We present a method to obtain the resting membrane potential ($\Delta\Psi$) from the dielectric behavior of a suspension of living cells by the use of dielectric spectroscopy. Since cells behave as conducting particles surrounded by low-conducting shells with surface charge densities, we can apply this technique to record the dielectric permittivity ϵ and conductivity σ of the suspension as a function of frequency. A previous theoretical model has correlated the relative dielectric permittivity ϵ of the suspension with resting membrane potential in the very low radio frequency regime (alpha). We use this model with our experimental results to obtain $\Delta\Psi$ for bacteria (E.Coli K12) and mammalian cell suspensions from HEK293-hERG line. We compare our value for $\Delta\Psi$ and its changes with the traditional methods-voltage sensitive dyes and patch clamping. For E. Coli measurements, resting membrane potential is changed by KCl addition to the suspension bath. As for mammalian cells, $\Delta\Psi$ changes are triggered by the use of various pharmaceutical compounds that act as HERG K^+ channel blockers and IC_{50} values are computed for each compound. Precise measurements of the dielectric permittivity ϵ and conductivity σ of live cells suspensions in the alpha frequency regime require prior elimination of the polarization errors. Polarization errors are caused by the ionic content of a buffer, and they affect the total impedance in the low frequency interval. We hereby present our approach of measure the polarization impedance then remove it by fitting both real and imaginary experimental curves with an ideal impedance $Z=d/i\omega\epsilon^*S$, where $\epsilon^*=\epsilon+1/i\omega\sigma$.

3423-Pos Board B470

Continuum Multi-dielectric Treatment Of Fluctuations And Breakdown In Membranes With Embedded Charges

Michael B. Partensky[1], Gennady V. Miloshevsky[2], Ahmed Hassanein[2], Peter C. Jordan[1].

[1]Brandeis University, Waltham, MA, USA, [2]Purdue University, West Lafayette, IN, USA.

Stabilization of protein charges due to their interaction with membrane fluctuations is a subject of growing interest, especially because of its possible implications for voltage gating. Two complementary mechanisms governing charge-fluctuation interactions are considered: (1) the *electroelastic mechanism (EM)* [Partenskii, et al., *Israel J. Chem.* **47**, 385 (2007)], where the membrane is treated as an elastic slab (smectic bilayer model); (2) the *hydrophobic mechanism* (*HM*), which accounts for water penetration into the membrane's hydrophobic core with a corresponding interfacial tension contribution. In both cases the linear Poisson-Boltzmann equation is solved using a multi-dielectric continuum model with arbitrarily shaped membrane-water interfaces and a point charge surrounded by a ''Born sphere'' of low dielectric constant. The *EM* often leads to large membrane thickness perturbations, far larger than are consistent with elastic model descriptions. We demonstrate that switching from *EM* to *HM* becomes energetically advantageous at intermediate perturbation amplitudes. We apply kinetic Monte Carlo Reaction Path Following [Miloshevsky & Jordan. *J. Chem. Phys.* **122**, 214901 (2005)] using the water dimple's amplitude or the z-coordinate of the charge as the reaction coordinate for determining the shape of the solvation cavity. The resulting picture confirms that of recent MD studies.

3424-Pos Board B471

Phosphatidylserines Transduce Cell-Penetrating Peptides

Kevin Cahill.

University of New Mexico, Albuquerque, NM, USA.

Certain short polycations, such as TAT and polyarginine, rapidly pass through cell membranes and pervade all intracellular compartments by an unknown mechanism called transduction. These cell-penetrating peptides (CPPs) when fused to biologically active peptides promise to be medically useful. I offer a simple model in which phosphatidylserine (and possibly other anions) transduce CPPs. The model also involves surface tension and the electrostatic field across the membrane of the cell. The model is consistent with the empirical upper limit on the cargo peptide of about 35 amino acids. More importantly, it also fits experimental data on how the transduction of a polyarginine-fluorophore into mouse C2C12 myoblasts depends on the number of arginines in the CPP and on the CPP concentration.

3425-Pos Board B472

Electrical Relaxation Experiments With Bilayer Lipid Membranes In The Presence Of Cationic Quinones

Tatyana Rokitskaya, Inna Severina, Vladimir Skulachev, **Yuri Antonenko**.

Belozersky Institute, Moscow State University, Moscow, Russian Federation.

Mitochondria-targeted antioxidants consisting of a quinone part conjugated with a lipophilic cation via a hydrocarbon linker were previously shown to prevent oxidative damage to mitochondria *in vitro* and *in vivo*. In the present work, we studied the permeation of a series of compounds of this type across a planar bilayer phospholipid membrane. For this purpose, relaxation of the electrical current after a voltage jump was measured. All compounds studied exhibited slow relaxation kinetics in the time range from seconds to minutes. With respect to the characteristic time of the relaxation, hydrophobic cations can be ranked in the following series: 10(plastoquinonyl) decylrhodamine 19 (SkQR1) > 10-(6′-plastoquinonyl) decyltriphenylphosphonium (SkQ1) > 10-(6′-methylplastoquinonyl) decyltriphenylphosphonium (SkQ3) > 10-(6′-ubiquinonyl) decyltriphenylphosphonium (MitoQ). The relaxation was strongly dependent on the redox state of the quinone part of the molecule being substantially suppressed in the reduced form. Surprisingly, the kinetics of relaxation of several compounds depended not only on the phospholipid composition of the bilayer but also on the pH of the bathing solution.

3426-Pos Board B473

Impact Of $Na_V1.7$-PEPD Missense Mutations That Slow The Rate Of Inactivation On Sensory Neuronal Resurgent Sodium Currents

Brian W. Jarecki, Andrew D. Piekarz, James O. Jackson II, Theodore R. Cummins.

Indiana University School of Medicine, Indianapolis, IN, USA.

Voltage-gated sodium ($Na_v1.1$-9) channels are dynamic transmembrane proteins that, in response to changes in the potential across the lipophilic cell membrane, undergo specific conformational (gating) modifications, between ion-conducting (open) and non-conducting (closed and inactivated) states, to selectively conduct sodium ions through their aqueous pore. Importantly, changes in these voltage-dependent gating properties can impact action potential (AP) characteristics. TTX-sensitive sodium channels in cerebellar neurons can produce resurgent currents (Raman & Bean, 1997), intriguing currents that are reactivated during intermediate repolarizations following strong, but short, depolarizations. We observe resurgent currents in some DRG neurons and found that wild-type $Na_v1.6$ but not wild-type $Na_v1.7$ channels can generate resurgent currents in DRG neurons (Cummins *et al.*, 2005). It has been demonstrated that, in cerebellar neurons from $Na_v1.6$-null mice, slowing inactivation of the remaining Na_v current can induce resurgent currents (Grieco & Raman, 2004). Interestingly, single-point missense mutations in the SCN9A gene that encode for $Na_v1.7$, implicated in paroxysmal extreme pain disorder (PEPD), slow the rate of $Na_v1.7$ inactivation (Jarecki *et al.*, 2008). Therefore, we hypothesized that slowing of $Na_v1.7$ by PEPD mutations might induce abnormal resurgent currents, thus altering AP properties. To explore this hypothesis, we transiently transfected adult rat DRG neurons with a TTX-resistant form of human $Na_v1.7$-wild-type or PEPD mutant cDNA and rat $Na_v1.8$-targeted shRNA. Voltage-dependent properties were observed using whole-cell voltage-clamp electrophysiology and AP generation was tested using current-clamp electrophysiology. Recordings were made in the presence and absence of extracellular TTX. These experiments should yield insight into (1) the mechanism of resurgent sodium current generation in DRG neurons, (2) a potential additive effect in channel dysfunction observed in PEPD, and (3) how these mutant channels contribute to alterations in AP characteristics.

Cardiac Electrophysiology II

3427-Pos Board B474

Effects Of Mitochondrial Depolarization On Cardiac Electrical Activity In An Integrated Multiscale Model Of The Myocardium

Lufang Zhou, Natalia Trayanova, Gernot Plank, Miguel Aon, Sonia Cortassa, Brian O'Rourke.

Johns Hopkins University, Baltimore, MD, USA.

Metabolic or oxidative stress can trigger the abrupt collapse or oscillation of mitochondrial membrane potential, which activates KATP current and alters the cardiac action potential. This mechanism can introduce both temporal and spatial dispersion of electrical excitability in the form of a ''metabolic sink'', leading to heterogeneous conduction in the tissue, reentrant tachyarrhythmias, or fibrillation. To quantitatively study this mechanism, a 2D monodomain model of the myocardium (5×5cm2; 200 micron resolution) was developed, comprised of 63,000 nodes, each representing an integrated cellular model of cardiac excitation-contraction coupling, mitochondrial energetics, and ROS-induced ROS release (ECME-RIRR). Oxidative stress was initiated in a central circular zone of the tissue by increasing the fractional mitochondrial ROS production (shunt) during oxidative phosphorylation from 2% to 10%. Model simulations show that mitochondrial dynamics bifurcate during stimulation at 1 Hz and complete depolarization of $\alpha\Psi m$ ensues in the sink zone. Within the metabolic sink, sarcolemmal KATP currents increase, action potentials dramatically shorten, and the refractory period is abbreviated. These effects are enhanced by increasing the KATP density. In addition, fast and irregular electric activity (ventricular fibrillation) in the electrically paced tissue is observed when an S2 stimulus is introduced within or near the border of the

metabolic sink, but only when the sink exceeds a critical size (r>0.4cm). Phase singularity analysis indicates that the fibrillatory activity is initiated at sites close the border zone. The results emphasize the power of integrating cellular electrophysiological, Ca2+ handling, and metabolic subsystems into a multiscale model to simulate emergent macroscopic phenomena in the heart. Moreover, the results provide a proof-of-concept of the metabolic sink hypothesis and a new tool to study its role in arrhythmogenesis and sudden cardiac death.

3428-Pos Board B475

Action Potential Modelling Predicts Electrophysiological and Pharmacological Features of Human Embryonic Stem Cell-derived Cardiomyocytes

Stefano Severi[1], Laura Sartiani[2], Marisa E. Jaconi[3], Alessandro Mugelli[2], Elisabetta Cerbai[2].
[1]University of Bologna, Cesena, Italy, [2]University of Florence, Florence, Italy, [3]University of Geneva, Geneva, Switzerland.

Human embryonic stem cell-derived cardiomyocytes (hES-CM) represent a promising tool for cell therapy and drug screening. Their functional properties must be assessed.

We characterized hES-CM action potentials (AP) at two developmental stages with a combination of electrophysiological, RT-PCR and modelling tools. The AP was simulated on the basis of a model of human adult ventricular cell. The model was modified to incorporate experimentally assessed stage-dependent modifications of ionic currents (e.g. f-current, I_f, inward rectifier, I_{K1}, and delayed rectifier currents, I_{Kr}). Effects of current blockers were simulated by selectively reducing the current maximum conductance.

As we previously showed, changes in AP occur during in-vitro maturation (Early vs. Late): increase in AP duration and amplitude, decrease of slope of diastolic depolarization and rate of spontaneous beating. AP modelling reproduces: (i) experimentally observed changes in AP profile and differential effects of I_{Kr} blockade by E4031 at Early vs. Late stages (Figure A-B); (ii) effects of Ba^{2+} and zatebradine (I_{K1} and I_f blockers, respectively) (Figure C-D). These results suggest that our novel mathematical model can serve as a predictive approach to interpret and refine in-vitro experiments on hES-CM.

3429-Pos Board B476

Stability and Oscillations in a Ventricular Cardiomyocyte Model Studied Using the Tools of Dynamic Systems Analysis and Bifurcation Theory

Marin N. Popescu[1], Gheorghe Nistor[1], Adelina Georgescu[1], Alexandru D. Corlan[2], **Bogdan P. Amuzescu**[3], Cezar I. Barbu[3], Maria-Luiza Flonta[3].
[1]Faculty of Mathematics, University of Pitesti, Pitesti, Romania, [2]University Emergency Hospital of Bucharest, Bucharest, Romania, [3]Faculty of Biology, University of Bucharest, Bucharest, Romania.

Although ventricular fibers of the working myocardium *in vivo* or *in vitro* do not feature pacemaker activity in normal conditions, getting depolarized and contracting only upon receiving current input from the surroundings, in certain pathological states they become prone to generate sustained oscillations. In order to get an insight into the mechanisms underlying these rhythm disturbances, we studied the dynamics and bifurcation behavior of a simple mathematical model of ventricular cardiomyocyte, the Luo-Rudy I model, using numerical and analytical methods, as described by Kurata *et al.* For different configurations of parameters and initial conditions, we found equilibrium points (states where the field of variables vector vanishes). These were further used to compute the eigenvalue vector of the linearized system of differential equations at various values of stimulus current (I_{stim}) in the range of −5 to +5 uA/uF. Doubling the time-dependent potassium conductance (g_{kt}) resulted in sustained self-oscillations in a narrow interval of I_{stim}: (-0.7,+0.3) uA/uF for a reversal potential of the background current e_b=0 mV, and (-3.0,-2.1) uA/uF for the default value e_b=-59.87 mV, while for normal g_{kt} the system reached stable equilibrium over the entire I_{stim} range with either of the e_b values tested. We also demonstrated that, for a given set of parameters, the system admits a maximum of two different equilibrium points, with the same potential but different intracellular calcium concentrations.Acknowledgements to Prof. K. Mubagwa and A. Gwanyanya, PhD from KULeuven for help in initiating experiments on cardiomyocytes in Bucharest.

3430-Pos Board B477

Feasibility of Estimating Maximum Ion Conductance Parameters from the Shape of the Action Potential. A Simulation Study

Alexandru D. Corlan[1], **Bogdan P. Amuzescu**[2], Ivan Milicin[3], Gheorghe Nistor[4], Marin N. Popescu[4], Adelina Georgescu[4], Marcel O. Vlad[5].
[1]University Emergency Hospital of Bucharest, Bucharest, Romania, [2]Faculty of Biology, University of Bucharest, Bucharest, Romania, [3]Scalacalc Laboratory, Quattro Electronics Design SRL, Bucharest, Romania, [4]Faculty of Mathematics, University of Pitesti, Pitesti, Romania, [5]Dept. of Chemistry, Stanford University, Stanford, CA, USA.

Simultaneous measurement of ion currents and transmembrane potential (V(t)) is difficult. We verified whether the V(t) of a myocardial action potential (AP) is sufficient to estimate the cell's ion currents densities. We built a database of 45000 simulated APs by running the Luo-Rudy dynamic model simulator (LRd) on uniform randomly generated parameter sets comprising the maximum conductances for 12 major ion current components in the range of 0.5–2.0 times the default. A 'data' action potential (V_d(t)) was generated randomly in the same parameter interval and we tried to estimate its parameters. Each AP in the database was assigned the same prior probability at step 0. Then, at each of 50 steps spaced at 5 ms, a posterior probability was computed that was used as the prior probabily for the next step. We considered for each step t(j) the difference DV(i,j) between the i'th action potential in the database and the 'data' action potential. Using a normal noise model we calculated the non-conditional probability of DV(i,j), then the post-probability for step j given the prior obtained in step j-1. The highest posterior probability finally obtained identified our estimated parameters in the database.

RESULTS. In 100 such simulated experiments we found a RMSD of 4.14 ± 1.15 mV (mean $\pm$ SD) between estimate V(t) and data, corresponding to a very close resemblance. However, the absolute differences in parameters were large, ranging from 0.30 ± 0.31 for I_Kr to 0.9 ± 0.5 for I_Na.

CONCLUSION. There appears to be insufficient information in the single AP recording to simultaneously estimate the maximum conductances for 12 ion currents, as the same AP can be reconstructed from quite different parameters. Further progress will need taking into account other measurable experimental data.

3431-Pos Board B478

Characterization Of Human Embryonic Stem Cell-derived Cardiomyocyte Action Potentials And Channel Conductances Using A Theoretical Model

Brian E. Carlson[1], Gisela F. Wilson[2], Jianhua Zhang[2], Timothy J. Kamp[2], Daniel A. Beard[1].
[1]Medical College of Wisconsin, Milwaukee, WI, USA, [2]University of Wisconsin-Madison, Madison, WI, USA.

Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) can provide insights into the development of human myocardium and provide a powerful cellular system to investigate the electrical properties of human cardiomyocytes. In this study, we examined the action potentials (APs) of early developing hESC-CMs studied in spontaneously contracting EB outgrowths after 12-15 days of differentiation and modeled the channel conductances/activities responsible for the APs. Intracellular recordings using sharp KCl microelectrodes reveal cellular APs that are similar in basic form to those of early embryonic human cardiomyocytes. Comparison of the AP duration, AP upstroke slope and mean diastolic potential (MDP) show three distinct AP classes: nodal, embryonic-ventricular and embryonic-atrial. To gain a better understanding of the differences in channel activity underlying each AP class and to allow comparison to adult human cardiomyocytes, we used a modified version of a previously developed computational model of the adult cardiomyocyte. The main modification is the addition of a hyperpolarization-activated Na/K channel to represent the observed slow depolarization in diastole. The channels in this model are represented with a Hodgkin-Huxley formalism including parameters describing channel conductance, as well as inactivation and activation gating voltage and time constants. AP time courses are reproduced with this model by varying the various channel conductances (fast Na, rapid delayed rectifier K, etc.) In this manner the three differentiated hESC-CM classes have been characterized in terms of their relative channel conductances for the 12-15 day in culture developmental time point. Our results show that a more active background Na channel is required to adjust for the less polarized MDP seen in the recordings and the slow delayed rectifier K channel activity is greater in the nodal class of APs than is seen in the embryonic-ventricular class.

3432-Pos Board B479

A Novel Computational Model of the Human Ventricular Action Potential and Ca transient

Eleonora Grandi[1], Francesco S. Pasqualini[2], Jose L. Puglisi[1], Donald M. Bers[1].
[1]University of California Davis, Davis, CA, USA, [2]Polytechnic University of Marche, Ancona, Italy.

We have developed a detailed mathematical model for Ca handling and ionic currents in the human ventricular myocyte. Our objective was to implement a model that: 1) accurately reflects Ca-dependent Ca release; 2) uses repolarizing K currents with realistic amplitude; 3) comes to steady state; 4) simulates basic excitation-contraction coupling phenomena; and 5) runs on a normal desktop computer. The model relies on the framework of the rabbit myocyte