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.

2485-Pos Board B455

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.

2487-Pos Board B457

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