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.

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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.

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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

hormones on QT interval in males versus females, we constructed ''male'' and ''female'' cell models using Faber-Rudy model of the guinea pig myocyte. The female model incorporated physiological concentrations of 17β-estradiol and progesterone measured in the follicular and luteal phases of the menstrual cycle, and predicts changes in APD at different stages of the menstrual cycle that are consistent with clinically observed QT interval fluctuations. The male model was developed to reflect changes induced by physiology concentrations of Testosterone. The models suggest protective effects of testosterone and progesterone to prevent APD prolongation and reduce QT interval, while estrogen significantly increase QT and susceptibility to drug-induced arrhythmias.

3442-Pos Board B489

Regression Analysis for Constraining Free Parameters in Electrophysiological Models of Ventricular Cells

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One of the challenges of building mathematical models is constraining free parameters. Parameter adjustments that have desirable effects on a given model output sometimes cause unexpected changes to other aspects of model behavior. Here, we extend a novel method for parameter sensitivity analysis and show that this procedure can uniquely define ionic conductances in a simple model of the human ventricular action potential (AP). We randomized ionic conductances in this model, ran repeated simulations, then collected the randomized parameters and simulation results as ''input'' and ''output'' matrices, respectively. Outputs included measures to characterize AP morphology as well as more abstract quantities such as the minimum pacing rate to induce AP alternans. We subjected the results to partial least squares regression, thereby deriving a regression matrix B. The elements of B indicate how changes in ionic conductances affect the model outputs. We show here that the matrix B can be inverted when 1) the number of inputs equals the number of outputs, and 2) outputs are linearly independent. The inverted matrix B^{-1} can then be used to specify the ionic conductances that would be required to generate a particular combination of model outputs. When we applied this procedure to our simulation results, we found that most ionic conductances could be specified with fairly high precision ($R^2 > 0.70$ for six out of eight conductances). This procedure therefore shows tremendous promise as a tool for constructing new models. The success of our approach suggests that if several physiological characteristics of cell are known, this information can be used to constrain the model parameters.

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Interactions Of Calcium Clocks And Membrane Voltage Clocks Enhance Robustness And Flexibility In A Novel Numerical Model Of Cardiac Pacemaker Cell Function

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Recent studies in sinoatrial node cells (SANC), have demonstrated strong interactions between the classical sarcolemmal voltage oscillator (membrane clock) and intracellular Ca^{2+}cycling (Ca^{2+}clock). We numerically explored possible advantages of this pacemaker system featuring mutual entrainment of both clocks. In our novel numerical model of rabbit SANC sarcoplasmic reticulum (SR) spontaneously and rhythmically generates subsarcolemmal Ca^{2+}releases during the late diastolic depolarization. These Ca^{2+}clock ''ticks'' generate Na^+/Ca^{2+}exchanger ''ignition'' currents that accelerate the diastolic depolarization. Grading Ca^{2+}clock ticking speed, by varying SR Ca^{2+}pumping rate, broadly modulates the pacemaker rate (-40% to+53% from 3Hz basal rate), as experimentally demonstrated with cyclopiazonic acid and milrinone, respectively. A physiological rate reduction (~-50%) is achieved by muscarinic receptor stimulation via the synergism of moderate Ca^{2+} pumping rate reduction and moderate I_{KACh} activation. When the Ca^{2+}clock is disabled, the membrane-delimited model generates dysrhythmic action potentials (APs) which can be converted to rhythmic APs by increasing I_{CaL} and/or I_f. However, our model without its Ca^{2+} clock, like many previously published SANC models, featuring the enhanced membrane clock function, has a substantially smaller range of AP rate modulation. For example, g_{CaL} doubling combined with a +8 mV I_f activation shift results in only a 12% rate increase; a 30% g_{CaL}decrease combined with −8 mV I_f activation shift results only in a 14% rate decrease. **Conclusion:** Our numerical SANC model of interacting Ca^{2+} and membrane clocks is substantially more flexible and robust than the classical membrane-delimited clock: Rhythmic ticks of the Ca^{2+}clock and their resultant ignition currents insure function of the pacemaker system within much wider ranges of rates and reserve in sarcolemma function, embracing smaller I_{KACh}, I_{CaL}, and I_f, including those, at which membrane clock, operating alone, fails.

Ion Channels, Other

3444-Pos Board B491

VSOP Protein Lacking the C-terminal Half of S4-like Segment Retains Proton Permeation

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VSOP/Hv1 is a protein that contains the voltage sensor domain but not pore domain [1, 2]. It exhibits properties of native voltage-gated proton channels reported in phagocytes and microglia. Addressing how proton permeates and how voltage-dependent gating is achieved in VSOP/Hv1 will lead to critical clues to understand mechanisms of voltage sensor operation and ion permeation. The putative fourth transmembrane segment (S4) of mouse VSOP (mVSOP) has three positively charged residues in a pattern similar to those conserved in other voltage-gated channels. We have previously shown that VSOP/Hv1 forms dimer and a version lacking the cytoplasmic region (V216X) expressing mainly as monomer exhibits robust voltage-dependent proton currents, suggesting that monomer constitutes proton permeation pathway [3]. However, V216X still contains some cytoplasmic stretch and it remained unknown whether a remaining stretch downstream of S4 segment is essential for proton channel activities. To address this, a series of deletion constructs of mVSOP were expressed in tsA201 cells and whole cell patch recording and western blot were performed. Surprisingly, voltage-gated outward currents were elicited in constructs with stop codon at sites upstream to the third arginine. Proton permeation was verified by measuring intracellular pH using the pH-sensitive fluorescent dye, simultaneously with whole-cell patch clamping. Therefore, mVSOP retains functions of voltage-gated proton channel only with a truncated S4 segment, neglecting some possible mechanisms of proton permeation. To gain more insights, we are currently trying to biochemically map the topology of S4 using the cysteine-targeting reagent.

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3445-Pos Board B492

Mammalian Spermatozoa Possess A Voltage-Gated Proton Channel

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Mature mammalian spermatozoa are stored in quiescent state in the male reproductive tract. Upon ejaculation and during their transit through the female reproductive tract, they acquire progressive motility and undergo other important functional changes that enable them to reach and fertilize the egg. Sperm intracellular pH controls intracellular Ca^{2+} concentration, membrane potential and motility of the axoneme, and appears to be a key regulator of the sperm functional changes in the female reproductive tract. Unfortunately, the mechanisms controlling sperm intracellular pH remain poorly understood. Here we applied the whole-cell configuration of the patch-clamp technique to identify and characterize these mechanisms. In human sperm, when pH of the pipette and bath solutions was 6.0 and 7.4 correspondingly, we observed a robust voltage-gated proton current with a half-activation voltage +13 mV. Similar to voltage-gated proton channels found in other cell types, the sperm proton channel (sHv) was strongly up-regulated by unsaturated fatty acids and potently blocked by Zn^{2+} with IC_{50} = 340 nM. Millimolar concentrations of Ca^{2+} and Mg^{2+} slowed down sHv activation kinetic but did not significantly reduced its amplitude. The amplitude of the voltage-gated proton current observed in human sperm was one of the highest among different cell types, with average current density ~ 50 pA/pF at +100 mV; however in mouse sperm the amplitude of the voltage-gated proton current at the same conditions was only about 5 pA/pF. Intracellular alkalinization induced by sHv can lead to activation of pH-sensitive CatSper calcium channel resulting in well-known phenomenon of voltage-gated Ca^{2+} entry into the sperm cell. Here we present a model of how sHv may regulate sperm motility and discuss its role in male fertility and contraception.

3446-Pos Board B493

Electron Current and Proton Current in Activated Human Monocytes - Strong Glucose Dependence of the Electron Current

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Monocytes play multiple roles in the immune system, among other things, linking innate to adaptive immunity. Despite their biological importance, monocytes alone among all other phagocytes have not been investigated during