independent cerebrovascular constriction (Liu et al., 2004). On the other hand, high cholesterol levels, which inhibit vascular smooth muscle BK channels (Bolotina et al., 1989), decrease vascular compliance, favoring vasoconstriction (Bukiya et al., 2008). Synergistic inhibition of cerebrovascular BK channels by cholesterol and ethanol would certainly have a profound negative impact on vascular compliance and dilation. Remarkably, such synergism on channel function has not been studied. Thus, we cloned BK subunits (channel-forming cbv1 and accessory, smooth muscle-abundant β1) from rat resistance-size cerebral arteries, reconstituted the channel complex into 1-palmitoyl-2-oleoyl phosphatidylethanolamine/1-palmitoyl-2-oleoyl phosphatidylserine (POPE/POPS) bilayers, and studied cholesterol modulation of ethanol action on channel steady-state activity (NP_o). Acute exposure to 50 mM ethanol mildly yet significantly decreased BK NPo (−4 ± 0.8 % from control) without modifying channel unitary conductance. In the same bilayer type, incorporation of cholesterol at levels found in cell membranes (15 % w/w) also reduced BK NPo (-8.78 ± 5.2 % from control). Remarkably, 50 mM EtOH added to the cholesterol-containing bilayer resulted in a robust decrease in BK NP_o (−36 ± 8.4 % from control). These data unveil a multiplicative inhibition of BK channel activity by alcohol and cholesterol. The kinetic and biophysical mechanisms of such synergism are currently being investigated.

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Regulation Of The Slo2.2 Channel By Na+ Ions And Phosphatidylinositol 4,5 Bisphosphate

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A sodium coordination loop has been shown to bind sodium and switch the sensitivity of Kir channels to phosphatidylinositol-4,5-bisphosphate (PIP2) (Rosenhouse-Dantsker et al., Nat. Chem. Biol. 2008 4:624-631). The large conductance potassium channel Slo2.2 (Slack) is activated by intracellular Na+ and is regulated by Gq-coupled receptor stimulation. Here we investigated whether the molecular switching induced by Na+ in Kir channels operated also in Slack channels. First, by using polylysine and PIP2 in the inside-out patch configuration, we demonstrated that the Slack channel activity can be regulated by PIP2. Second, we screened the intracellular domains of Slack for potential Na+ sites and found that a coordination site similar to the one found in Kir channels controls the sensitivity of Slack channels to Na+. Mutation of an Aspartate located in the RCK2 domain of Slack decreased Na+ sensitivity by 4-5 fold, while it had no influence on Cl- sensitivity. Our preliminary results suggest that the Slack channel shares with Kir channels a similar mechanism of Na+ activation that is likely to modulate its sensitivity to PIP2.

2486-Pos Board B456

Role of Charged Residues in the S1-S4 Domains of Slo2.1 K+ Channels

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Slo2.1 is a weakly voltage-dependent, large conductance K^+ channel activated by intracellular Na^+. Unlike the typical Kv channel, the S4 transmembrane domain of human Slo2.1 contains two basic residues (K174, R186) whose charge is partially offset by two acidic residues (E178, D183). The N-terminal residue of the putative S45 linker contains a single charged residue, R190. In addition, Slo2.1 has two basic residues in S1 (R80, K70) and a single acidic residue each in S2 (E118) and S3 (E143). We examined the effects of mutation of individual charged residues to Ala. Human Slo2.1 channels were expressed in *Xenopus* oocytes and whole cell currents were measured using the two electrode voltage clamp technique. In normal extracellular solution, Slo2.1 channels were closed, but could be activated by bathing oocytes in a K^+-free solution for 10-15 minutes (to increase $[Na^+]_i$) or by exposure to 1 mM niflumic acid (NFA). The $V_{1/2}$ for activation of wild-type Slo2.1 channels activated by NFA was −5 mV; effective valence, $z = 0.56$. Point mutations of the charged residues in S1-S4 induced relatively small changes in voltage dependence of activation (max $\Delta\Delta G$=0.25). R190E Slo2.1 channels were constitutively active (current not enhanced by NFA) and the $V_{1/2}$ was shifted to −63 mV; $z = 0.55$. Introduction of a second site mutation (R190E/D183K) reverted channels to wild-type gating mode (closed under control conditions, but activated 10-fold by NFA). Thus, an electrostatic interaction between D183 in S4 and R190 in the S45 linker may stabilize the closed state of Slo2.1 channels.

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Amino-termini Isoforms Of Slack K(Na) Channel Differentially Influence The Rate Of Neuronal Adaptation.

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The rates of activation and unitary properties of Na+-activated K+ currents, K(Na) currents have been found to vary substantially in different types of neurons. One class of K(Na) channels are encoded by the Slack gene. We have now determined that alternative RNA splicing gives rise to at least five different transcripts for Slack, one class of K channels which produce Slack channels that differ in their predicted cytoplasmic amino-termini and in their kinetic properties. Two of these, termed Slack-A channels, contain an amino-terminus domain closely resembling that of another class of K(Na) channels encoded by the Slick gene. Neuronal expression of Slack-A channels and of the previously described Slack isoform, now called Slack-B, are driven by independent promoters. Slack-A mRNAs were enriched in the brainstem and olfactory bulb and detected at significant levels in four different brain regions. Slack-A channels activate rapidly upon depolarization and, in single channel recordings in Xenopus oocytes, are characterized by multiple subconductance states with only brief transient openings to the fully open state. In contrast, Slack-B channels activate slowly over hundreds of milliseconds, with openings to the fully open state that are ~6 fold longer than those for Slack-A channels. In numerical simulations, neurons in which outward currents are dominated by a Slack-A-like conductance adapt very rapidly to repeated or maintained stimulation over a wide range of stimulus strengths. In contrast, Slack-B currents promote rhythmic firing during maintained stimulation, and allow adaptation rate to vary with stimulus strength. Our data suggest that alternative promoters of the Slack gene differentially modulate the properties of neurons. Supported by NIH Grants NS61479 and DC01919.

2488-Pos Board B458

Single channel studies of heteromer formation between Slick and Slack K(Na) subunits

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Slack (Slo 2.2) and Slick (Slo 2.1) encode sodium-activated K^+ channels (K_{Na}). Native K_{Na} currents may enhance the phase locking of action potential firing at high frequencies, adaptation to prolonged stimulation and are believed to protect both excitable and non-excitable cells from hypoxic injury. Slack and Slick resemble native K_{Na} channels in their Na^+ sensitivity and large unitary conductances (140 and 180 pS in 130 mM KCl, respectively). Two alternative isoforms have been described for the Slack gene; Slack-A and Slack-B. The Slick channel differs from Slack in its opposite regulation by PKC, the presence of an ATP binding site, sensitivity to intracellular Na^+ and Cl^- ions and channel kinetics. We have obtained direct electrophysiological evidence for Slick and Slack-B heteromer formation at the single channel level by constructing a Slick* Q276E, Y279E (Slick*EE) mutant and coinjecting it with Slack-B in Xenopus oocytes. Introducing these negatively charged residues in the inner pore S6 helix resulted in a dramatic increase in the unitary conductance of the Slick*EE homomeric channel from 140 pS to ~ 450 pS (140 mM KCl). In a 1:1 cRNA injection of Slick*EE and Slack-B we identifed conductances of ~330 pS. In contrast, we found no evidence for heteromer formation between Slick and Slack-A at the single channel level. These findings support previous studies demonstrating that Slick and Slack-B, but not Slack-A, subunits can be co-immunoprecipitated from rat brain and from co-transfected HEK cells.

2489-Pos Board B459

Soluble β-amyloid oligomers alter biophysical properties of Kv1.3 channels

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The aggregation of amyloid peptides in brain tissue is a hallmark of Alzheimer's disease. Amyloid beta (Aβ) exists in several assembly states, which may play different physiological or pathophysiological roles. The effects of Aβ on voltage-dependent ion channels in neurons and microglia were implicated in early stages of neurodegeneration. We tested the effect of soluble oligomers (Aβ1-42) of amyloid precursor protein (APP) on voltage-dependent potassium channels Kv1.3. Potassium current was measured during whole-cell recording from L929 cells, stably expressing Kv1.3. Acute application of Aβ1-42 reversibly reduced peak current amplitudes and affected kinetics of current activation, inactivation and deactivation in a voltage- and a dose-dependent manner. The time constant of K^+ current activation during

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.

2491-Pos Board B461

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