Kv4.3-L. In contrast, 70-amino acid KChIP2d was permissive for PKC modulation with respect to both CSI and current expression, while allowing the fast recovery from open-state inactivation characteristic of other KChIP2 isoforms. Additionally, the KChIP2d effects on CSI in Kv4.3-L were dependent on the presence of a putative PKC phosphorylation site in the C terminus. These data suggest a different physiological role for KChIP2d than the other KChIP2 isoforms, and suggest that the longer forms of KChIP2 interact with the regions of Kv4.3 affected by PKC, while KChIP2d interacts with the channel in a manner that allows PKC modulation while still accelerating recovery.

2766-Pos

Regulation of NALCN Sodium Leak Channel by UNC79 and UNC80

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In addition to the selective ion channels such as voltage-gated K^+ (K_V), Na^+ (Na_V) and Ca^{2+} (Ca_V) channels, neurons also express a voltage-independent, non-selective cation channel NALCN. NALCN contributes to the TTX- and Cs^+ -resistant Na^+ leak conductance in neurons and is activated by neuropetides substance P and neurotensin via a G-protein independent pathway that requires the Src family of tyrosine kinases. The pore-forming subunit of NALCN resembles that of the 24 transmembrane spanning (24TM) Ca_Vs and Na_Vs, but lacks some of the charged residues in the S4s and has a unique sequence signature in the selectivity filter region. Unlike those of other ion channel families, the subunit composition of NALCN is not known. Recent studies in *Drosophila melanogaster* and *C. elegans* suggest genetic interaction between *Nalcn* and two novel genes *Unc79* and *Unc80.* We have now analyzed the mammalian homologs of UNC79 and UNC80 from mouse brain. UNC79 and UNC80 encodes large proteins (~3,000 amino acids), have no obvious domains with defined function, and are well conserved from humans "down" to the placozoan *Trichoplax adhaerens*. UNC79 and UNC80 form a complex with NALCN in the brain, where UNC79 indirectly associates with NALCN through its interaction with UNC80. While UNC79 and UNC80 are not required for the basal Na^+ leak current through NALCN, UNC80, but not UNC79, is essential for the channel's regulation by GPCRs. These data suggest that UNC79 and UNC80 are likely "auxiliary subunits" of the NALCN channel complex.

2767-Pos

One Sumo is Sufficient to Silence the Dimeric Background Potassium Channel K2P1

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SUMO, a 101 residue peptide well-known to regulate nucleocytoplasmic trafficking and function of transcription factors, was recently shown to reversibly regulate the activity of K2P1 channels in cell surface membranes (Rajan et al, Cell **121**; 2005). Thus, K2P1 channels are silenced by interaction of SUMO with lysine at position 274 (K274) and activated by SUMO-specific protease (SENP1). As such, channels with K274 altered to glutamine (K274Q) are constitutively active and insensitive to SUMO and SENP1. Here we report that, like wild-type K2P1 channels (WT), channels formed by two subunits linked in tandem (WT-WT) are silent at baseline and activated by exposure to SENP1 when studied in CHO cells by patch-clamp recording. Suggesting that channel silencing requires only one SUMO, channels bearing one wild-type subunit (WT-K274Q and K274Q-WT) behave like WT. To test this hypothesis, GFP-labeled subunits were studied using total internal reflection microscopy and stepwise decreases in fluorescence due to single-particle photobleaching (SPPB) to count the number of fluorophores per channel. Validating the method, two bleaching steps are recorded with GFP fused to WT or K274Q subunits because K2P channels are dimeric (Lopes et al., 2001. JBC **276**:24449-52; Kollewe et al., 2009. JGP **34:**53-68) and four steps seen with GFP on Kv2.1 subunits that form tetrameric channels. Next, GFP-SUMO was observed in discrete plasma membrane particles when expressed with WT but not K274Q subunits. Finally, SPPB was used to identify two GFP-SUMO with each WT or WT-WT channel but only one with WT-K274Q or K274Q-WT channels. The data show K2P1 channels to assemble with two SUMO subunits but a single SUMO to be sufficient for silencing.

2768-Pos

Identification of K_v Subunits Underlying the Delayed Rectifier K^+ Current (I_K) in Small Cultured DRG Neurons

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Voltage-gated potassium (Kv) channels serve a wide range of functions in both excitable and non-excitable cells. In neurons these include the regulation of the resting membrane potential and control of the shape, duration and frequency of action potentials. The large number of Kv subunits presents a challenge to determine the molecular composition of the native currents. We attempted to identify the Kv subunits underlying the delayed rectifier current (I_K) in cultured small mouse dorsal root ganglia (DRG) neurons. Using extracellular Stromatoxin (ScTX) and intracellular Kv2.1 antibodies we recently reported that approximately 60% of the I_K current in these DRG neurons is carried by both homotetrameric Kv2.1 and heterotetrameric Kv2.1/silent Kv channel complexes. The 40% of I_K remaining after ScTx (100 nM) pretreatment, was virtually abolished with 1 mM extracellular TEA ($n = 6$) indicating that this part of the I_K current could be represented by the Kv subunits Kv1.1, Kv1.6, Kv3.1, Kv3.2 and/or Kv3.3, and possibly a fraction of KCNQ2 and KCNQ2/3 channels, which underlie the M-current in small DRG neurons. Using channel specific toxins we determined the contribution of each channel to the remaining 40% of I_K. Furthermore, we detected the presence of Kv3.1, Kv3.2 and Kv3.3 mRNA using RT-PCR in freshly isolated DRG. These observations support a substantial role of at least the Kv3.x subunits in small DRG neurons which are visceral and somatic sensory neurons that conduct information about temperature, pressure and touch.

Ion Channels, Other I

2769-Pos

Tubulin-Binding Drugs Thiocolchicoside and Taxol Permeabilize Lipid Bilayer Membranes by Forming Ion Channels

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The purpose of this study was to examine the possibility of ion channel formation by two tubulin-binding drugs: thiocolchicoside (TCC) and taxol (TXL). Both these compounds, in addition to interaction with tubulin, are known to have other cellular targets. For example, TCC has been shown to interact with $GABA_A$ and strychnine-sensitive glycine receptors, while TXL interferes with the normal breakdown of microtubules during cell division, inducing mitotic block and apoptosis and found significant chemotherapeutic applications in breast, ovarian and lung cancer. In order to better understand the diverse mechanisms of TCC and TXL actions, we examined their effects on phospholipid bilayer membranes formed by applying the lipid cocktail of POPE: POPS: POPC (5:3:2, v/v/v). Our electrophysiological recordings across membranes constructed in NaCl aqueous phases consisting of ~ 50 μχg/ml of TCC/TXL suggest that both of these drug molecules induce stable (possibly toroidal type) ion channels in membranes. The discrete conductance events appear with conductances (~0.01-0.1 pA/mV) and lifetimes (~5-30 ms) falling in the average orders observed in gramicidin A and alamethicin channels. The channel formation probability increases linearly with TCC/TXL concentration and transmembrane potential and is not affected by pH (5.7 - 8.4). Results suggest that TCC/TXL can partition through membranes and perhaps act at cellular levels. This novel finding may help to understand the biophysical properties of these two important drug molecules and similar ones which will hopefully assist in developing novel drugs to treat health problems related to muscular spasms, rheumatologic, orthopedic, traumatologic disorders in addition to a broad spectrum of cancers.

2770-Pos

Towards Simultaneous Single Channel Current and Fluorescence Recordings in Planar Lipid Bilayer

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The ion conducting pore of K^+ channels has two gates – one at the selectivity filter and another at the helical bundle crossing. Both gates have to open for ions to pass through the pore. Opening of the helical bundle crossing, which is linked to the voltage sensor movement in voltage-dependent channels, is thought to occur in one cooperative step of all four subunits. On the other hand, subconductance levels, which are states of lower conductance than the normal conducting state, are suggested to be caused by a partial (i.e. not all four subunits) opening of the channel. It remains unknown whether full opening is necessary or whether partial opening is sufficient to get ion conduction. Here, we present our development towards studying the correlation between the subunits' opening and the associated current of the channel. We are investigating purified KcsA channels fluorescently labeled at the helical bundle crossing. The channels are reconstituted at low concentration in lipid vesicles and inserted into horizontal planar lipid bilayer until single channel current is observed. In the horizontal bilayer configuration, we have optical access and electrical control simultaneously. Using fluorescence spectroscopy techniques, we