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.

3443-Pos Board B490

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

the ''respiratory burst'' - the coordinated activation of NADPH oxidase and proton channels. Voltage gated proton current and NADPH oxidase generated electron current were measured simultaneously in human monocytes in perforated patch configuration after one to three days in culture. Upon stimulation by 60 nM phorbol myristate acetate (PMA), electron current (reflecting NADPH oxidase activity) appeared and the proton current amplitude increased. PMA slowed the kinetics of tail currents, sped the activation of outward proton current, and shifted the g_H-V relationship negatively. The NADPH oxidase inhibitor diphenylene iodonium (DPI) inhibited the electron current but affected solely the tail current kinetics of the proton current. Thus, although monocytes differentiate from a different lineage than granulocytes, their responses to PMA resemble those of other phagocytes. Hydrogen peroxide (H_2O_2) production was recorded fluorometrically. Zinc, at concentrations that inhibit proton current, reduced H_2O_2 production in monocytes. This Zn^{2+} sensitivity resembles that seen in neutrophils and eosinophils. The dogma that extracellular glucose is necessary to support the oxidative burst of human monocytes was tested in electrophysiological and fluorescence measurements. Electron current measured in patch clamp experiments was increased 2.5 fold by adding glucose to the bath solution; proton current was unaffected. Correspondingly, H_2O_2 production was strongly increased and more sustained in the presence of glucose. In summary, the electrophysiological events during activation of monocytes resemble those in other phagocytes, but NADPH oxidase is more acutely dependent on the presence of glucose.

3447-Pos Board B494

Determining the Functional Core for Proton Transport, Ion Selectivity and Amantadine Sensitivity of the A/M2 Protein from Influenza A Virus

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Influenza continues to be an epidemic and pandemic disease. The M2 protein from influenza A virus is a pH-activated proton channel. Its function is essential for efficient replication of the virus. Moreover, the M2 protein is the target of the antiviral drug amantadine, which is one of few available antiviral drugs that inhibit influenza A replication. Although M2 protein is only a 97-amino acid protein, it possesses multiple roles by its different domains in different stages of virus life cycles. In spite of the importance of the ion channel function of the M2 protein, the part of the protein that possesses its central role-proton channel-has not been defined clearly. Moreover, recent structural studies used truncated constructs that have not yet been evaluated for proton channel function. Here we report findings from experiments designed to investigate the functional core for proton transport, ion selectivity and amantadine sensitivity of M2 ion channel protein. We constructed a series of truncation mutants, measured low-pH activated, amantadine sensitive current in oocytes of *Xenopus laevis* and also determined the relative M2 surface expression on the *Xenopus laevis* oocyte membrane. We found that a construct of residues 21-61 (''shortie''), which includes the TM domain and 18-residues of the cytoplasmic tail, has the ion channel activity indistinguishable from that of the full length M2 protein. Functional reconstitution vesicle assay also showed that this construct was sufficient for proton channel function. Further truncated peptides (residues 22-46 and residues 22-50) showed amantadine-sensitive proton fluxes similar to ''shortie'' M2 (residues 19-62), however these peptides displayed a lower proton-selectivity and some potassium ion flux.

3448-Pos Board B495

Conformational Heterogeneity Of The M2 Proton Channel: A Model For Channel Activation

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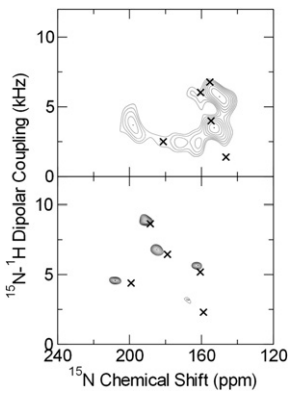
The M2 protein of influenza A is a proton selective ion channel activated by low pH. Recent structures determined by X-ray crystallography and solution NMR suggested models for open and closed states. However, these models are based on limited data and other important functional states need to be characterized. Indeed, solid-state NMR data demonstrate that the M2 protein possesses significant conformational heterogeneity. Here, we report MD simulations of the M2 transmembrane domain in the absence and presence of the anti-viral drug, amantadine. The ensembles of MD conformations for both apo and bound forms reproduce the PISEMA data well (Figure). The helices kink around Gly34, where a water molecule penetrates deeply into the backbone. The bound form exhibits a single peak around 10° in the distribution of helix kinking angle, but the apo form exhibits two peaks, around 0° and 40°. Conformations with the larger kinking angles have a wider opening around the primary gate formed by His37 and Trp41, reproducing some of the key observations on the low-pH activated state by ^{19}F NMR. We propose that this population is stabilized by low pH and leads to proton conductance.



3449-Pos Board B496

Reversibility of Amantadine Inhibition in the M2 Proton Channel of Influenza A Virus

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The M2 protein from influenza A virus forms a pH-activated proton channel that mediates acidification of the interior of viral particles entrapped in endosomes. M2 is the target of the anti-influenza drug amantadine. Many observations have shown that amantadine inhibits the channel only when applied extracellulary, probably by residing within the channel pore and disrupting the gating mechanism. The effect of amantadine on AM2 channel activity was found to be slowly reversible in tissue culture cells and oocytes, but the reversibility kinetics of the drug on recombinant AM2 channels has never been directly addressed. In the current work we provide evidence for significant differences in the rate of amantadine reversibility among several heterologous expression systems. We characterized the channel activity, the amantadine sensitivity and reversibility of the AM2 protein expressed in CHO-K1 cells using novel solid supported membrane technology (SURFE2R, IonGate Biosciences GmbH). SURFE2R technology allows time resolved measurements of the electrogenic activity of slowly conducting channels and provides a valuable compliment to electrophysiological studies. We also tested amantadine reversibility in AM2 expressing CHO-K1 cells using pH sensitive green fluorescent protein microscopy and in AM2 expressing oocytes using TEVC technique. We show that amantadine inhibition in AM2-expressing mammalian cells is rapidly reversible. However in oocytes the reversibility of amantadine inhibition is much slower and depends on the channel properties. These findings should be taken into consideration for future investigation of the mechanism of amantadine inhibition and show once again that the properties of recombinant proteins may be significantly influenced by the properties of the expression system.

3450-Pos Board B497

Study Of Gating Mechanism Of Two Pore Domain K+ Channels Gated By Extracellular Alkalinization: TASK-2 And TASK-3

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Potassium channels share a common selectivity filter that determines the conduction characteristics of the pore. Diversity in K+ channels is given by how they are gated open. TASK-2 and TASK-3 are two-pore region (2P) KCNK K+ channels gated open by extracellular alkalinization. We have explored the mechanism for this alkalinization-dependent gating using molecular simulation and site-directed mutagenesis followed by functional assay. We show that the side chain of a single arginine residue (R224) near the pore senses pH in TASK-2 with an unusual pKa of 8.0, a shift likely due to its hydrophobic environment (Niemeyer, et al., PNAS, 2007; 104(2):666-71). While, TASK-3 sense the pH through a histidine residue located at the outer part of the pore adjacent to the selectivity filter (GYG-H-) (Rajan, et al., JBC, 2000; 275(22):16650-7). R224 (TASK-2) and H98 (TASK-3) would block the channel through an electrostatic and structural effect on the pore, a situation relieved by its deprotonation by alkalinization. In this work we show a complete experimental and theoretical study about how the environment stabilizes the neutral or charged state of the sensor residue. Our free-energy profile, determinated using an Adaptive Biasing Force, together with a host of site-directed mutagenesis experiments illustrate in a physiological context the principle that the hydrophobic environment drastically modulate the pKa of charged amino acids within a protein.

3451-Pos Board B498

Dual Effect of Wogonin on TREK-2 Expression and Channel Activity

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