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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2490-Pos Board B460
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.

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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