Na channel can both be described (over a wide range of solutions and concentrations) by the same reduced model with the same unchanging two parameters (dielectric coefficient and diameter) in which side chains are spheres (Ca channel = EEEE or EEEA; Na channel = DEKA). In the EEEE channel, Ca\textsuperscript{2+} selectivity is driven by charge/space competition in which selectivity arises from a balance of electrostatics and the excluded volume of ions in the crowded selectivity filter. Electrostatics selects Ca\textsuperscript{2+} over monovalent cations. Excluded volume selects Ca\textsuperscript{2+} over larger divalent cations. All these combine to create depletion zones in the ion density profiles that are crucial determinants of the current carried by each ion species.

2666-Pos

Energetic Variational Analysis EnVarA of Ions in Calcium and Sodium Channels
Robert S. Eisenberg, Yun Kyong Hyon, Chun Liu
Department of Molecular Biophysics, Rush University Medical Center, Chicago, IL, USA, Institute of Mathematics and its Applications, University of Minnesota, MN, USA, Department of Mathematics, Pennsylvania State University, State College, PA, USA.

Selective binding in both calcium and sodium channels can be described (in many solutions and concentrations: Biophysical Journal (2007) 93:p.1960) by the same reduced model and unchanging two parameters (dielectric coefficient and diameter) in which side chains are spheres. The very different primary structure of the two proteins (Ca channel EEEE/EEEE; Na channel DEKA) and properties, even though amino-acid side-chains (E, D, etc.) are represented only as charged spheres. Monte Carlo MC simulations, reported in ~30 publications, work well (we think) because they do not specify structure as an input, independent of conditions. Rather, MC calculates the structure as an output, as a self-organized system, as the complex of ions (and vice-versa). Structure is different in different solutions in self-organized systems. Self-organized systems can be powerfully analyzed using the calculus of variations, specifically, energetic variational analysis (EnVarA). We optimize both action and dissipation integrals (Least Action and Maximum Dissipation Principles), motivated by Rayleigh, then Onsager who optimized just one, or the other. The resulting systems of coupled partial differential equations automatically satisfy the First and Second Laws of Thermodynamics and electrostatic Poisson equations, with physical boundary conditions that can produce flow. EnVarA extends Navier-Stokes equations to complex fluids containing deformable droplets (Journal of Fluid Mechanics (2004) 515:p.293). EnVarA provides a seamless extension of conservative Hamiltonian systems (perhaps at thermodynamic equilibrium) to dissipative systems. EnVarA is a field theory of ions in channels and solutions with entropy, friction, and flow. EnVarA computes current where MC computes only binding. EnVarA applied to EEEE/DEKA channels gives binding like real calcium/sodium channels. Time dependent currents computed with EnVarA resemble time dependent currents in either voltage activated sodium or potassium (squid axon) channels (depending on parameters), although the EnVarA model has only one unchanging conformation.

2667-Pos

3D Structure of a Recombinant DHPR Expressed in Mouse
John Szpyt, Claudio F. Perez, Nancy Lorenzo, Ethan Norris, PD Allen, Kurt Beam,Montserrat Samso
Brigham & Women’s Hospital/Harvard Medical School, Boston, MA, USA, University of Colorado-Denver, Aurora, CO, USA, University of Denver-Colorado, Aurora, CO, USA.

The dibhydropridine receptor (DHPR) is an L-type Ca\textsuperscript{2+} channel that acts as the voltage sensor for excitation-contraction coupling in skeletal muscle by tightly controlling the intracellular Ca\textsuperscript{2+} channel RyR1. Because previous 3D electron microscopic studies have largely not resolved the spatial organization of the DHPR subunits (x1, x2b, x2d, x1, and x2g), we constructed mice expressing a x1a subunit with YFP and a biotin acceptor domain attached to its N- and C- termini respectively. This engineered x1a sustains a functional DHPR-RyR1 interaction and viable animals in a x1a null background. DHPRs were purified from mice by means of the (biotinylated) biotin acceptor domain, negatively stained and imaged with electron microscopy. 8,662 individual DHPRs were analyzed using single-particle image processing algorithms. Multivariate statistical analysis, classification, and multi-reference alignment yielded distinct 2D class averages corresponding to different orientations of the macromolecule. The 3D reconstruction, with 25 A resolution, shows two distinct parts: a main body shaped like an irregular pentagon (~150x125x75 Å) with distinct corners, and a hook-shaped feature that extends ~60 Å from the main body. Consistent with the considerable conservation of membrane topology among voltage-gated channels, a good part of the main body can be closely fitted with an atomic structure of a full-length potassium channel, suggesting that the main body contains the x1a subunit. Besides the fitted potas-
sium channel the main body has extra volume that can accommodate the YFP atomic coordinates and other subunits.

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2668-Pos

Ca\textsubscript{2,3} Calcium Channels Inactivate from the Open State with Partial Charge Immobilization and Altered Deactivation Kinetic

Gustavo Contreras, Alan Neely
1Doct. en Neurociencia. U. de Valparaiso, Valparaiso, Chile, 2C. Inderdisc. de Neurociencia de Valpo. U. de Valparaiso, Valparaiso, Chile.

Voltage-dependent ion channels undergo inactivation following activation. In the sodium and potassium channels, the molecular determinants that govern the mechanism of inactivation involve pore blocking by a cytoplasmic particle. In calcium channels the conventional model is that the intracellular loop joining the first two homology domains of the pore forming subunit contributes to the inactivation gate or hinged-lid. To investigate key features of this model, we expressed Cav\textsubscript{2,3} channel in Xenopus oocytes without auxiliary subunits and recorded ionic and gating currents using the cut-open voltage-clamp technique. Ionic current were recorded in either Ba\textsuperscript{2+} or Ca\textsuperscript{2+}. Here we report that consistent with a hinge-like mechanisms, charge movement at the end of a depolarizing pulse decreases up to a 50% with channel inactivation. In contrast with a previous report by Patil et al (1998, Neuron 20:1020) that co-expresses auxiliary subunit with Cav\textsubscript{2,3}, trains of pulse elicited minimal inactivation. It appears then that when expressed alone, Cav\textsubscript{2,3} channels inactivate mostly from the open state. We also found that as channels inactivate, a slow component emerges in tail-current recordings. This component contributes to about 20% of the tail currents in 80% inactivated currents and can be accounted for with a classic allosterically-coupled model provided that channels can re-open multiple times from the last closed state and that some closed-inactive channels re-open during membrane repolarization.

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2669-Pos

Action Potential Hyperpolarization Kinetics Abets the Modulation of Alpha1b T-Type Calcium Channels by KLHL1 Mutants Lacking the Actin-Binding Domain (ΔKelch)
Kelly A. Aromolaran, Erica S. Piedras-Renteria.
Loyola University Chicago, Maywood, IL, USA.

The Kelch-like 1 protein (KLHL1) is a neuronal actin-binding protein that increases the current density and channel number of Ca\textsubscript{2,3} calcium channels via actin-F mediated increases in recycling endosomal activity. Removal of the actin-binding kelch motif (ΔKelch) prevents the increase in x1a current density seen with wild-type KLHL1 stimulated with square pulse protocols but not the increase in calcium influx seen during action potentials (AP).

Here we set out to dissect the kinetic properties of AP that confer the mutant kelch the ability to interact with x1a and induce an increase in calcium influx. Square waveforms (black trace) following the AP did not significantly increase calcium influx (25%, p>0.05) compared to the AP (red). We investigated the effects of altering the slope of the repolarization (1), the length of hyperpolarization (2), the slope of depolarization (3) and the duration of depolarization (4) on the modulation of x1a by ΔKelch. Our results show that the slope of repolarization from hyperpolarization induces the conformational changes that allow the channel to properly interact with ΔKelch, leading to increased Ca influx.

2670-Pos

C-terminal Alternative Splicing Modules Single-Channel Gating of Ca\textsubscript{1,3} L-Type Calcium Channel
Wanchana Jangsangthong, Jan Petran, Anamika Singh, Jörg Strissig, Stefan Herzg, Alexandra Koschak
1University of Cologne, Cologne, Germany, 2University of Innsbruck, Innsbruck, Austria.

We have recently discovered a novel mechanism of channel modulation in Ca\textsubscript{1,3} channels enabling cells to tightly control gating by C-terminal alternative splicing. The absence of a C-terminal modular motif (CTM) within a short splice form facilitates Ca\textsubscript{1,3} channel activation at lower voltages and induces pronounced Ca\textsuperscript{2+} dependent-inactivation (CDI) (Singh et al., JBC 2008). Intriguingly, whole-cell measurements revealed a significant