control ionic conditions in the cell and energise osmotic potentials, secondary transport schemes and ionotropic signalling.
A surprising finding from the Na+,K+-ATPase structure was the docking of two conserved tyrosine residues at the C-terminus of the alpha subunit into the transmembrane domain, hinting that this was a previously unidentified regulatory element. Several mutations causing human neurological syndromes have subsequently been mapped to the C-terminal structure element, also clearly indicating that conservation of the structure is important for pump function.
Mutational analysis confirmed this and prompted our further analysis by electrophysiology and molecular dynamics simulations, which have shown a profound effect of the C-terminus on the electrogenic transport properties. We further propose that the C-terminal region forms a binding pocket that can be exploited for pharmacological intervention in cardiovascular and neurological disease.

1103-Symp
Alternating Access Mechanism of Glutamate Transporters
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In the central nervous system, glutamate transporters are responsible for the glutamate clearance following rounds of neurotransmission. They are molecular pumps, which utilizes the energy of pre-existing electrochemical gradients of ions to drive substrate uptake against steep concentration gradients. Sodium coupled aspartate transporter from Pyrococcus horikoshii, GltPh, is a homologue of the mammalian transporters and has served as a model system, within which to understand the molecular details of transport. The previously determined crystal structures of GltPh revealed the substrate and sodium binding sites located near the extracellular solution leaving the question of how they reach the cytoplasm unanswered. Recently, we have determined the crystal structure of a double cysteine mutant of GltPh, captured by cross-linking in a novel conformational state. In this state the substrate-binding sites are near the cytoplasmic surface of the protein. These findings suggest a novel and unexpected mechanism, by which GltPh and, by analogy mammalian glutamate transporters catalyze trans-membrane transport of their substrates.

1104-Symp
Alternating Access of the Maltose Transporter
Jue Chen.
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No Abstract.

Platform R: Channel Regulation & Modulation

1105-Plat
Photopharmacology: Controlling Native Voltage-Gated Ion Channels with Light
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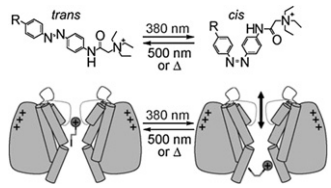
Optical control of proteins provides critical advantages for studying cell signaling and offers great promise in biotechnology and biomedical research. We have developed a series of photochromic ligands (PCLs) that target voltage-gated ion channels. They possess an azobenzene photoswitch connected on one side to a quaternary ammonium ligand (internal blocker for potassium, sodium and calcium channels) and on the other side to a variety of chemical groups. The azobenzene photoisomerizes between cis and trans configurations using different wavelengths of light, thereby repetitively turning on and off ion flow.
Alteration of the R group makes our approach very modular. First, increasing hydrophobicity allows better membrane penetration and therefore greater potency of the PCL. Second, PCLs with a charged R group require hydrophilic pathways to cross cell membranes and can be specifically targeted to cells expressing entry-route proteins. Third, selectivity for certain ion channels can be attained, allowing a more precise control over cellular excitability. Fourth, some PCLs act as cis blockers, offering the advantage of being silent in the dark. Finally, modifying the R group can be used to tune the spectral characteristics of the PCL, with potential interest for vision restoration.



1106-Plat
Receptor and Subunit Specificity in AKAP79/150 Actions On M-Type(KCNQ) K^+ Channels
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A-kinase-anchoring protein (AKAP)79/150 mediated PKC phosphorylation of M-type (KCNQ) channels is involved in M current (I_M) suppression by muscarinic M_1, but not bradykinin B_2 receptors (Hoshi *et al.* Nat. Cell Biol. 7:1066-73). In this study, we first explored the involvement of AKAP79/150 in muscarinic suppression of KCNQ currents by co-transfecting AKAP79 with KCNQ1-5 subunits in CHO cells stably expressing M_1 receptors. Expression of AKAP79 sensitized KCNQ2-5 and KCNQ2/3, but not KCNQ1, channels to suppression by the M_1 receptor agonist oxotremorine (oxo-M). Mutation of the PKC phosphorylation site on KCNQ4 (T553A) eliminated the effect of AKAP79, confirming the role of PKC. Co-transfection of wild-type, but not dominant negative, calmodulin abolished the effect of AKAP79 on KCNQ2/3 channels. We asked if purinergic and angiotensin suppression of I_M in superior cervical ganglion (SCG) sympathetic neurons involves AKAP79/150, since purinergic P2Y receptors depress I_M in SCG neurons via a similar mechanism to that of bradykinin, involving IP_3-mediated Ca^{2+} signals, whereas angiotensin AT_1 receptors depress I_M via a similar mechanism as M_1 receptors, by depletion of PIP_2. Transfection of ΔA-AKAP79, which lacks the A-domain necessary for PKC binding, did not affect I_M suppression by the purinergic agonist UTP (2 μM), nor by bradykinin (100 nM), but did reduce I_M suppression by oxo-M (1 μM) and angiotensin II (500 nM). We also tested association of AKAP79 with M_1, B_2, $P2Y_6$ and AT_1 receptors via FRET experiments on CHO cells under TIRF microscopy, which revealed weaker FRET between AKAP79 and $P2Y_6$ or B_2 receptors than for M_1 and AT_1 receptors. Our data suggest AKAP79/150 action generalizes to KCNQ2-5 subtypes, is disrupted by calmodulin, and is involved in angiotensin, but not in purinergic, suppression of neuronal M current. Supported by NIH grants R01 NS043394 and R01 NS065138.

1107-Plat
Potassium Channel Modulation by A Toxin Domain in Matrix Metalloprotease 23
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Peptide toxins found in a wide array of venoms block K+ channels causing profound physiological and pathological effects. Here, we describe the first functional K+ channel-blocking toxin domain in a mammalian protein. Matrix metalloprotease 23 (MMP23) contains a domain (MMP23TxD) that is evolutionarily related to peptide toxins from sea anemones. MMP23TxD shows close structural similarity to the sea anemone toxins BgK and ShK, and the domain blocks K+ channels in the nanomolar to low micromolar range (Kv1.6 > Kv1.3 > Kv1.1 = Kv3.2 > Kv1.4 in decreasing order of potency), while sparing other K+ channels (Kv1.2, Kv1.5, Kv1.7, KCa3.1). Full-length MMP23 suppresses K+ channels with a pattern of inhibition consistent with MMP23TxD activity. Our results provide clues to the structure and function of the vast family of proteins that contain domains related to sea anemone toxins. Evolutionary pressure to maintain a channel-modulatory function may contribute to the conservation of this domain throughout the plant and animal kingdom.

1108-Plat
Differential Redox Regulation of ORAI Channels: A Mechanism to Tune T-Cell Responses
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Phagocytes play an essential role in host defence against pathogens by generating reactive oxygen species (ROS). Effector T helper (Th) cells migrating to sites of infection will be exposed to this highly oxidative environment. Here we show how Th-cells respond and adapt to ROS. Oxidation affects different Ca^{2+}-signalling pathways essential for T-cell function. ORAI1 channels are inhibited with an IC_{50} of ~40 μM H_2O_2, but ORAI3 channels are insensitive. We identify cysteine (C195) of ORAI1, absent in ORAI3, as the major redox