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.

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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

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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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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