

abundant in V than in A (E1 ratio ~ 1:2 - 3; E2 ratio ~ 1:2), (3) the E3 Ab recognizes a specific 20 kDa band in A but not in V. Patch clamp data: (1) I_{Ks} current density is much higher in LA than in LV myocytes, (2) I_{Ks} half-maximal activation voltage is more negative in LA than in LV myocytes, (3) I_{Ks} activates faster in LA than in LV myocytes. **Conclusion:** Q1 and E3 are more abundant in A than in V, while E1 & E2 have the opposite expression pattern. The uneven protein expression patterns can enhance I_{Ks} contribution to atrial action potential repolarization by generating a higher I_{Ks} density, that can reach a higher degree of activation in the action potential plateau range, than its counterpart in the ventricles.

1740-Pos

CaMKII Regulation of the Dynamic L-Type Ca^{2+} Current and Na^{+}/Ca^{2+} Exchange Current During Action Potential in Cardiac Myocytes

Tamas Banyasz^{1,2}, Leighton T. Izu¹, Ye Chen-Izu¹.

¹UC Davis, Davis, CA, USA, ²Univ. of Debrecen, Debrecen, Hungary.

The L-type Ca^{2+} current ($I_{Ca,L}$) and the Na^{+}/Ca^{2+} exchange current (I_{NCX}) are the main inward currents that contribute to the depolarization during cardiac action potential (AP) plateau and later phases. Pathological changes of $I_{Ca,L}$ or I_{NCX} can cause early or delayed afterdepolarization (EAD, DAD). The steady-state kinetics of $I_{Ca,L}$ and I_{NCX} have been characterized in previous studies. However, the non steady-state dynamics of $I_{Ca,L}$ and I_{NCX} during the AP cycle still remain unclear. Here we report the new data on the dynamic $I_{Ca,L}$ and I_{NCX} during the cell's AP recorded using the *self AP-clamp* method. **Results:** (1) The I_{NCX} was isolated using its specific inhibitor SEA0400 at 3 μ M. The data show that I_{NCX} is an inward current during most of the AP cycle. Importantly, I_{NCX} is the dominant contributor to a pronounced inward foot current at AP phases-3&4. This foot current is important because it depolarizes the cell at the late phases of AP and directly links to EAD or DAD. (2) Furthermore, the foot current is abolished by Ca^{2+} -calmodulin dependent kinase II (CaMKII) inhibition. (3) The $I_{Ca,L}$ was isolated using 10 μ M nifedipine. The dynamic $I_{Ca,L}$ takes the form of a spike at AP phase-1 and a dome at phase-2. (4) Both $I_{Ca,L}$ and I_{NCX} during the AP are affected by using EGTA to buffer the SR Ca^{2+} release and prevent the CaMKII activation. **Conclusion:** Here we show for the first time the dynamic $I_{Ca,L}$ and I_{NCX} currents during the cell's AP in physiological milieu. CaMKII modulation of the foot current might explain, in part, the effect of elevated CaMKII activity on promoting arrhythmias in the hypertrophied and failing hearts.

1741-Pos

KCNQ1/KCNE1 K⁺ Channels Associated with Long QT Syndrome are Expressed in Early Stage Human Embryonic Stem Cell-Derived Cardiomyocytes

Cecile Terrenoire¹, George K. Wang¹, Eric Adler², Aaron D. Kaplan³, Kevin J. Sampson¹, Jonathan Lu¹, Robert S. Kass¹.

¹Columbia University, New York, NY, USA, ²OHSU, Portland, OR, USA,

³Mount Sinai School of Medicine, New York, NY, USA.

Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) are not only a potential source of functional cardiac tissue that can be utilized as a drug screening platform or agents for cell-based therapy but also offer great potential in studies of heritable cardiac arrhythmias known as channelopathies. Many major cardiac ion channels have been reported to be expressed in hESC-CMs. However, the presence of KCNQ1/KCNE1 (I_{Ks}) K^{+} channels critical to cardiac repolarization particularly during sympathetic nerve stimulation and associated with the most common variant of congenital Long QT syndrome (LQT1), to date has not been reported. Here we report investigation of the cellular electrophysiological properties of hESC-CMs during the first 34 days of cytokine directed differentiation with a focus on I_{Ks} channels. All beating hESC-CMs studied had action potentials with cardiac phenotypes and expressed L-type calcium channels (n=26) and pacemaker channels (n=27) while 68% of cells (n=11 out of 16) expressed I_{Kr} , the potassium current associated with LQT2, defined as E4031-sensitive outward current measured during prolonged depolarization. I_{Ks} , the potassium current associated with LQT1, was identified by its biophysical and pharmacological properties: recorded in 29% of cells (n=5 out of 17), I_{Ks} was defined as an outward current slowly activating during prolonged depolarization, insensitive to E4031 (5 μ M) and blocked by Chromanol 293B (30 μ M). qPCR experiments confirmed the presence of I_{Ks} channels α - (KCNQ1) and β - (KCNE1) subunits in these hESC-CMs. This is the first report of I_{Ks} channel expression in hESC-CMs providing strong evidence in support of their use in mechanistic and pharmacological investigations of LQT1 and other heritable arrhythmia syndromes linked to mutations in the genes coding for I_{Ks} channel subunits and/or accessory proteins.

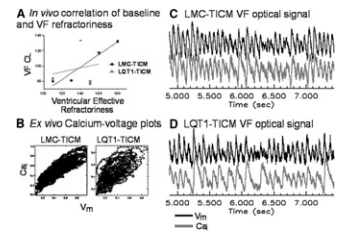
1742-Pos

LQT1 Genotype in Tachypaced Cardiomyopathy Causes Discordance of Baseline and VF Refractoriness

Ohad Ziv¹, Emily Lau¹, Lorraine Schofield¹, Lenny Chaves¹, Xuwen Peng², Bum-Rak Choi¹, Gideon Koren¹.

¹Rhode Island Hospital, Providence, RI, USA, ²Pennsylvania State University, Hershey, PA, USA.

Reduction of the slow outward rectifier (I_{Ks}) and calcium dysregulation accompany tachypacing induced cardiomyopathy (TICM). While TICM I_{Ks} downregulation prolongs APD, its effect on refractoriness during VF is less clear. We used a transgenic rabbit model of Long QT 1 (LQT1) to investigate the effect of loss of I_{Ks} on VF refractoriness in TICM. Five LQT1 and littermate control rabbits underwent rapid RV pacing followed by *in vivo* electrophysiological studies and VF inductions. Dual voltage-calcium epicardial optical mapping was performed on whole hearts at baseline and in VF. *In vivo*, a strong correlation for ventricular effective refractoriness and VF interval was seen in LMC-TICM, but not in LQT1-TICM ($r = 0.83$ vs $r = 0.36$; $p < 0.05$). Optical mapping demonstrated APD prolongation in LQT1-TICM compared to LMC-TICM (224 ± 18 ms vs. 191 ± 15 ms), but surprisingly higher VF frequencies in LQT1-TICM (15.7 ± 0.8 vs 12.6 ± 0.7 Hz; $p < 0.05$). In spatial VF frequency maps, LMC-TICM showed a negative VF frequency-APD map correlation (-0.43 ± 0.24), while LQT1-TICM demonstrated a paradoxical positive correlation (0.22 ± 0.14 ; $p < 0.05$). Calcium-voltage discordance was increased in LQT1-TICM compared to controls (see fig). LQT1-TICM leads to dissociation between baseline and VF refractoriness demonstrating high frequency VF associated with calcium-voltage discordance.



1743-Pos

Unique Molecular Profile of Transient Outward Potassium Current (I_{to}) Subunits in Cardiac Purkinje Fibers

Ling Xiao, Balazs Ordog, Maya Marmabachi, Ange Maguy, Stanley Nattel. Montreal Heart Institute, Montreal, QC, Canada.

Background and objective: Cardiac Purkinje-fiber (PF) tissue plays a key role in conduction and arrhythmogenesis. The transient outward K^{+} -current (I_{to}), an important cardiac repolarizing conductance, has unusual kinetic and pharmacological properties in PF cells (PCs), suggesting a distinct and presently-unknown molecular basis. The present study addressed the differential expression of putative I_{to} -subunits in PF versus left-ventricular (LV) myocardium.

Methods: I_{to} was recorded with whole-cell voltage-clamp at 36°C from isolated PCs or LV cardiomyocytes before and after the K^{+} -channel blocker TEA. The regional mRNA expression-levels of I_{to} α -subunit (Kv4.3, Kv3.4) and β -subunit (KChIP2, NCS-1, Kv β 1, KChAP, KCNE1-5, and DPPX_S/_L) candidates were determined by real-time PCR.

Results: I_{to} from PCs was more sensitive to TEA than LV: 10 mM TEA reduced I_{to} by $53 \pm 10\%$ ($N = 5$) in PCs versus $-4 \pm 8\%$ ($N = 5$) in LV cells, $P < 0.01$. The mRNA levels of I_{to} α -subunits Kv4.3 and Kv3.4 were significantly higher (by about 2.7 and 159-fold respectively: e.g., epicardium versus PF 1.67 ± 0.38 vs $3.58 \pm 1.14 \Delta\Delta$ -Ct units, $P < 0.05$ for Kv4.3, 0.0001 ± 0.0001 vs 0.044 ± 0.020 , $P < 0.05$ for Kv3.4; $N = 9$ /group) in PF-tissue than in LV. KChIP2 was much richer in LV epicardium (2.91 ± 0.73) and midmyocardium (0.92 ± 0.26) than in LV endocardium (0.09 ± 0.02 , $P < 0.01$) and PF (0.07 ± 0.03 , $P < 0.01$). NCS-1 was abundantly expressed in PF-tissue (1.95 ± 0.68), at about 400 \times LV values (epicardium 0.10 ± 0.03 , midmyocardium 0.16 ± 0.07 , endocardium 0.15 ± 0.04). KCNE1 and KCNE3-5 mRNA levels were significantly higher (eg, by 2.8, 2.9, 3.4 and 6.0-fold vs epicardium) in PF than in all LV zones, whereas Kv β 1, KCNE2, KChAP and DPPX_S/_L subunits were similarly expressed among these four regions.

Conclusion: Cardiac PF-tissue has a unique expression profile of I_{to} -subunits that may account for its unusual properties. Expression studies are under way to determine the precise biophysical mechanisms.

1744-Pos

Decreased Phosphorylation of the Gap Junction Protein Connexin43 and Increased Anisotropy of Conduction as a Consequence of Myofilament Ca^{2+} Sensitization

Raghav Venkataraman¹, Sung In Kim¹, Veniamin Y. Sidorov¹, James D. Potter², Bjorn C. Knollmann¹, Franz Baudenbacher¹, Sabine Huke¹.

¹Vanderbilt University, Nashville, TN, USA, ²University of Miami, Miami, FL, USA.