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

monitor the opening of single subunits while single channel current is recorded. Fluorescence intensity changes of FRET pairs or environment-sensitive dyes allow us to distinguish the movement of the four subunits (*Blunck et al., 2008*). We successfully imaged the diffusion of single channels in the bilayer using an EMCCD camera and now seek to correlate their fluorescence intensity, which is associated to a partial or a full opening, with the occurrence of subconductance levels.

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2771-Pos

Functional Incorporation of KcsA into Tethered Lipid Bilayer Membranes

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Tethered lipid bilayer membranes (tBLMs) are solid-supported lipid bilayers separated by a ~ 2 nm thick hydrated layer from the solid interface. In comparison to cell membranes, they are simpler in their chemical composition and can therefore be quantitatively studied with a variety of experimental techniques. In comparison to free-standing or vesicle membranes, they are much more long-term stable. tBLMs can be formed by rapid solvent exchange,[1] which leads to highly electrically insulating, defect-free bilayers,[2] or by vesicle fusion, which results in membranes with higher residual conductance but makes protein reconstitution more straightforward. While we studied the structure and function of tBLMs produced by rapid solvent exchange extensively in the past,[2] we have more recently optimized vesicle fusion protocols for tBLM formation and observed with neutron reflectometry that the membranes resulting from the two preparation methods are very similar in their molecular structure. Fluorescence correlation spectroscopy shows that lipid-label diffusion is identical in both cases. In this work, we incorporate the tetrameric potassium-selective channel KcsA from *Streptomyces lividans* into tBLMs through vesicle fusion. KcsA is reconstituted into POPE/POPG lipid vesicles that are spread onto the solid support to form the membranes. The functionality of the reconstituted channel is confirmed by electrochemical impedance spectroscopy (EIS), where we observe that the resistance of tBLMs with KcsA is 3 to 10 times smaller than the resistance of neat tBLMs in the presence of K^+ ions, and with various blockers of the potassium channel.

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[1]Cornell, B.A, *et al.* 1997. *Nature* 387:580-583.
[2]Valincius, G., *et al.* 2008. *Biophys. J.* 95:4845-4861.

2772-Pos

Energetic Coupling Between Amino Acids in the pH-Sensing Region of the KcsA Channel

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The bacterial potassium channel KcsA is gated by high concentrations of intracellular protons, allowing the channel to open at pH < 5.5. Replacing key ionizable residues from the N and C termini of KcsA with residues mimicking their protonated counterparts with respect to charge renders the channel open up to pH 9.0 (Thompson et al., 2008). We proposed that these residues function as the proton-binding sites. At neutral pH they form a complex network of inter- and intrasubunit salt bridges and hydrogen bonds near the bundle crossing, stabilizing the closed state. At acidic pH, these residues change their ionization state, thereby disrupting this network, favoring channel opening. To gain insight into the interactions that govern channel opening, we performed a thermodynamic analysis of the residues in the pH-sensing region. Individual mutations of most residues in this region result in modest shifts in the pH dependence of channel opening. However, pair-wise mutations of a subset of these amino acids show a large shift on the pH dependence of the channel opening suggesting these amino acids interact to open the channel with protons.

2773-Pos

Membrane Trafficking Controls K2P1/TWIK1 Channel Expression at the Cell Surface

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Two-P-domain potassium (K2P) channels produce background conductances involved in neuronal excitability and cell volume regulation. In contrast with other K2P channels, little is known about TWIK1 (K2P1), despite the fact that it has been the first K2P channel cloned and expressed (Lesage et al, EMBO J. 1996, 15, 1004-1011). Functional studies on TWIK1 have been impeded by the fact that it produces only modest current upon heterologous expression in Xenopus oocytes, and that so far, no currents similar to TWIK1 have been reported in native cells. It has been proposed that K2P1 was present at the cell surface but silenced by conjugation of a SUMO peptide to an unconventional sumoylation site (Rajan et al, Cell. 2005, 121, 37-47). However, we did not observe any quantitative sumoylation of TWIK1 in vivo or even in vitro. Also, we have shown that inactivation of the putative sumoylation by a conservative lys to arg mutation was without effect on the level of TWIK1 current (Feliciangeli et al, Cell. 2007, 130, 563-569). We now provide new evidence demonstrating that the lack of measurable current upon TWIK1 expression in mammalian cells is caused by its active endocytosis from cell surface and retention in intracellular recycling endosomes. Inactivation by point mutation of an unusual endocytosis signal sequence produces a mutated TWIK1 channel that is expressed at the cell surface and produces measurable currents in all the cell types that have been tested.

2774-Pos

Helix C Regulates Surface Expression of KCNQ2 (kv7.2) Channels

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KCNQ2 (Kv7.2) subunits is one of the main subunits that give rise to the M-current, which play a prominent role in the control of neuronal excitability. Little is known regarding how the density of KCNQ2 channels at the plasma membrane is controlled. We have used the Tac membrane protein (also known as CD25/interleukin-2 receptor) as a reporter for the identification of critical traffic determinants. Fusion of helix C to Tac prevented trafficking to the plasma membrane. Within helix C, we identified the sequence RIK as a key player in the process. After deletion or neutralization to AIA or NIN, the surface expression increased, suggesting that this motive may function as a retention/retrieval signal. A natural existing mutant at this site, R553Q, is associated with neonatal epilepsy (BFNC), reflecting an important role of this sequence on KCNQ channel physiology.

2775-Pos

Lack of Clinically Important hERG Channel Block by the Antipsychotics Tiapride and Sulpiride

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The human *ether-a-go-go*-related gene (*hERG*) channel is important for repolarization in human myocardium and is a common target for drugs that prolong the QT interval. We studied the effects of two antipsychotics, tiapride and sulpiride on hERG channels expressed in *Xenopus* oocytes and also in the delayed rectifier K^+ current of guinea pig cardiomyocytes. The amplitude of the hERG outward currents measured at the end of the pulse showed no concentration-dependent change with increasing either tiapride or sulpiride concentration (3-300 μM). Also, the amplitude of hERG tail currents did not show concentration-dependent changes with increasing either tiapride or sulpiride concentration (3-300 μM). However, our findings showed that tiapride increased the values of the potential for half-maximal activation ($V_{1/2}$) at 10 - 300 μM, on the contrary, sulpiride increased the maximum conductance (G_{max}) at 3, 10, 100 μM. In guinea pig ventricular myocytes, bath applications of 100 and 500 μM tiapride at 36°C blocked rapidly activating delayed rectifier K^+ current (I_{Kr}) by 40.3% and 70.0%, respectively. Also, sulpiride at 100 and 500 μM blocked I_{Kr} by 38.9% and 76.5%, respectively, but tiapride and sulpiride at the concentrations did not significantly block slowly activating delayed rectifier K^+ current (I_{Ks}). Our findings suggest that the concentrations of the antipsychotics required to evoke a 50% inhibition of the I_{Kr} were well above reported therapeutic plasma concentrations of free and total compound. None of tiapride and sulpiride was a potent blocker of the hERG channel.

2776-Pos

Biologically Closed Electrical Circuits and Voltage Gated Ion Channels in Plants

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