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

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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 μM. 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 μM 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

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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 μM) and blocked by Chromanol 293B (30 μM). 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

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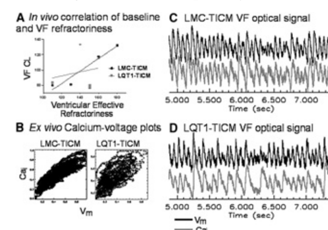
Reduction of the slow outward rectifier (I_{ks}) and calcium disregulation accompany tachypacing induced cardiomyopathy (TICM). While TICM I_{ks} downregulation prolongs APD, its effect on refractoriness during VF is less clear. We used a transgenic rabbit model of Long QT 1 (LQT1) to investigate the 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 ± 18ms vs. 191 ± 15ms), but surprisingly higher VF frequencies in LQT1-TICM (15.7 ± 0.8 vs 12.6 ± 0.7Hz; 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

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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, Kvβ1, 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 ± 10% (N = 5) in PCs versus −4 ± 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 ± 1.14 ΔΔ-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×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, 3.4and 6.0-fold vs epicardium) in PF than in all LV zones, whereas Kvβ1, 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 Ca2+ Sensitization

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Increased myofilament Ca^{2+} sensitivity is associated with an increased susceptibility for arrhythmias, as previously demonstrated in mice expressing Ca^{2+} sensitizing troponin T (TnT-I79N) mutations and after acute application of the Ca^{2+} sensitizer EMD57033. We hypothesized that the arrhythmia risk is increased due to altered regulation of connexin43 (Cx43), subsequently leading to a slowing of cardiac conduction.
Methods: Fast (longitudinal) and slow (lateral) conduction velocity (CV) was calculated using epi-fluorescence maps from isolated hearts by plotting local CVs against orientation. Phosphorylated Cx43 (P1, P2) from these hearts migrated slower in SDS PAGE and at least three distinct bands could be separated (P0,P1,P2).
Results: The lateral CV, but not the longitudinal CV, was significantly reduced in TnT-I79N mice compared to control (16.9 ± 0.8 cm/s (n = 11) vs. 21.5 ± 1.4 cm/s (n = 11) respectively, $p<0.05$). As a direct consequence the anisotropy of conduction (fast/slow) was increased in TnT-I79N hearts (to 3.2 ± 0.2 vs 2.3 ± 0.1 in control). This change in CV was associated with decreased Cx43 phosphorylation (un-phosphorylated P0-Cx43 increased to 146 ± 20% of control, $p<0.05$). Blebbistatin, a Ca^{2+} desensitizer and contractile uncoupler, prevented ventricular arrhythmias in TnT-I79N hearts. Strikingly, blebbistatin also prevented the increase in anisotropy and the decrease in Cx43 phosphorylation, while it had no effect in control hearts. Conversely, acutely increasing Ca^{2+} sensitivity with EMD decreased Cx43 phosphorylation (P0-Cx43 491 ± 147%, n = 4, $p< 0.05$) and rapidly slowed lateral conduction velocity (to 18.2 ± 1.3 vs 22.9 ± 1.1 cm/s in control, $p<0.05$) thereby increasing anisotropy (to 2.64 ± 0.1 vs 2.17 ± 0.07 in control) and rendered control hearts susceptible to arrhythmia induction.
Conclusion: These data suggest that decreased Cx43 phosphorylation and increased conduction anisotropy is at least in part responsible for the increased arrhythmia susceptibility associated with myofilament Ca^{2+} sensitization.

1745-Pos
Electron-Conformational Model of SR-Based Ca^{2+} Clock Mode
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Most of the calcium that activates cardiac contraction comes from the sarcoplasmic reticulum (SR) from where it is released through the Ryanodine Receptors (RyRs). It is well known that the SR overload results in the release of Ca from the SR in the form of waves driving some cardiac arrhythmias. Recently it has been experimentally documented that the isolated SR is capable to spontaneously and rhythmically release Ca^{2+} (SR-based Ca^{2+} clock). This self-sustained intracellular Ca^{2+} oscillator contributes substantially to the late phase of the diastolic depolarization of cardiac pacemaker cells under normal physiological conditions. Interaction of ''a surface membrane oscillator'' and ''an internal oscillator'' with ''cycles of Ca^{2+} uptake and release by the SR'' can drive normal cardiac automaticity. To describe the SR-based Ca^{2+} clock mode we propose a simple, physically-reasonable electron-conformational (EC) model for the RyR and present a theory to describe the RyR lattice dynamics. Each RyR is modelled with a single open and closed electronic state. In addition to the fast electronic degree of freedom, RyR channels are characterized by a slow classical conformational coordinate, which specifies the RyR channel conductance. The RyR gating implies a conformational Langevin dynamics, Ca^{2+}-induced electronic transitions, quantum tunneling and thermal transitions. The cooperativity in the RyR lattice is assumed to be determined by the inter-channel conformational coupling. Model simulations of the of 11x11 RyR cluster revealed different regimes depending on the cis- and trans-Ca concentrations and parameters of EC-model. The SR overload is shown to result in RyR lattice auto-oscillations with spontaneous RyR channel openings and closures. We have studied this Ca^{2+} clock mode, in particular, its stability, under different model suggestions as regards the RyR conformational potential (diabatic and adiabatic regimes), EC-model parameters.
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1746-Pos
Design of a Fluorescence-Based System for Measurement of Transmembrane Potential Variations of Electrically and Mechanically Stimulated Cardiomyocytes
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Cardiomyocytes are electrically-active heart cells whose electrical properties vary with the local electrical and mechanical environment. Variations in myocytes electrical properties are known to play a role on abnormal rhythms.
The purpose of the project is to design a fluorescence-based photodetection system for measurement of transmembrane potential variations by combined electrical and mechanical stimulations in post-culture cardiomyocytes.
The isolated cardiomyocytes are seeded on a 10mm x 10mm x 0.127mm silicon sheet held by a pair of pliers, coupled to a stretcher apparatus made of two linear guide systems and two computer controlled linear stepper motors. The cells are kept in bubbled Tyrode solution and are electrically stimulated during the 10 minutes staining by the voltage-sensitive dye di-8-Anepps (Invitrogen) at 5 μmol/L concentration. Field electrical stimulation is done by a pair of parallel carbon electrodes with grounded anode. The cathode voltage is supplied by a bipolar isolation amplifier circuit whose input is a set of pulses from the digital-to-analog converter of a National Instruments card (NI USB-6221). The light source is a green LED array (wavelength = 523nm, NTE Electronics Inc.), with intensity controlled by a Darlington array receiving TTL signals. The emitted fluorescence is filtered ($\lambda > 610$nm), converted to voltage with a fast photodiode (S1226-5BK, Hamamatsu), and amplified by an instrumentation amplifier (AD524ADZ, Analog Digital Inc). The voltage is then digitized with a National Instruments card, filtered and saved for post-experiment analysis.
Each sub-system has been successfully validated. Testing the whole system with cardiac-derived HL1 cells allowed final improvement on the signal-to-noise ratio and optimization of excitation intensity. This ready to use bioinstrument will play a key role in further studies on cultured cardiomyocytes.

1747-Pos
In Vitro Cardiac Safety Profiling of a Novel Benzyl-Ethylamine Compound
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When developing novel compounds for any clinical indication, the possibility of untoward cardiovascular effects must be addressed. To evaluate the cardiac electrophysiologic effects of A-674563 ((S)-1-benzyl-2-[5-(3-methyl-1H-indazol-5-yl)-pyridin-3-yloxy]-ethylamine), a novel benzyl-ethylamine, we profiled the compound in a series of cellular and tissue assays. In canine Purkinje fibers (30 min exposure; 2 sec BCL), A-674563 elicited concentration dependent depolarization with shouldering of the terminal repolarization phase (20 μM) and increased abnormal automaticity (60 μM). In papillary muscles, concentration dependent depolarization was also seen, but the effect was much less potent; 60 μM induced shouldering of the terminal phase of repolarization. Contractility was assessed using percent changes in fractional shortening of sarcomere length (FS) in rabbit left ventricular myocytes. A-674563 reduced FS in a concentration dependent manner; 10% at 2 μM and 47% at 20 μM. Effects on ionic currents were further evaluated using heterologously expressed cardiac ion channel cell lines. A-674563 inhibited Cav1.2, expressed in HEK cells, with an IC_{50} of 20 μM, suggesting that the decreased contractility seen in native cells is due to L-type calcium channel block. The compound reduced Nav1.5 (HEK cells) by 50% at 20 μM and inhibited Kir2.1 (tSA201 cells) with an IC_{50} of 35 μM. Block of these channels would be expected to reduce the upstroke velocity, depolarize the cellular membrane and lead to abnormal automaticity as was seen in the tissue assays. Although A-674563 caused minimal prolongation of action potential duration in tissues up to 60 μM, it inhibited hERG (HEK cells) with an IC_{50} of 0.7 μM. This potent block was most likely offset by the concomitant block of multiple cardiac ion channels. In conclusion, A-674563 affects multiple cardiac ion channels to elicit depolarization and increased automaticity in native cardiac tissues.

1748-Pos
Stretch-Sensitivity of Stretch-Activated BK_{Ca} Channels in Post-Hatch Chick Ventricular Myocytes
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We have previously reported the electrophysiological properties of stretch-activated BK_{Ca} (SAKCA) channels cloned from cultured chick embryonic ventricular myocytes. However, the physiological role of SAKCA channels in the post-hatch heart *in situ* is not clear. We have investigated the effects on the SAKCA current of cell length changes, applied axially using a pair of carbon fibers attached to opposite ends of an isolated ventricular myocyte of a 2 week-old chick. Whole-cell currents were recorded using the patch-clamp technique, while the cells were either held at resting length, or stretched to cause a 10% increase in sarcomere length. Stretch did not affect whole-cell currents immediately after the stretch was applied. However, sustained stretch for 3 minutes significantly increased outward currents. This stretch-induced change was reversed by applying 10 nM Iberiotoxin, a specific BK_{Ca} channel blocker, or a Na^{+}/Ca^{2+} free environment. These results were reproduced in a computer simulation study, suggesting that stretch does not activate SAKCA channels directly, but does so in a secondary manner via a stretch-induced increase in the cytosolic Na^{+} concentration followed by an increased Ca^{2+} influx.