model developed by Shannon et al. in our laboratory, and includes a subsarcolemmal compartment (in addition to the other two commonly formulated cytosolic compartments, junctional and bulk) where the ion channels sense ion concentrations that differ from the bulk. Ion channels and transporters have been modeled on the basis of the most recent experimental data obtained in our group and from the literature. In particular, novel formulations of the rapidly and slowly inactivating components of Ito have been implemented and utilized to differentiate between endocardial and epicardial myocytes. The model has been validated against a wide set of experimental data including action potential adaptation and restitution properties, frequency dependent inotropy and intracellular sodium staircase. It also correctly predicts the effect of pharmacological intervention on K currents (e.g. chromanol 293 B and dofetilide administration) on ventricular repolarization. We conclude that this model is more robust than previously existing models and provides a useful framework to explore excitation-contraction coupling mechanisms and repolarization abnormalities at the single cell level. To overcome the substantial limitations to experimental studies involving human cardiac tissue, due to its low computational cost this model is suitable to be integrated into multi-scale models of tissue and/or heart.

3433-Pos Board B480

Ventricular reentrant arrhythmia due to regional differences - A computational Study

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For many years the most accepted hypothesis is the mechanism of fibrillation in turn leads to arrhythmia was that the anatomical and electrophysiological heterogeneity in cardiac tissue. Regional differences in action potential duration (APD) and changes in depolarization in the heart favor re-entrant arrhythmia. With the help of mathematical model of ventricle cell, the role of sodium on 2D grid of cells in establishing arrhythmia is studied. First a homogeneous tissue of 60×60 was considered with all the parameters are identical. Next the heterogeneity in the tissue has been formed with the help of small squares by varying sodium conductance (g_{Na}) values from its nominal. Also spatial heterogeneity in the tissue has been formed by setting g_{Na} values of some of the squares at deviated values from its nominal. Due to heterogeneity among the cells in the tissue, the action potential (AP) propagation in the tissue is totally arrhythmic. The regional variation in g_{Na} at the center square showed that cells in that region where g_{Na} is varied gets disturbed (i.e. not able to depolarize). Next study, the regional differences in g_{Na}[[Unsupported Character - Codename]] is increased to three squares in diagonal wise. It is observed that the activity pattern of AP propagation in the tissue almost gets disturbed and spiral waves start originating from the center of the squares. Next analysis the number of squares increased to five. Compared to all previous cases, the variation in activity pattern of AP is totally gets collapsed in this case. Further, it is observed that multiple spirals are formed in the tissue around the region where regional differences are made. This multiple spirals further propagated to the entire tissue and causes re-entrant arrhythmia.

3434-Pos Board B481

Drug-induced Brugada ECG Changes Associated With A Novel SCN5A Mutation In A Patient With Atrial Arrhythmias

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Background: Subclinical mutations in the genes associated with inherited arrhythmias may cause unexpected pharmacologic responses in antiarrhythmic therapies.

Methods: The administration of pilsicainide, a class Ic antiarrhythmic agent, caused marked Brugada-type ST-elevation and frequent PVCs in a 66-year-old Japanese male who had presented with paroxysmal atrial fibrillation and type I atrial flutter. The patient has structurally normal heart, with no family history of Sudden Cardiac Death (SCD) or syncope. Genetic screening using PCR/direct sequencing identified a novel *SCN5A* mutation, V1328M. Biophysical characteristics of WT and V1328M-*SCN5A* were studied using patch-clamp techniques.

Results: The whole-cell sodium current densities were comparable between WT and V1328M. While V1328M did not significantly affect the voltage-dependent activation kinetics, V1328M was found to rightward shift the voltage-dependency of the peak currents by 10 mV and the steady-state inactivation by 7 mV (inactivation V_h: WT, -100.2 ± 0.8 mV, n = 10; V1328M, -93.1 ± 0.7 mV, n = 10, $p<0.01$). The pharmacologic responses of WT and V1328M to pilsicainide were studied .Pilsicainide (25 μM) caused similar extent of the tonic block reduction of sodium currents induced by a low frequency pulse protocol (q15s) in WT and V1328M. On the contrary, V1328M significantly enhanced the use-dependent block (2Hz) by pilsicainide (25 μM) compared to the WT (%block: V1328M, 62.0 ± 1.7, n = 6; WT, 42.6 ± 1.0, n = 6, $p<0.001$). In addition, intracellular pilsicainide (500 μM) did not block both WT and V1328M currents.

Conclusion: Our findings suggest that a *SCN5A* mutation V1328M might predispose certain individuals in the antiarrhythmic pharmacotherapy to drug-induced Brugada ECG changes. Our data,also, suggests that the *SCN5A* mutation located in the intracellular side can affect the sodium channel blocking from the extracellular side.

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Inactivation In Kv1.4 Channels Involves Significant Intracellular Structural Rearrangements Mediated By A Proline Hinge

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Several voltage gated channel families share a common structural motif in the intracellular side of their S6 segment: a Proline-Valine-Proline sequence known as a proline hinge. We studied the proline hinge in Kv1.4 channels which activate and then inactivate via two distinct inactivation mechanisms, N- and C-type. We made several point mutations to the two prolines in the P-V-P hinge of Kv1.4 channels, most of which did not result in a functional channel. Two mutations did result in a functional channel: a glycine or alanine for the second proline. These mutations were studied in the presence and absence of the N-terminal to separate the effects on N and D-type inactivation (Kv1.4[P558A], Kv1.4[P558A]ΔN, Kv1.4[P558G], and Kv1.4[P558G]ΔN Both of these S6 mutations slowed or removed N- and C-type inactivation, and altered recovery from inactivation. The P558G mutation, which allowed more flexibility slowed N-type inactivation by nearly an order of magnitude and no C-type inactivation was observed in the abasensce of the N-terminal, consistent with our previous findings of a major structural rearrangement involving S6 in C-type inactivation. The P558A mutation was much more disruptive and slowed activation by more than an order of magnitude. No inactivation was observed in either N intact or deleted constructs, however activation in the presence of the N-terminal domain was biphasic and paradoxically slower for the P558A mutation. These results are consistent with our hypothesis that the proline hinge plays a significant role in inactivation and recovery, and that inactivation involves significant conformational changes of the intracellular side of Kv1 channels which is modulated by interaction with the lipophilic N-terminal ball and are closely linked with activation and deactivation.

3436-Pos Board B483

Cardiac Characteristics of a Mouse Model of Timothy Syndrome

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Timothy Syndrome (TS) is the only L-type Ca^{2+} channel (Cav1.2) defect linked to arrhythmias and sudden cardiac death (Splawski I et al. Cell 119: 19-31, 2004). Timothy SyTS results from a *de novo* gain-of-function mutation on the intracellular side of S6 from the first domain which affects the voltage dependent component of inactivation in Cav1.2 and results in prolongation of the QT interval.In addition to arrhythmogenesis, TS is associated with congenital heart disease, syndactyly and autism spectrum disorders. We created a knock-in mutation of TS in mice. Computer modeling suggested that the cardiac AP should be minimally affected under physiological conditions and in mice the arrhythmic potential should be observable only under pathophysiological conditions. The ECGs of conscious, unrestrained, unanesthetized mice and were performed double blinded. The QTc (38) in TS mice was prolonged, shifting from 44.3 ± 0.5 ms (n=8) for control mice to 47.2 ± 0.5 ms (n = 17) for mice expressing the TS mutation ($P<0.01$). Viewed qualitatively, many of the electrocardiograms from the TS mice showed a marked change in T-wave morphology. Other significant changes in conscious mice were also noted, the duration of the QRS complex shifted from 9.2 ± 0.4 ms (n=8) to 11.1 ± 0.2 ms (n = 17), heart rate showed a slight but not statistically significant increase and normalized heart rate variability showed a decrease from 5.1% ± 1.1 (n=8) to 2.6% ± 0.6 (n=17 $P<0.05$) indicating an increase in sympathetic tone in the TS mice. TS patients are particularly susceptible to arrhythmias in response

to anesthesia and TS mice showed increased sensitivity to anesthetic (ketamine) with much loner QT prolongation and arrhythmias such as premature beats and apparent AV block.

3437-Pos Board B484

Ranolazine Antagonizes The Effects Of Anemone Toxin-II On Intracellular Ca2+ Cycling In Whole Heart

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The late sodium current (INa,L) is pathologically enhanced in several cardiac disease states, including ischemia, causing increased intracellular Na+ and Ca2+ loading and cellular dysfunction. Ranolazine (RAN) is a blocker of INa,L and this mechanism is thought to underlie its effectiveness at reversing many of the cellular effects of ischemia. The goal of this study was to determine if RAN antagonizes the effect of anemone toxin II (ATX-II), an INa,L enhancer known to increase Na+ influx, to alter intracellular Ca2+ cycling in individual myocytes of intact heart. Langendorff-perfused rat hearts were loaded with fluo-4AM (15μM) and placed in a chamber on the stage of a confocal microscope (contractions abolished with cytochalasin-D and blebbistatin). ATX-II (1nM) prolonged the early phase[j1] of basal Ca2+ transients (CaTs) in cells of hearts paced at a rate of 2 Hz[j2]. ATX-II slowed the rate of recovery of cellular CaTs (i.e., restitution) and promoted the development of CaT alternans at slower pacing rates. RAN (10μM) partially reversed the effects of ATX on restitution and alternans, shifting both to shorter intervals[j3] . In addition, pre-treatment with RAN reduced the effects of subsequent exposure to ATX-II on both restitution and alternans development and blunted the ATX-induced changes in basal CaTs. These effects are consistent with an action of RAN to block $I_{Na,L}$, reducing Na^+ influx and resulting intracellular Ca^{2+} accumulation, and therefore suggest RAN treatment may reverse the effects of Ca^{2+} accumulation that occur in response to disease states in which $I_{Na,L}$ is enhanced (such as ischemia). Consequently, RAN may also reduce the arrhythmias that might result from repolarization gradients established by Ca2+ alternans and the resulting action potential duration alternans.

3438-Pos Board B485

NS5806 Activates the Transient Outward Potassium Current in the Canine Ventricle and Provides a New Model of the Brugada Syndrome

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Background: The Brugada syndrome (BrS) is characterized by elevated ST segments in the right precordial leads, ventricular tachycardia and sudden death. The syndrome has been linked to loss-of-function of sodium and calcium channels, however the transient outward potassium current (Ito) is thought to be central in the pathogenesis of BrS. We assessed the effects of Ito augmentation in a mammalian model using a novel activator of Ito, NS5806. Methods and Results: Voltage-clamp experiments were performed on midmyocardial cells isolated from the canine left ventricular wall. At 40mV NS5806 (10 μM) increased peak Ito by 79 ± 4 % and the time-course of inactivation was slowed (from Tau=12.6 ± 3.2 ms to 20.3 ± 2.9 ms). We next assessed the effect of increased Ito in the development of BrS phenotype using canine ventricular wedge preparations. NS5806 increased the epicardial action potential (AP) phase 1 magnitude, whereas the APs of endocardial cells were largely unaffected. The accentuated epicardial notch was associated with an accentuated J-wave on the ECG. Loss of the epicardial AP dome at some sites but not others, led to development of phase-2-reentry and polymorphic ventricular tachycardia. NS5806 was able to induce the BrS phenotype in wedges from both right and left ventricles of the canine heart; at 15 μM NS5806 BrS developed in 4/6 right ventricular preparations compared to 2/10 left ventricular preparations. Conclusion: The Ito agonist NS5806 recapitulates the electrographic and arrhythmic manifestation of BrS, providing evidence in support of its pivotal role in the genesis of the disease. Our findings suggest that a genetic defect leading to a prominent gain of function of Ito could explain variants of BrS in which ST segment elevation are evident in both right and left ECG leads.

3439-Pos Board B486

Chaos Synchronization in the Genesis of Cardiac Arrhythmias

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Afterdepolarizations resulting from interactions between membrane voltage and intracellular calcium cycling are considered to play a key role in arrhythmias in long-QT syndromes (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), heart failure and other conditions. Although the molecular pathophysiology of early afterdepolarizations (EADs) at the cellular level has been analyzed in many studies, how EADs lead to triggered activity in cardiac tissue remains a major unsolved question. Specifically, due to the source-sink mismatch, a single myocyte which is well-coupled to adjacent myocytes cannot manifest an overt EAD unless a critical mass of the adjacent myocytes also simultaneously decide to exhibit EADs, What then synchronizes EADs in a critical mass of myocytes? In this study, we present evidence from isolated myocytes exposed to hydrogen peroxide (H2O2) that EAD dynamics during periodic pacing are chaotic, rather than random. Using computer simulations, we demonstrate that electronic interactions between adjacent myocytes can cause local synchronization of chaotic EADs over a characteristic length scale, producing groups of myocytes with overt EADs next to groups of myocytes without EADs when the tissue exceeds a critical size. The resulting gradients in refractoriness allow EADs to propagate, which can then stimulate other regions to develop EADs, creating a tissue network of multifocal triggered activity. Local conduction block across refractory gradients can also initiate reentry. The electrocardiographic pattern is polymorphic ventricular tachycardia (PVT). In optically-mapped rabbit ventricles, we observed activation patterns during H2O2-induced EADs and PVT showing a mixture of focal activity and reentry, consistent with this chaos synchronization mechanism. Chaos synchronization is a novel mechanism for cardiac arrhythmogenesis which may account for lethal arrhythmias appearing suddenly during bradycardia.

3440-Pos Board B487

Assembling And Imaging Long Cables Of Live Cardiomyocytes For Validation Of Cable Theory Relationships

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Theoretical work on excitable tissue (heart, brain, muscle) often employs concepts from cable theory and resorts to one-dimensional models of wave propagation for capture of essential functional properties. We offer an experimental technique to spatially pack, image and computationally unpack quasi-one dimensional long cables (>10cm) of live excitable cells within the imaging field of view. This is achieved by micropatterning neonatal rat cardiomyocytes into Archimedean spiral topologies and imaging the whole cable at ultra-high resolution. We validate the method's applicability to studies of wave propagation assessing distortions due to curvature effects.

Specific demonstrations of the utility of the proposed method include experimental verification of the eikonal relationship linking the velocity of a wave in homogenous cardiac tissue and the radius of curvature seen by the wavefront. This is achieved by patterning thin cables with well defined linearly varying curvature. Furthermore, the technique is applied to validation of theoretical predictions regarding spatially discordant alternans - beat-to-beat alternations in cardiac signals that can be out-of-phase over space. Previous attempts to uncover mechanisms for spatially discordant alternans have utilized purely computational representations and fluorescence imaging of whole-heart preparations and two-dimensional cardiomyocyte monolayer networks. The cable-like geometry (~10cm) used here facilitate the direct comparison to analytical and numerical derivations done exclusively in 1D.

In conclusion, our experimental approach allows for systematic validation of different aspects of cable theory and various excitable tissue phenomena in a well-controlled setting, including wavefront-waveback interactions, implementations of distributed feedback control strategies etc.

3441-Pos Board B488

Gender Difference in Cardiac Repolarization: A Computational Study

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Multiple experimental studies have shown post-pubescent males have shorter QT intervals than females. Clinical studies have revealed that sex differences in QT interval become apparent from puberty, suggesting sex steroid hormones play a role in shortening QT intervals. Testosterone has acute non-transcriptional effects mediated by increased nitric oxide (NO) production, which results in increased slow delayed rectifier K^+ currents (I_{ks}) and reduced L-type Ca^{2+} currents ($I_{Ca,L}$). Like testosterone, progesterone modifies I_{ks} and $I_{Ca,L}$ currents via eNOs production of NO. On the other hand, 17β-estradiol inhibits I_{Kr} current according to very recent experimental results. To investigate effects of sex