

Brownian Dynamics. We modeled the carboxylate-rich selectivity filter of calcium channels with 8 independent half-charged oxygens confined in the central region of the pore. We computed ions' trajectories self-consistently evaluating the electrostatic forces acting on the ions at every timestep. Such forces were evaluated solving Poisson's equation with a Boundary Element Method to deal with dielectric boundaries, called Induced Charge Computation method (ICC). A transmembrane potential was included as a spatially constant component of the electric field, a good approximation to a fully consistent treatment, see Crozier et al. (Biophys. J. 81:3077) and Hollerbach and Eisenberg (Langmuir, 18:3626). Boundary conditions for ionic concentrations in the intra- and extra-cellular domain were imposed by a Grand Canonical-Monte Carlo algorithm. We simulated different concentrations of CaCl_2 added to NaCl solution only on one side of the membrane. Ion permeation was investigated under physiological conditions, using different sub-millimolar calcium concentrations and different transmembrane potentials. Channel selectivity and conductance were determined by electrostatic forces, steric repulsion due to charge crowding, and gradients of concentration and potential.

534-Pos Board B303

Real-Time Modulation of Zebrafish Cone Phototransduction by Whole-Cell Delivery of zGCAP3 and of its Monoclonal Antibody

Marco Aquila¹, Daniele Dell'Orco², Anna Fasoli¹, Mascia Benedusi¹, Ramona Fries³, Alexander Scholten³, Elisabeth Kremmer⁴, Karl W. Koch³, Giorgio Rispoli¹.

¹University of Ferrara, Department of Biology and Evolution, Section of Physiology and Biophysics, Ferrara, Italy, ²University of Verona, Department of Life Sciences and Reproduction, Section of Biological Chemistry, Verona, Italy, ³University of Oldenburg, Institute of Biology and Environmental Sciences, Biochemistry Group, Oldenburg, Germany, ⁴Institute of Molecular Immunology, Helmholtz Zentrum München, Munich, Germany.

Regulation of excitation and adaptation in photoreceptors of the vertebrate retina strongly depends on the cytoplasmic Ca^{2+} concentration and its interplay with Ca^{2+} sensor proteins like recoverin, calmodulin and the activating proteins (GCAPs) of guanylate cyclase (GC) (Scholten and Koch, 2011). Of the four GCAP isoforms exclusively transcribed in cones (zGCAP3, 4, 5 and 7), we investigated the physiological function of zGCAP3 in green-sensitive cones of zebrafish, by recording the effect on the photoresponse waveform by cytosol injection of exogenous zGCAP3 (to simulate "real time" protein over-expression), and its monoclonal antibody (to simulate protein knock-down). To identify a suitable antibody we screened several hybridoma fluids with respect to specificity and affinity towards zGCAP3, using immunoblotting and surface plasmon resonance (SPR) spectroscopy. The global fitting of an overlay of SPR sensorgrams obtained with increasing antibody concentrations gave a Ca^{2+} -independent K_D of 12 nM for the interaction of zGCAP3 with the antibody. Exogenous proteins were incorporated with a precise timing in the zebrafish cone cytosol by an internal perfusion system coupled to a pressure-polished patch pipette (Benedusi et al. 2011). Typical whole-cell recordings lasting even more than 20 min did not show any significant change in light sensitivity, dark current amplitude, response kinetics and light adaptation, proving also that the enzymatic cascade was not perturbed by the recording protocol. Injection of anti-zGCAP3 caused the complete shutdown of the dark current, indicating that zGCAP3 plays a major role in regulating GC. Injection of purified zGCAP3 did not alter the photoresponse, indicating that the target GC was already saturated with endogenous zGCAP3.

Benedusi M, Aquila M, Milani A and Rispoli G (2011). Eur Biophys J 40: 1215-23.

Scholten A, Koch KW. (2011). PLoS One 6(8):e23117.

Excitation-Contraction Coupling I

535-Pos Board B304

Effects of Redox Environment on Calcium Alternans in Isolated Rabbit Cardiomyocytes

Joshua N. Edwards, Lothar A. Blatter.

Rush University Medical Center, Chicago, IL, USA.

Cardiac alternans is a multifactorial phenomenon linked to cardiac arrhythmias. At the cellular level cardiac alternans is defined by beat-to-beat alternations in contraction amplitude (mechanical alternans), action potential duration (electrical or action potential duration alternans) and Ca transient amplitude (Ca alternans) at constant stimulation frequency. The aim of this project was to characterize the effect of changes in the cellular redox environment on Ca alternans in cardiac myocytes. Single myocytes (from New Zealand White

rabbits) were isolated enzymatically by retrograde Langendorff perfusion. Ca alternans were induced by incrementally increasing the pacing frequency (electrical field stimulation) until stable Ca alternans occurred. The frequency at which stable Ca alternans were observed varied from cell to cell and ranged from >1 to 2.5 Hz at room temperature. Global cytosolic Ca transients were measured with Indo-1. In some experiments, cytosolic Ca alternans and intra-SR Ca alternans were simultaneously measured with the fluorescent Ca indicators Rhod-2 and Fluo-5N, respectively. Confocal microscopy was used to measure Ca sparks with Fluo-4.

Reducing agents dithiothreitol and reduced glutathione partially abolished Ca and mechanical alternans by restoring diastolic Ca and Ca transient amplitudes. A decreased sarcoplasmic reticulum (SR) Ca release flux but not Ca content, together with a decreased Ca spark frequency, suggest that reducing agents normalized alternans through effects on the SR Ca release channel (ryanodine receptor type-2). Addition of a membrane permeant superoxide dismutase mimetic, Tempol, had little effect on Ca alternans, suggesting the possible role of dithiothreitol directly acting on the ryanodine receptor. These data highlight that the redox state of the cell may be important in the generation of Ca and mechanical alternans during oxidative stress.

536-Pos Board B305

Modeling the Effect of Unitary Calcium Current on Neighboring Ryanodine Receptors during Calcium Induced Calcium Release

Michael J. Yonkunas, Michael Fill, Dirk Gillespie.

Rush University Medical Center, Chicago, IL, USA.

In resting cardiac cells, the open probability (P_o) of single ryanodine receptors (RyRs) is very low and consequently little Ca^{2+} is released from the sarcoplasmic reticulum (SR). However, a stochastic RyR opening will cause diastolic local Ca^{2+} release from the SR that can activate neighboring closed RyRs. This inter-RyR Ca^{2+} -induced Ca^{2+} release (CICR) may generate diastolic Ca^{2+} sparks. It is known that elevating SR Ca^{2+} load above normal levels dramatically increases spark frequency and increases the unitary RyR Ca^{2+} current. It is this current that acts on neighboring RyRs through CICR. We have developed a simple model based on experimental single-channel RyR Ca^{2+} sensitivity to understand how unitary RyR Ca^{2+} current may control CICR within a group of neighboring RyRs (a Ca^{2+} release unit, CRU). The model predicts how the current carried by an open RyR influences the activity of neighboring RyRs in a CRU. These predictions match published experimental single and clustered RyR channel results obtained in bilayer studies.

537-Pos Board B306

On the Role of Endogenous Calmodulin in Excitation-Contraction Coupling in Skeletal Muscle

Erick O. Hernández-Ochoa, Martin F. Schneider.

University of Maryland, Baltimore, MD, USA.

In skeletal muscle, calmodulin (CaM) regulates excitation-contraction coupling, primarily via modulation of the role of ryanodine receptors. Here we aimed to further our understanding of the role of endogenous CaM in excitation-contraction coupling. Since systemic ablation of CaM in mice is difficult to achieve due to CaM's multiple functions, *in vivo* gene transfer via electroporation mediated transfection method was used to deliver plasmid coding for both cerulean and short-hairpin (sh)RNA targeting CaM (shRNA-CaM) to study the effect of CaM knockdown in adult mouse *flexor digitorum brevis* skeletal muscle. CaM protein expression levels were significantly reduced in shRNA-CaM fibers, which exhibited no evident morphological changes when compared to the shRNA-control fibers. After confirming the reduction of endogenous CaM expression, we used high-speed confocal microscopy and rhod2-based Ca^{2+} imaging to assess the consequence of CaM knockdown on action potential (AP)-evoked Ca^{2+} signals. Isolated single muscle fibers expressing shRNA-CaM exhibited decreased mean peak amplitude and slowed decaying phase of AP-induced Ca^{2+} transient when compared to the shRNA-controls, indicating compromised Ca^{2+} release and Ca^{2+} uptake. We also used a model for myoplasmic Ca^{2+} binding and transport processes to calculate AP-evoked sarcoplasmic reticulum Ca^{2+} release flux, which demonstrated decreased Ca^{2+} release flux and indicated suppressed Ca^{2+} uptake in shRNA-CaM fibers. Decreased Ca^{2+} release could reflect decreased coupling between Cav1.1 and ryanodine receptor, a reduction in expression of one or both proteins or a decreased store content, whereas a slowed decaying phase is consistent with compromised Ca^{2+} uptake. Our study shows the importance of endogenous CaM in the maintenance of excitation-contraction coupling in adult skeletal muscle and could provide new avenues to further explore the potential role of both CaM-dependent and CaM-independent pathways in skeletal muscle contractility and plasticity. Supported by NIH-NIAMS Grant R37-AR055099.