depolarizing voltage pulses from the holding membrane potential of −80 mV was reduced by Aβ1-42 at low (<10 mV) voltages, but was not changed at higher voltages. Slow C-type inactivation of K^+ current was significantly faster in the presence of Aβ1-42 with the effect being most prominent at −20 mV (lowest voltage measured) and diminishing with increasing voltage. The time constant of K^+ current deactivation was significantly reduced by Aβ1-42, and the effect progressively increased with voltage increase. Under the same conditions, the voltage sensitivity of Kv1.3 conductance was not significantly changed by Aβ1-42. Our results reveal acute effects of biologically active soluble β-amyloid oligomers on voltage-dependent potassium channels Kv1.3. Faster inactivation and deactivation of K^+ current in the presence of Aβ could impair regulation of the membrane potential, ultimately leading to pathophysiological changes in the cell.

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Structural Mechanism Of Redox Modulation In The Kv1-Kvβ Complex

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The *Shaker* type voltage-dependent K^+ channels (Kv1) are expressed in a wide variety of cells and essential to regulating membrane potential and cellular excitability. All Kv1 channels assemble with cytoplasmic β subunits (Kvβ) to form a macromolecule complex. Kvβ is a functional aldo-keto reductase that utilizes NADPH as cofactor, and in addition to being a functional enzyme, certain Kvβs have an N-terminal segment that blocks the channel by the N-type inactivation mechanism. The enzymatic activity and the N-type inactivation are functionally coupled: when the Kvβ-bound NADPH is oxidized, the N-type inactivation is inhibited and channel current increases as a result. Further studies showed that loss of the N-type inactivation is not due to dissociation of Kvβ upon NADPH oxidation. To understand the structural basis of the coupling mechanism, Kvβ was co-crystallized with either NADPH or $NADP^+$, and high-resolution data sets were collected. Since NADPH is easily oxidized, for the Kvβ-NADPH complex special cares were taken to preserve the reduced cofactor throughout the crystallization process. The redox state of the cofactor was also monitored during synchrotron data collection by a micro-spectrophotometer. Results obtained from both structural analysis and functional studies led us to propose a novel mechanism of channel modulation.

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Neuronal N-glycosylation Processing Modulates Voltage-gated Potassium Channel Activity

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The N-glycan pool contains high amounts of sialic acid with atypical linkage in mammalian brain. Sialoglycoconjugates are more alike in similar tissues from different mammals than in dissimilar organs from the same mammal. The intent of this study was to generate a cell model for examining the role of neuronal derived N-glycans of a voltage-gated K^+ channel, Kv3.1. Neuroblastoma B35 cells were utilized to heterologously express glycosylated (wild type Kv3.1) and unglycosylated (N220Q/N229Q) forms of Kv3.1 channels. Immunoband shift assays of partially purified wild type Kv3.1 protein digested with PNgase F indicated that both sites were utilized. Additionally, the attachment of *N*-linked sialooligosaccharides to the wild type Kv3.1 protein was shown by digestions with neuraminidase. Endoglycosidase N digestions demonstrated that an oligo/polysialyl unit with internal α2,8-linked sialyl residues was associated with the Kv3.1 glycoprotein. To date this unusual glycosidic bond for sialyl residues has not been identified on *N*-glycans of potassium channels. Whole cell current measurements of glycosylated and unglycosylated Kv3.1 channels revealed differences in channel activation, inactivation and deactivation properties. Channel density at the cell surface was also greatly reduced for the unglycosylated Kv3.1 channel compared to the glycosylated Kv3.1 channel. Based on the glycosidase specificities and the immunoband patterns, our results demonstrated that both *N*-glycosylation sites within the S1-S2 linker of Kv3.1 are highly available, and that at least one of the carbohydrate chains is capped with an oligo/polysialyl unit. These results also provide strong evidence that the S1-S2 linker of Kv3.1 is extracytoplasmic, and that *N*-glycosylation modulates the inactivation and activation kinetics of the Kv3.1 channel. Given the above observations, we suggest that neuronal *N*-glycosylation processing of the Kv3.1 channel is crucial in regulating and fine tuning the excitable properties of neurons in the nervous system.

2492-Pos Board B462

Interactions of the S4 Helix of a Kv Channel with a Lipid Bilayer: Free Energy Calculations via Coarse-Grained Molecular Dynamics Simulations

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The S4 helix is a major element of the voltage-sensor of voltage-sensitive ion channels. This helix contains an array of positively charged sidechains and yet adopts a transmembrane orientation within the voltage sensor of a voltage-gated channel. Thus, from both mechanistic and a biosynthetic perspectives, the question of how the S4 helix may be stabilized in a membrane environment is of some importance. We have performed coarse-grained (CG) molecular dynamics (MD) simulations to calculate: (1) the free energy of insertion of a S4 helix; and (2) the free energy cost of driving a S4 helix through an angular motion in model membranes. Our results suggest that it is possibly to meta-stably insert a S4 helix in a TM orientation in a lipid bilayer. In this orientation, the helix is stabilized local bilayer deformation and by snorkelling of the sidechains of the positively-charged residues of S4 to interact with lipid phosphates and waters.

2493-Pos Board B463

Probing Voltage Sensors In Nonphospholipid Bilayers

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Recent studies have identified that the phosphodiester groups in phospholipid bilayers play a critical role in the voltage-dependent gating of voltage-gated potassium channels. The nature of such lipid-protein interaction is still not well understood. We have developed assays to check the conformational state of the voltage sensor domain in a voltage-gated channel reconstituted in lipid bilayers without the phosphate groups. Using cysteine accessibility assay we are examing the state of both the voltage sensor domain and the pore domain in such membranes. We also are investigating whether the phosphate groups are mainly for interacting with the first two arginine residues on the S4 of the voltage sensor and supporting the voltage sensor function.

2494-Pos Board B464

Voltage Sensors: Diverse sequences but common bilayer interactions?

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Voltage sensors are accessory protein domains which regulate the activity of ion channels with regard to the membrane potential, therefore coupling membrane ion-permeation to membrane depolarization. Recently, they have been found to associate with other functional domains, such as phosphatase enzymes or even to form stand-alone proton channels. They show substantial sequence diversity, thus leading to the question of whether they share common mechanisms of action. We have constructed homology models for a number of VSs from voltage-gated K+ and Na+ channels as well as other stand-alone VS proteins. We have also explored the known experimental structures of VSs from the voltage-gated potassium channels KvAP, Kv1.2 and the Kv1.2-Kv2.1 chimera. We have performed coarse-grained molecular dynamics (CG-MD) simulations of the interactions of these various proteins with a palmitoyl oleoyl phosphatidylcholine (POPC) bilayer. Analysis of lipid bilayer distortion during the simulations suggests that that asymmetric perturbations of the membrane bilayer leaflets are shared by most homologues. Such perturbation seems to be enhanced in the intact Kv channel structures relative to the isolated VS domains. The possible relationship of such bilayer perturbations to VS function will be explored.

2495-Pos Board B465

Water-filled Cavities in the Voltage-Sensing Domain of a Potassium Channel Embedded in Lipid Bilayers

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S1-S4 voltage-sensing domains (VSD) are conserved structural modules found in a large variety of voltage-sensitive membrane proteins. We investigated the topology, hydration properties and protein-lipid interactions of the VSD from KvAP in lipid membranes using neutron diffraction and solid-state NMR techniques. Neutron diffraction experiments demonstrate that the VSD changes the water distribution and profiles of POPC:POPG bilayers. To explore the topology of the VSD in the membrane, we uniformly deuterated the protein and incorporated it into lipid bilayers. A significant fraction of the protein scattering length density is observed in the head-group region of the bilayer. The protein

density and water distribution within the bilayer largely overlap, suggesting a high degree of protein hydration within the membrane. Solid-state NMR magnetization transfer data are consistent with deep penetration of water molecules into bilayer-embedded VSDs. We hypothesize that VSDs have water filled cavities, which may be essential for VSD function.

2496-Pos Board B466
Voltage and proton gradient sensing in H_v1 proton channels
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H_v1 voltage-gated proton channels appear to conduct H^+ through a voltage sensor domain (VSD) that is homologous to that found in voltage-dependent cation channels and phosphatases. A conserved S4 transmembrane helix that contains a series of at least three Arg residues is integral to the voltage sensing function of all VSD proteins. In contrast to other VSD-containing proteins, voltage-gated proton channels possess an additional unique biophysical property: coupling of the transmembrane pH gradient to voltage dependent activation. For both native voltage-gated H^+ currents and expressed H_v1 channels, the apparent voltage threshold for H^+ current activation (V_{thr}) shifts linearly ~40 mV per log([H^+]) over at least five pH units. The molecular mechanism of coupling between voltage and the pH gradients represents one of the central mysteries of proton channel function. What constitutes the pH sensor in proton channels and how does it interact with the voltage sensor?
DeCoursey and colleagues previously proposed a model for H^+ channel gating wherein protonation of discrete sites that are alternatively exposed to either the extra- or intra-cellular milieu regulates the voltage-dependence of channel opening (Cherny et al., 1996); the required first step in this model is deprotonation of an extracellular H^+ binding site. In order to identify residues that mediate pH-dependent regulation of voltage sensitivity in H_v1, we performed site-directed mutagenesis to convert each of the candidate H^+ acceptors in the H_v1 VSD to either neutral (alanine or asparagine), basic (arginine) or H^+-titratable (histidine) amino acids. Mutant channels were expressed in HEK-293 cells and V_{thr} was determined under a variety of imposed pH gradients using whole-cell voltage clamp. Surprisingly, charge-neutralizing mutations failed to abrogate pH gradient sensing in H_v1. Our findings are interpreted in the context of the Cherny and DeCoursey model for proton channel gating.

2497-Pos Board B467
Voltage-Dependent Conformational Changes of the Voltage Sensor of KVAP Measured with LRET
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The opening and closing of voltage-dependent ions channels depend upon conformational changes initiated in the voltage-sensors. The nature and extent of this rearrangement has been widely investigated in K^+ channels, using various techniques, but so far, there is no agreement on this fundamental mechanism in K^+-channel gating.
We investigated the voltage-sensor operation using a LRET technique employing Tb^{3+} ions bound to several Lanthanide-Binding-Tags (LBT) genetically encoded at the top of the S3 and S4 segments of the KvAP channel. A fluorescently-labeled pore-blocking toxin, the Agitoxin-2, was conveniently used as an acceptor placed at a non-mobile position near the pore-axis. Three mutations, S179G, K181D and P176E, were systematically introduced in the pore region to increase toxin binding. Various fluorophores were covalently attached to cysteines individually inserted at positions N5, Q13 and D20 of the agitoxin. After purification, mutant channels were reconstituted into proteoliposomes and submitted to a Nernst-clamp procedure combining the use of valinomycin and a K^+ chelator. This method allowed us to stably clamp the liposomal electrical potential at negative and positive potentials. Thus, this allowed us to stabilize the voltage-sensor in its closed state and in its open-inactivated state during the data acquisition period. Channels were then blocked with the fluorescent toxins and LRET measurements were recorded. Sensitized emissions of the acceptors were fitted with a square-base pyramidal multi-exponential model (Posson and Selvin, 2008) allowing the extraction of the four distances separating the position of the acceptor near the pore and the position of the donors located further away in the four subunits. The voltage-dependent coordinates of the LBTs indicate a rotational movement of the top of S4, with little participation of the top of the S3 segment.
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2498-Pos Board B468
Down-State Model of the KvAP Voltage-Sensing Domain
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Voltage-gated potassium (Kv) channels play a crucial role in the generation and propagation of electrical impulses in excitable cells by controlling the flow of K+ ions into the cell membrane in response to changes in transmembrane potential. The voltage-sensing domains (VSD) of these channels have at least two distinct conformations, the so-called up- and down-states, corresponding to the open and resting/closed states of the channel. To date, structures of three different Kv channels have revealed the up-state of the VSD; in the down-state, there is a great deal of functional data, but no crystal structures have been reported. We report here a model of the KvAP VSD in a down-state. The model was generated by molecular dynamics simulations (MD) of the VSD in a lipid bilayer in excess water using harmonic constraints to steer an equilibrated up-state sensor into a configuration that is consistent with the biotin-tethered avidin accessibility measurements of Ruta et al. (2005, Cell 123: 463). We have run a MD simulation of the system for 30 ns in the absence of constraints. The system is stable in the new configuration, and is consistent with newly reported avidin accessibility measurements (Banerjee and MacKinnon, 2008, JMB 381: 569) that were not used to generate the model. In addition, we have estimated the gating charge transfer between the up- and down-state configurations. Compared to the Kv1.2 model by Pathak et al. (2007, Neuron 56: 124), we see slightly larger transmembrane displacements for the S3 and S4 helices with significant differences in their orientation.

2499-Pos Board B469
Structural models of NaChBac: Does the secondary structure of S4 change during gating?
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NaChBac is a prokaryotic 6TM tetrameric voltage-gated sodium channel with a locus point in homology space connecting channels from all major voltage gated channels superfamilies. The voltage-sensing domain of NaChBac exhibits the familiar RxxRxxR motif of S4 and conserved negative residues on S2 and S3. Thus, the voltage sensing mechanism of NaChBac is probably shared with other voltage gated channels. We have used the crystal structure of the Kv1.2/2.1 chimera to model NaChBac's open conformation and that of the MlotiK channel to model its closed conformation. In the closed MlotiK structure the first part of S4 forms a 3_{10} helix and the last part forms an α helix, whereas in the open Kv1.2/2.1 structure the first part is an α helix while the rest is a 3_{10} helix. This elastic type of transition between secondary structures during gating can explain some apparent discrepancies regarding the magnitude of S4 motion reported for several potassium channels. However, this type of transition alone is not sufficient to explain the large gating charge movement reported for NaChBac and other channels. To account for this, we have incorporated the α-3_{10} transition into the "helical screw model" in which the α-helix part of S4 moves in a screw-like fashion while the 3_{10} part of S4 moves in a simple axial translation. In our models four positively charged residues of S4 moves outwardly during activation across a transition barrier formed by highly conserved hydrophobic residues on S1, S2, and S3. S4 movement is coupled to opening of the activation gate formed by S6 through interactions with the segment linking S4 to S5. Consistencies of our models with experimental studies of the NaChBac and Kv channels will be discussed.

2500-Pos Board B470
Modulation of HCN Channel Deactivation Kinetics by CAMP and Depolarization Can Be Amplified by Mode Shift
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HCN ion channels belong to the voltage-gated K-channel superfamily, but their voltage-sensing (S4) helices produce an unusual hyperpolarization-activation mechanism. This hyperpolarization-activation can be enhanced by (a) cyclic AMP (cAMP) binding to a cytoplasmic C-terminal region, and (b) a voltage-independent "mode-shift" after gate-opening that forms a secondary open state ("Mode II"). We previously [*Biophys. J.* **94**, 1400-Pos.] found that a mutation (K381E) within S4 produced a cAMP-dependent ''ultra-sustained activation'' phenotype without disrupting voltage-activation. Cyclic AMP applied to K381E channels in excised inside-out patches greatly slows deactivation kinetics, increasing decay time constants beyond 6 s at −40mV. In this study, we activated K381E channels with hyperpolarizing pulses too short for significant mode shift to occur, thus isolating open channels predominantly in the Mode I state. Deactivation transients were well-described with a sum of three exponential components, consistent with three subpopulations of open states. The three time constants (τ_{fast}, τ_{medium}, and τ_{slow}) were widely separated in the absence of cAMP, enabling the rapidly deactivating Mode I kinetics (τ_{fast} ~50 ms) to be clearly delineated from those of the more stable Mode II states (τ_{medium} ~300