

2757-Pos Board B527**A Cardiac Specific Inducible Mouse Model of Timothy Syndrome 2**

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Timothy syndrome (TS) is an extremely rare human disorder resulting from a single point mutation (G406R) in the intracellular part of the S6 transmembrane segment of domain 1 of Cav1.2. This region is encoded in a mutually exclusive manner by exons 8/8a.

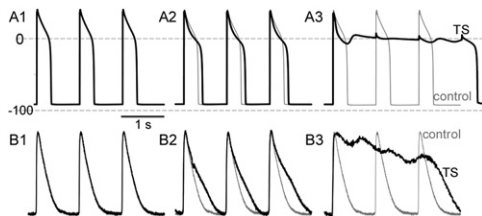
The identical glycine to arginine mutation occurs in both the 8a and 8 human splice variants resulting in TS1 and TS2. Heterozygous expression of the TS2 defect is lethal to mice. However, the TS2 defect is tolerated when suppressed through transcriptional interference by the NEO promoter (TS2-NEO mouse). By crossing TS2-NEO with a cardiac-specific tamoxifen-inducible CRE we can upregulate expression of the mutant TS2 calcium channel.

We extracted mRNA from cardiac left ventricle and probed with PCR primers to exon 7 and exon 9. Individual PCR products were incorporated into plasmid DNA and individual clones were isolated. This method detected exon 8a:8 ratios in normal mice as 0:58 and 1:56 in TS2-NEO mice suggesting very low level expression of the exon related to TS1. Only 1 in 56 TS2-NEO colonies expressed mutant channels in heart suggesting that the NEO cassette effectively suppressed mutant exon 8 isoforms. TS2-NEO mice were crossed with a cardiac specific promoter regulating a CRE recombinase which can be induced with tamoxifen. When activated with tamoxifen (to remove the NEO cassette and suppression of the TS2 mutation) the ratio of mutant to non-mutant exon increased from 1:34 to 29:72. Removal of the NEO cassette induced a strong slowing of inactivation in isolated myocytes. Tamoxifen-induced removal of suppression of the TS2 mutant exon increased QT duration from 33.1 ± 1.8 ms to 74.0 ± 5.5 ms ($n=5, p<0.01$). These results demonstrate the critical role of the L-type calcium channel inactivation in repolarization.

2758-Pos Board B528**Nonlinear Threshold Behavior in the Induction of Arrhythmias by Channels Bearing Timothy Syndrome Mutations**

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Timothy Syndrome (TS) is a multisystem disorder, featuring cardiac action-potential prolongation with paroxysms of life-threatening arrhythmias. The underlying defect is a single point mutation in Cav1.2 channels. Interestingly, recent clinical studies hint that overt disease manifestation might be mitigated by diminished expression of mutant channels. To gain insight, we undertook in-depth biophysical characterization of TS channels, and used this profile to refine a widely-utilized ventricular myocyte model (*AJP* 292:H2854). Simulations of variable levels of TS channels suggested a remarkable behavior. As expected, a modest increase in TS channels produced moderate action-potential prolongation (**A1**, control; **A2**, TS). By contrast, only a minute further increase in TS channels induced flagrant disturbances of excitability (**A3**), suggesting a nonlinear threshold for induction of frank arrhythmias. To explore this experimentally in cell networks, we variably expressed TS channels in monolayers of cultured guinea-pig ventricular myocytes. Indeed, conservative expression of TS channels yielded graded action-potential prolongation (**B1**, control; **B2**, TS), but small further elevation begat severe disturbances (**B3**). If like behavior exists in the heart, limited changes in TS expression may generate dramatic alterations in arrhythmogenic potential, an important principle for therapeutics.

**2759-Pos Board B529****Effect of Acidosis on Ventricular L-Type Calcium Current during Action Potentials**

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Changes in extra- and intracellular pH (pH_o and pH_i) accompany myocardial ischemia and protons have major effects on ventricular ion currents. However, the separate action of low pH_o and low pH_i on L-type Ca^{2+} current ($I_{Ca,L}$) during ac-

tion potentials is unresolved. $I_{Ca,L}$ was measured (at 37°C) by applying action potential voltage-clamps (AP-clamp) to adult rabbit ventricular myocytes bathed in CO_2/HCO_3^- -free solution, with sarcolemmal sodium-hydrogen exchange blocked (30 μM cariporide). pH_i and Ca^{2+}_i were measured with SNARF-1 and fluo-4, respectively. Starting from control conditions (pH_o 7.4; pH_i 7.2) each type of acidosis was applied for 2 min: low pH_o (pH_o 6.5, pH_i 7.1), low pH_i (pH_o 7.4; pH_i 6.7, achieved with 80mM acetate superfusion) and combined acidosis (pH_o 6.5; pH_i 6.7). In current-clamped cells, low pH_o shortened AP duration, whereas low pH_i or combined acidosis lengthened AP duration and slowed phase 1 repolarization. During AP-clamps, initial peak $I_{Ca,L}$ was reduced by each type of acidosis. For low pH_o , this effect was mediated largely by direct channel pore block. For intracellular and combined acidosis, it resulted largely from: a) increased steady-state inactivation of $I_{Ca,L}$ produced by an H^+ -induced rise in diastolic Ca^{2+}_i and b) by slowing of phase 1 repolarization. Net Ca^{2+} influx via $I_{Ca,L}$ was reduced by low pH_o but markedly increased by low pH_i or combined acidosis, the latter two effects mediated by proton-induced slowing of $I_{Ca,L}$ inactivation kinetics. Whole-cell modeling successfully predicted the AP changes induced by low pH_o and low pH_i plus many of the accompanying changes in Ca^{2+} signaling. We conclude that the pH_i -versus- pH_o control of $I_{Ca,L}$ will exert a major influence on electrical and Ca^{2+} -dependent signaling during acid-base disturbances in the heart.

2760-Pos Board B530**Effects of Anandamide on Electrical Activity, Calcium Transients and Contraction in Guinea Pig Cardiac Ventricular Myocytes**

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Anandamide is an endocannabinoid which reduces cardiac contractility. Although the effects of endocannabinoids are generally attributed to their association with G-protein-coupled cannabinoid CB_1 and CB_2 receptors, the identity of the receptor(s) or target(s) responsible for the effects of anandamide in the heart are unknown.

Anandamide (10 μM) reduced action potential (AP) duration and contraction in guinea pig ventricular myocytes stimulated to fire APs using an intracellular micro-electrode. In addition, anandamide reduced peak amplitude of L-type Ca^{2+} currents over the range -30 to $+60$ mV (switched voltage clamp, step depolarisations from -40 mV), with a reduction in peak current at 0 mV of $57 \pm 11\%$ ($n=6, p<0.01$). Ca^{2+} transients measured in field-stimulated cells using Fluo-5F were also reduced. The reduction in AP duration in response to anandamide was partially inhibited by the CB_2 receptor antagonist AM 630 (1 μM), but effects appeared non-competitive, and were smaller than predicted based on a K_d value of 31 nM for CB_2 receptors. The CB_1 receptor antagonist AM 281 (1 μM), the TRPV1 receptor antagonist capsazepine (1 μM), and O-1918 (an antagonist of a reported non CB_1 /non CB_2 cannabinoid receptor; 1 μM) failed to inhibit responses to 10 μM anandamide. Furthermore, the selective CB_1 receptor agonist ACEA, and the selective CB_2 receptor agonist HU-308 (both 10 μM) were without significant effect on AP duration.

The effects of anandamide can, at least in part, be accounted for by an inhibition of L-type Ca^{2+} currents. However, additional effects on other ion channels or intracellular targets cannot be ruled out. It appears that if a cannabinoid receptor mediates these effects, the characteristics of this receptor are not those expected of conventional CB_1 or CB_2 receptors.

2761-Pos Board B531**The Role of Caveolae and Membrane Conformation in Load-Induced Ventricular Conduction Slowing**

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Mechanical load has been shown to alter cardiac electrophysiology, but the underlying mechanisms are still not completely understood. Conduction slowing has been observed during increased ventricular load in the isolated heart and has been associated with changes in intercellular coupling and membrane capacitance. The goal of this work was to investigate the effect of increased ventricular pressure on cardiomyocyte membrane conformation and caveolar structure, and the role of these changes in conduction slowing associated with load. Murine hearts were isolated, retrogradely perfused, and pressure loaded through a cannula inserted into the left ventricular cavity. In the pressure loaded wild-type heart, electron microscopy revealed a tautening of excess membrane folds, fewer sub-sarcolemmal caveolae, and an apparent opening of the neck of sarcolemmal caveolae compared to the unloaded state. Optical mapping revealed a significant 15-18% slowing of conduction velocity with load in both the directions of fastest and slowest conduction (CVmax and CVmin, respectively). Hearts isolated from caveolin-3 deficient mice have no caveolae on the cardiomyocyte membranes, and control hearts treated with methyl- β -cyclodextrin (M β CD) have a diminished number of caveolar structures, as assessed by electron microscopy. These hearts lacked changes in

sub-sarcolemmal caveolae and caveolar structure with pressure load, and conduction slowing was attenuated. Cardiomyocyte caveolar localization and conformation change with increased ventricular load, and this alteration appears to play an important role in load-induced conduction slowing, possibly by contributing to changes in effective membrane capacitance.

2762-Pos Board B532

Ionic Mechanism for the Formation of Excitable Scroll Wave Filaments

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Scroll waves revolving at high frequency in the heart are responsible for fatal arrhythmias. Some mechanisms of arrhythmias require scroll waves to revolve at high frequency around an excitable filament. Recent ventricular cell models including calcium dynamics cannot reproduce this phenomenon. We address this problem revising sodium current kinetics and key phase II inward currents. We perform a nonlinear analysis of the sodium current gathered in canine cardiac myocytes. Despite an extensive data set, our nonlinear analysis shows that several Hodgkin-Huxley formalisms, i.e., a model family, reproduce the voltage clamp data. We incorporate formalisms taken from this family in the latest version of the Luo and Rudy cell model (LRd) and explore the parameter space for scroll wave dynamics. The simulations are performed on a monolayer of cells (3cm x 3cm) and portions (about 2/3) of the left ventricular free wall with realistic representation of the microanatomy. The Bidomain equations are solved at a resolution of 100um in space and 100us in time and are carried out on a supercomputer of the Texas Advanced Computer Center.

Our bifurcation analysis shows that sodium current formalisms associated with higher threshold and slightly slower rate of rise during early depolarization may be more realistic. When combined with relatively minor revision of phase II plateau inward currents, the LRd model can produce scroll waves revolving around an excitable filament. The revised model exhibits a rotation period significantly briefer than the original one, and even briefer than the refractory period measured on a plane wave. This mode of excitation allows us to study mechanisms of cardiac death. In conclusion, revision of sodium current kinetics and phase II inward currents of the LRd model allows to realistically reproduce scroll wave revolving around unexcited filament.

2763-Pos Board B533

Computational Predictions of Tissue-Specific Consequences of Long QT Mutations: Role of the Purkinje Fiber

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The long QT syndrome (LQTS) is a heritable cardiac disorder that leads to prolongation of ventricular repolarization, episodes of ventricular arrhythmia, and sudden cardiac death. Mounting evidence has implicated the Purkinje fiber (PF) conduction system in the genesis of ventricular arrhythmias. This study assesses for tissue-specific consequences of the biophysical alterations induced by LQTS-related mutations, using computational models of ventricular and PF cells. Mutations causing LQT1 and LQT2 (in KCNQ1 and HERG, respectively) are first simulated by reducing density of the respective components of the delayed rectifier current. These mutations prolong action potential duration (APD) in ventricular myocytes more than in their PF counterparts, due to differences in the conductance and activation of plateau potassium currents between the two cell types. Next, the canonical LQT3 mutation delKQP (in the cardiac NaV 1.5 sodium channel, encoded by SCN5A) is modeled in both tissue types (as described previously, by increasing entry into a bursting mode of channel gating, producing substantial late non-inactivating inward current). Marked APD prolongation is confirmed in both tissue types, exacerbated by slow stimulation rates or pauses in pacing. Simulation of another SCN5A mutation, F1473C, which clinically produces severe QT prolongation and heavy arrhythmia burden, is shown to have a markedly larger effect on PF cells than ventricular myocytes (including a propensity for repetitive early afterdepolarizations), owing to a depolarizing shift in the mutant channel availability and lower plateau potential in PF. Finally, the interactions between PF and ventricular cells at the tissue level are investigated in the context of these mutations in a cable model representing a section of ventricular wall. In conclusion, the biophysical alterations induced by LQT mutations may have significant tissue-specific consequences, with important implications for arrhythmia and its therapy.

2764-Pos Board B534

Optical Screening of Electrical, Mechanical, and Signaling Function on Adult Cardiac Myocytes as Alternative QT-Screen

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QT interval prolongation is a biomarker for ventricular tachyarrhythmias and a risk factor for sudden death. It is well known that some drugs can prolong the QT interval. QT-interval screens are recommended by FDA for nearly all

new molecular entities. Preclinical QT screens include animal experiments and patch-clamp based methods solely screening for alterations in heterologously expressed hERG channel conductance in cell lines. The aim of the present study was to explore the recording conditions and define settings which allow the optical screening of multiple electrophysiological parameters in isolated cardiac ventricular myocytes as an alternative to traditional QT-screens. Here we have shown that modern calcium and voltage fluorescent probes allow for high temporal resolution (under 3 ms) of cytoplasmic calcium and membrane potential transients in beating adult cardiac myocytes. A novel approach allowing for simultaneous measurements of intracellular calcium and cell contractions using fast line scanning mode of laser confocal microscope is described. Concomitant ratiometric detection of voltage sensitive probes fluorescence allowing for motion artifacts correction is also discussed.

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Impact of Multiple Ionic Changes in Arrhythmic Risk Biomarkers in Human Ventricular Electrophysiology

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Electrophysiological variability represents an important challenge in evaluating cardiac response to disease and drug action. Our goal is to quantitatively evaluate the sensitivity of biomarkers to the simultaneous alteration of several ionic current in human cardiomyocytes. Computer simulations of human ventricular electrophysiology are conducted using an action potential (AP) model proposed by Carro et al.

Ionic current conductances and kinetics were simultaneously varied by +/-30%. Model output was evaluated by quantifying biomarkers including AP duration (APD) and triangulation. Statistical techniques based on experimental design along with a second order response surface model were used.

Results reveal that simultaneous alteration of several ionic properties results in nonlinear effects in biomarkers response. APD is more sensitive than triangulation to changes in ionic properties. 30% changes in sodium/potassium pump and in calcium inactivation result in APD values outside the physiological range (287-350ms; Fig. 1A).

In contrast, 30% changes in most ionic properties result in triangulation within physiological range (70-86ms). However, simultaneous reduction of GKr and GCaL by 30% significantly increases triangulation (Fig. 1B).

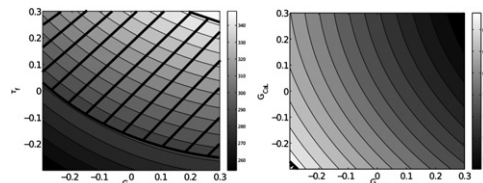


Figure 1. A) AP duration, B) AP triangulation. Marked area indicates physiological range

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Action Potential Duration Maps in Homogeneous Cardiac Ventricular Tissue Slices are Independent of Changes in Stimulation Site

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Cardiac slices are an increasingly popular model system for cardiac electrophysiology research, as they combine ease of handling with patho-physiologically relevant cell-type representation and distribution. The revival of the cardiac slice technique, first established in the 1980's¹ has been linked to improved techniques for preparation and monitoring. It now offers a well-posed target for computational modelling of cardiac structure-function interrelations.

This study used slices from the New Zealand white rabbits (female, ~1kg, n=12), cut tangentially to the left ventricular wall surface (350um thick, ~1x2cm large), offering a simplified 'pseudo-2D' experimental model. Slices, loaded with Di-4-ANBDQPQ (voltage sensitive dye; 10uM) to optically monitor action potential duration (APD), were stimulated at four different sites with concentric-bipolar point-electrodes, using a biphasic rectangular pulse 50% above activation threshold and, for comparison, with field stimulation.

APD at 50% and 80% repolarization (APD50 and APD80, respectively), was used to establish APD-maps in each slice. Locally-resolved average APD patterns were obtained for pacing frequencies of 1-4Hz. In addition, at each slice location the