inhibitors, the 4-anilinoquinazolines/4-anilinoquinolines, to introduce vibrational probes of electric field, which measure electrostatics via the vibrational Stark effect, into the ATP-binding sites of different kinases. The results yield direct insight into how chemical changes within this inhibitor class influence the selectivity for particular kinases, potentially yielding a method for rationally designing selective inhibitors.

2088-Plat
Specificity, Structure and Dynamics of Tiam1 PDZ Domain Ligand-Bound Complexes
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PSD-95/DlgA/ZO-1 (PDZ) domains are among the most abundant protein-protein interaction domains in the human proteome and typically bind the 4-10 C-terminal residues of its interaction partner with exquisite specificity. We used two homologous PDZ domains from the Tiam-family of guanine nucleotide exchange factors to investigate PDZ specificity. The Tiam1 and Tiam2 PDZ domains have overlapping but distinct ligand binding specificity, and this is exemplified by their unique preferences for C-terminal peptides derived from the syndecan1, Caspr4 and neurexin1 adhesion proteins. The Tiam1 PDZ domain binds syndecan1 and Caspr4 but not neurexin1, while the Tiam2 PDZ domain binds Caspr4 and neurexin1 but not syndecan1. Amino acid sequence comparison of Tiam-family PDZ domains revealed that four residues critical for ligand specificity are not conserved. Remarkably, substitution of these four residues in the Tiam1 PDZ for those found in the Tiam2 PDZ domain switched ligand specificity. To understand the structural and dynamic basis for this change in specificity we used X-ray crystallography and solution NMR methods, respectively. We determined the crystal structures of wild type Tiam1 PDZ domain bound to syndecan1 and phosphorylated syndecan1 peptides and the Tiam1 PDZ quadruple mutant (QM) bound to Caspr4 and neurexin1 peptides. Comparison of the crystal structures of the Tiam1 PDZ-syndecan1 and PDZ-phosphorylated syndecan1 showed that a distinct specificity pocket is used to accommodate the phosphoryl group. The crystal structure of the Tiam1 QM PDZ domain showed a unique side chain stacking interaction between aromatic residues in the PDZ domain and the Caspr4 ligand. Side chain methyl relaxation experiments revealed distinct patterns of dynamics in the Tiam1 PDZ-syndecan1 and PDZ-Caspr4 complexes. Collectively, the structures and dynamics of physiologically-based PDZ domain complexes are contributing to understanding the origin of PDZ specificity and function of Tiam-family PDZ domains.

Platform: Channel Regulation & Modulation

2089-Plat
Structural Basis For Alcohol Modulation of Pentameric Ligand-Gated Ion Channels
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Despite its long history of use and abuse in human culture, the molecular basis for alcohol action in the brain is poorly understood. The recent determination of the atomic-scale structure of GLIC, a prokaryotic member of the pentameric ligand-gated ion channel (pLGIC) family, provides a unique opportunity to characterize the structural basis for modulation of these channels, many of which are alcohol targets in brain. We observed bimodal modulation of GLIC by n-alcohols, similar to some eukaryotic pLGICs: methanol and ethanol weakly potentiated proton-activated currents in GLIC, whereas n-alcohols larger than ethanol inhibited them. Mapping of residues important to alcohol modulation of ionotropic receptors for glycine, GABA, and acetylcholine onto GLIC revealed their proximity to transmembrane cavities that may accommodate one or more alcohol molecules. Site-directed mutations in the pore-lining M2 helix allowed the identification of four residues that influence alcohol potentiation, with the direction of their effects reflecting helical structure. At one of the potentiation-enhancing residues, decreased side chain volume converted GLIC into a highly ethanol-sensitive channel, comparable to its eukaryotic relatives. Covalent labeling of M2 positions with a methanethiosulfonate reagent further implicated residues at the extracellular end of the helix in alcohol binding. Molecular dynamics simulations elucidated the structural consequences of a potentiation-enhancing mutation and suggested a structural mechanism for alcohol potentiation via interaction with a transmembrane cavity previously termed the "linking tunnel." These results provide a unique structural model for independent potentiating and inhibitory interactions of n-alcohols with a pLGIC family member.

2090-Plat
Ceramide Channel Regulation by Bcl-2 Family Proteins: Molecular Insights
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The sphingolipid, ceramide can self-assemble in phospholipid membranes to form large channels capable of translocating proteins through membranes. Electronmicroscopic visualization and electrophysiological measurements reveal a range of pore sizes with the most frequent being 10 nm in diameter. The channel size and the propensity to form channels are controlled by the apoptosis-related Bcl-2 family proteins. Bcl-xL favors channel disassembly whereas Bax favors channel growth. By using ceramide analogs we have identified regions of the ceramide molecule that are recognized by these proteins and through which the proteins exert their influence. The ability of Bax to enhance ceramide channel formation was sensitive to the stereochemistry of the ceramide head group and the hydrogen bonding ability of the amide nitrogen whereas the ability of Bcl-xL to favor channel disassembly was highly sensitive to the length of the fatty acyl chain length of ceramide. It is likely that the N-acyl chain is binding to the hydrophobic pocket on Bcl-xL because the action of Bcl-xL is blocked by 2-methoxyantimycin A3, ABT-737 and ABT-263, inhibitors that specifically bind at this site. The results are consistent with the conclusion that the highly specific binding of these proteins to the ceramide channel results in structural changes that propagate throughout the channel in an allosteric manner resulting in a disturbance of the dynamic equilibrium between ceramides in the channel and ceramides in other forms in the membrane. Depending on the influence on the dynamic equilibrium channel growth or disassembly may be favored. (Supported by NSF grant: MCB-1023008)

2091-Plat
Activation of M-Type Potassium Channels by Different Membrane Phospholipids and Analogs
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M-type (Kv7.2/7.3) channels are activated by the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PI(4,5)P_2) [Li et al., 2005: *J.Neurosci*., 25,9825], through interaction with a cluster of basic residues in the C-terminus [Hernandez et al, 2008: *J.Gen.Physiol*., 132,361]. However, little is known of the phospholipid specificity and requirements for this activation. We have explored this using inside-out membrane patches from CHO cells stably-expressing Kv7.2 and Kv7.3 subunits and held at a constant voltage *ca.* −20 mV. The dioctanoyl mono-, di- and tri-phosphatidylinositides DiC8-PI(4)P, DiC8-PI(4,5)P_2 and DiC8-PI(3,4,5)P_3 all produced biphasic P_{open}-concentration curves, maximizing at P_{open} ~0.8. EC_{50}s for the 'high-affinity' component 1 (maximum P_{open} ~0.2) were similar at ~1 μM; 'low-affinity' EC_{50}s were inversely proportional to the number of phosphates (DiC8-PI(4)P ~100 μM, DiC8-PI(4,5)P_2 ~50 μM, DiC8-PI(3,4,5)P_3 ~35 μM). In contrast, the inositol phosphates I(1,4,5)P_3 and I(4,5)P_2 neither activated nor inhibited the channels up to 300 μM, suggesting a crucial role for the lipophilic moiety. This was tested further using sphingosine-1-phosphate (S-1-P), fingolimod phosphate (FTY720-P) and 1-oleoyl lysophosphatidic acid (LPA). All three activated the Kv7.2/7.3 channels, to P_{open} values at 100 μM of ~0.8 (LPA), 0.15 (S-1-P) and 0.022 (FTY720-P). In each case 'high' and 'low' affinity components to the activation curves could be discerned, with EC_{50}s of 1.5 and 40 μM (LPA), 3 and 160 μM (S-1-P) and 0.5 and 61 μM (FTY720-P). No channel activation was observed using membrane lipids devoid of phosphate groups (D-erythrosphingosine, fingolimod, phosphatidylglycerol and phosphatidylcholine, all at 100 μM). Thus, M-channels can be activated in a rather similar manner by a range of membrane lipids, the minimal requirements at concentrations tested being one or more terminal phosphates and a lipophilic domain. Supported by Wellcome Trust grant 085419.

2092-Plat
BK Channel Modulation by Leucine-Rich Repeat Containing Proteins
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Molecular diversity of ion channel structure and function underlies variability in electrical signaling in nerve, muscle, and non-excitable cells. Regulation by variable auxiliary subunits is a major mechanism to generate tissue- or cell-specific diversity of ion channel function.Mammalian large-conductance, voltage and calcium-activated potassium (BK, K_{Ca}1.1) channels are ubiquitously expressed with diverse functions in different tissues or cell types, consisting of the pore-forming, voltage- and Ca^{2+}-sensing α-subunits (BKα), or together