overload in healthy human myocytes. With the advent of induced pluripotent stem cell-derived cardiomyocytes (iPS-CM), human myocytes are becoming more readily available. However, it remains unclear if these myocytes faithfully recapitulate all aspects of adult cardiomyocyte physiology. In order to determine if iPS-CM will be a useful platform to study the causes and consequences of Ca\(^{2+}\) overload, we developed methodology to examine Ca\(^{2+}\) overload in beating clusters of iPSC-CM and in isolated myocytes. Spontaneous and field stimulated action potentials were measured with high resistance microelectrodes in spontaneously beating clusters of iPSC-CM while measuring cluster con-
taction with simultaneous video edge detection. Free intracellular Ca\(^{2+}\) was measured with fluo-4 and confocal microscopy in beating clusters and in isolated iPSC-CM. Ca\(^{2+}\) overload was induced in both preparations by treatment with ouabain (2.5-5 uM or with isoproterenol (1 uM) plus 5.4 mM extracellular Ca\(^{2+}\)). Both maneuvers produced a rise in diastolic Ca\(^{2+}\) as well as the appearance of oscillatory action potentials, putative delayed afterdepolarizations and triggered activities in beating clusters. These results indicate that iPSC-derived cardiomyocytes provide a useful platform to study the mechanisms of Ca\(^{2+}\) overload-induced arrhythmias as well as possible treatments. However, given the spontaneous nature of iPSC-derived cardiomyocytes, these cells may more readily recapitulate the effects of Ca\(^{2+}\) overload on sinoatrial or latent pacemaker cells.

1526-Pos  Board B418
Characterization of a Transient Outward K\(^{+}\) Current in Hips-Derived Cardiomyocytes
Jonathan M. Cordeiro, Vladislav V. Nesterenko, Robert J. Goodrow, Serge Sicouri, Jacqueline A. Treat, Michael X. Doss, Charles Antzelevitch, Jose M. Di Diego.
Masonic Medical Research Laboratory, Utica, NY, USA.
Background: Human induced pluripotent stem cell (hiPS)-cardiomyocytes can be used to create in vitro models of genetic disease such as Brugada Syndrome (BrS). Central to the development of BrS is the Ca\(^{2+}\)-independent transient outward K\(^{+}\) current (I_{to}). In this study, we characterized I_{to} in single hiPS -cardiomyocytes and determined its functional role in beating clusters.

Methods: Embryoid bodies (EBs) were made from a hiPS cell line reprogrammed with Oct4, Nanog, Lin28 and Sox2. Whole cell patch clamp was used to record I_{to}, in single action potential (AP) record-

ings from spontaneously beating clusters (BCs) were made using sharp micro-
electrodes. All recordings were done at 36°C.

Results: BCs exhibited spontaneous APs with an average rate of 54.9 ± 30.1 bpm and maximum diastolic potential (MDP) of -65.6 ± 9.3 mV (n=122). A small phase 1 repolarization which could be blocked by 4-AP (1 mM) was observed in 14/149 BCs suggesting the presence of I_{to}. Interestingly, in single dissociated hiPS cardiomyocytes, patch clamp analysis revealed a robust I_{to} (13.4 ± 7.9 pA/µF at +40 mV, n=14) in the majority of cells studied. Recovery of I_{to} (at -80 mV) showed a fast and slow phase as follows: i=1=+271.9 ± 93 ms and 2=+2697 ±103 ms (n=8 cells). These observations demonstrate that I_{to} is present but the slow recovery suggests minimal contri-
bution during the course of an action potential. Mathematical modeling of APs from hiPS-CMs confirmed these observations.

Conclusion: There is a disconnect between the presence of I_{to} in cells and the absence of phase 1 repolarization in BCs. In BCs the depolarized MDP and fast spontaneous AP rate suggests negligible contribution of I_{to} to phase 1 repolar-
izazation. Our results point to an important deficiency of hiPSC-CMs in recapit-
lating the phenotype of adult native myocytes.

1527-Pos  Board B419
Analysis of Zolpidem-Induced Long Qt Syndrome in Recombinant hERG Channels and Stem Cell-Derived Human Cardiomyocytes
Julian Jehle1, Xiaoping Wan2, Isabelle Deschenes2, Dierk Thomas3, Eckhard Ficker2.
1Medical University Hospital, Heidelberg, Germany, 2Case Western Reserve University, Cleveland, OH, USA.
Zolpidem, a short-acting hypnotic drug prescribed to treat insomnia, has been clinically associated with acquired long QT syndrome (acLQTS) and torsade de points tachyarrhythmias (TdP). Because acLQTS is most often precipi-
tated by a reduction of hERG I_{Kr} currents, we have studied acute hERG block by zolpidem in HEK cells using patch clamp recordings. We found that zol-
pidem reduced hERG currents with an IC_{50} value of 65.5 ± 4.5 µM (n=3-6). In marked contrast to many other hERG blockers, hERG surface expression was not impaired on long-term drug exposure. To determine whether zol-
pidem effects in an expression system are relevant to cardiac electrophysiology, we studied human stem cell-derived cardiomyocytes (iCells, CDI, Madison, WI). In human cardiomyocytes, zolpidem prolonged APD_{90} significantly from 321.2 ± 45.5 ms (n = 9) under control conditions to 375.8 ± 55.5 ms (n=9) and 414.1 ± 54.8 ms (n=9) in the presence of 10 µM and 30 µM zolpidem, respectively. Because zolpidem produced TdP in combina-
tion with the antiarrhythmic drug amiodarone, we investigated whether both drug effects were additive. When amiodarone was applied separately, APD_{90} prolonged from 287.5 ± 30.8 ms (n=8) under control conditions to 349.6 ± 50.4 ms, and 403.0 ± 59.1 ms (n=8) with 100 nM and 1 µM amio-
darone (n=7), respectively. In contrast, zolpidem and amiodarone were co-administered, APD_{90} was further prolonged from 341.0 ± 45.3 ms (n=8) during amiodarone treatment to 381.3 ± 38.1 ms with 10 µM (n = 8) and 450.3 ± 50.2 ms with 30 µM zolpidem (n = 8). Taken together, our results indicate that acute zolpidem administration blocks hERG channels and induces cardiac action potential changes that are consistent with clinically observed QT prolongation, particularly in patients treated with multiple medications.

Primary Transporters & Exchangers
1528-Pos  Board B420
Time Resolved FRET in the SR Ca-ATPase
University of Minnesota, Minneapolis, MN, USA.
We have detected structural dynamics of the sarcoplasmic reticulum Ca-ATPase (SERCA) using time-resolved fluorescence spectroscopy. The Ca-ATPase from fast-twitch skeletal muscle (SERCA1a isoform) was labeled with cyan fluorescent protein (CFP) at the N-terminus in the actuator domain (A) and fluorescein iso-thiocyanate (FITC) at Lys-515 in the nucleotide-

binding domain (N). Time-resolved FRET was detected between CFP (donor in A domain) and FITC (acceptor in N domain) for SERCA in ligand-

stabilized states, including calcium-free (E2), calcium-bound (E1), and actively-cycling phosphoenzyme (EP). Lifetime fitting and molecular model-
ing were used to interpret fluorescence decays, thereby identifying a dynamic distribution of structural states within the cytoplasmic headpiece of SERCA
1529-Pos  Board B421  Screening for SERCA Activators using a High-throughput Time-Resolved FRET Assay  

**Ji Li**, Holly Langer¹, Kurt C. Peterson², Joseph M. Muretta¹, Gregory D. Gillespie¹, Razvan L. Corna³, David D. Thomas¹²

¹University of Minnesota, Minneapolis, MN, USA; ²Fluorescence Innovations, Inc., Minneapolis, MN, USA.

We used a prototype time-resolved fluorescence lifetime microscope plate reader to carry out a high-throughput screen designed to identify compounds that interact with the sarcoplasmic reticulum calcium ATPase (SERCA). SERCA is essential for the Ca homeostasis in many cell types. Insufficient SERCA activity leads to cardiovascular disease, muscular dystrophy, skin disease, and diabetes. Our goal is to discover activators of SERCA that can be developed into drugs to treat diseases in which Ca transport is deficient. The fluorescence lifetime plate reader was made possible by our recent development of fast time-resolved fluorescence by direct waveform recording, which achieves 105 higher throughput than the conventional single-photon counting technology. Using this plate reader, we detected fluorescence resonance energy transfer (FRET) between IAEDANS-labeled SERCA and nucleotide analog TNPADP in native sarcoplasmic reticulum membranes. This assay was designed to detect compounds that interact with SERCA and modify either the enzyme’s structure or the binding affinity of TNP-ADP. Initial hit compounds were further analyzed in functional assays. Upon screening a small (1300 compound) library, we determined that the time-resolved microscope reader has at least 10x higher precision than a conventional intensity-based microscope reader, raising the quality index (z’), of our assay from marginal, to the intensity reader, to excellent. A 384-well plate is read with high precision in 2 min, which allows screening of thousands of compounds/day. An important advantage of the time-resolved fluorescence measurement is that it provides detailed structural information, thus enabling discovery of multiple classes of compounds during the primary screen.

1530-Pos  Board B422  In-Cell FRET as a Tool to Develop SERCA Activators for Drug or Gene Therapy  

**Simon J. Gruber¹**, Kurt C. Peterson¹, Bengt Svensson¹, Seth L. Robia², David D. Thomas¹

¹University of Minnesota, Minneapolis, MN, USA; ²Loyola University, Chicago, IL, USA.

We are using in-cell FRET methods (Photobleaching, FLIM, time-resolved FRET in a plate reader) to study the relationships among structure, dynamics, and function of the sarcoplasmic reticulum Ca-ATPase (SERCA) and its cardiac regulator phospholamban (PLB), with the goal of designing activators of SERCA for treatment of heart failure (HF) and muscular dystrophy (MD). Ca²⁺ drives muscle contraction, and relaxation is accomplished by the sequestration of Ca²⁺ by the sarcoplasmic reticulum Ca-ATPase (SERCA), which is inhibited by phospholamban at submicromolar [Ca²⁺] in cardiac muscle. SERCA activity is frequently reduced in HF, and many current therapeutic strategies aim to increase cardiac Ca²⁺ cycling activity. We are designing LOF-PLB mutants (PLBM) that can compete with WT-PLB (PLBW) and thus relieve SERCA inhibition. Our ideal mutant is partial loss-of-function, binds tightly to SERCA2a, and remains phosphorylatable via β-adrenergic pathways. The effects of PLBM on Ca-ATPase activity and FRET are measured to determine the mutant’s ability to compete with PLBW, both physically and functionally. Optimal LOF mutants are being tested in a rat model of heart failure.

In addition to this SERCA-PLB FRET competition assay, we are using SERCA2a labelled with two different fluorescent probes (GFP and RFP) to screen for small molecule activators of SERCA in living cells. Time-resolved FRET measurements of RFP-GFP-SERCA2a in 96- or 384-well plates have shown the extraordinary precision and sensitivity necessary to probe SERCA’s structure-function relationship. New drug screening efforts are active and small molecules from previous screens are being developed as promising drugs for HF therapy (collaboration with Celladon, Inc.). Many of these drugs are direct SERCA activators and are being investigated for their ability to rescue the muscular dystrophy phenotype in dystrophic mice.

1531-Pos  Board B423  EPR Detects Changes in the Transmembrane Region of the SERCA-Phospholamban Complex upon Ser16 Phosphorylation  

**Zachary M. James**, Jesse E. McCaffrey, Christine B. Karim, David D. Thomas.

University of Minnesota, Minneapolis, MN, USA.

We have used site-directed spin labeling and electron paramagnetic resonance (EPR) spectroscopy to investigate the effects of Ser16 phosphorylation on phospholamban’s interaction with the sarcoplasmic reticulum Ca-ATPase (SERCA). Muscle contraction is signaled by the release of Ca from the sarcoplasmic reticulum (SR), while SERCA mediates muscle relaxation by actively pumping Ca back into the SR lumen. Cardiac SERCA is regulated by phosphorylated (PLB), a single-pass transmembrane protein that inhibits the Ca pump unless phosphorylated at Ser16.

We have shown that Ser16 phosphorylation does not dissociate PLB from SERCA to relieve inhibition, but instead induces a structural change in PLB’s transmembrane (TM) helix that restores SERCA Ca sensitivity. To characterize this change, we have attached spin labels along the TM helix and performed EPR accessibility measurements to determine the effects of Ser16 phosphorylation on TM helix topology within the regulatory complex. Our results show that upon phosphorylation, the TM helix undergoes a vertical shift that could break inhibitory interactions between SERCA and PLB. Complimentation studies employ these spin-labeled PLB constructs to determine changes in (a) the orientation of PLB and (b) distances to probes on SERCA, with the goal of obtaining a detailed structural model for the regulatory transitions within the SERCA-PLB complex.

1532-Pos  Board B424  Orientation of Phospholamban in Lipid Bicelles Detected by Electron Paramagnetic Resonance  

**Jesse E. McCaffrey**, Zachary M. James, Christine B. Karim, David D. Thomas.

University of Minnesota: Twin Cities, Minneapolis, MN, USA.

We have used electron paramagnetic resonance (EPR) to probe the structural dynamics of the integral membrane protein phospholamban (PLB), as a function of phosphorylation and addition of its regulatory target, the sarcoplasmic reticulum calcium ATPase (SERCA). We found previously that PLB remains bound to SERCA after phosphorylation, suggesting that a structural transition within the SERCA-PLB complex is responsible for relief of inhibition. Our current goal is to elucidate this mechanism through orientation and accessibility EPR, in order to support rational design of therapies to improve calcium transport in muscle cells. We used the monomeric mutant AFA-PLB, with the rigid electron spin label TOAC incorporated within the transmembrane domain, and reconstituted the protein in lipid bicelles and vesicles. EPR showed that the accessibility of PLB spin labels to paramagnetic relaxation agents changed upon phosphorylation, indicating vertical (parallel to the membrane normal) movement of PLB. EPR on aligned bicelles showed that PLB changes its tilt relative to the membrane upon phosphorylation. Results will also be reported on structural changes in the presence of SERCA. This work was funded by grants from NIH (R01 GM27906 and T32 AR007612).

1533-Pos  Board B425  Probing Cardiac Membrane Proteins with Fluorescence Resonance Energy Transfer  

**Xiaojong Dong**, Ji Li, Christine B. Karim, David D. Thomas.

University of Minnesota, Minneapolis, MN, USA.

We have used time-resolved fluorescence resonance energy transfer (TR-FRET) to study the structural basis of regulation of sarcoplasmic reticulum Ca²⁺ ATPase (SERCA) by a single-pass transmembrane protein, phospholamban (PLB). The most prominent feature of heart failure is calcium mishandling, which is largely due to impaired activity of sarcoplasmic reticulum calcium ATPase (SERCA), which actively transports Ca²⁺ from cytosol into the SR to relax the muscle. Unphosphorylated phospholamban inhibits SERCA by decreasing its apparent Ca²⁺ affinity. This inhibition can be relieved by either micromolar Ca²⁺ or by phosphorylation of PLB. The structural basis for relief of inhibition remains controversial. Cross-linking studies support the canonical dissociation model. However, recent EPR, NMR and FRET studies support the subunit model, in which subtle structural rearrangements are required to...