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Site-Directed Spin Labeling Reveals Pentameric Ligand-Gated Ion Channel Gating Motions
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Pentameric ligand-gated ion channels (pLGICs) are neurotransmitter-activated receptors that mediate fast synaptic transmission. In pLGICs, the flexible loops in each subunit that connect the extracellular binding domain (loops 2, 7, 9) to the transmembrane channel domain (M2-M3 loop) are essential for coupling ligand binding to channel gating. Comparing the recent crystal structures of two bacterial pLGIC homologues, ELIC and GLIC, suggests channel opening is associated with rearrangements in these loops but the specifics of these motions remain unknown. Here, using site-directed spin labeling electron paramagnetic resonance (SDSL EPR) spectroscopy, we measured proton-induced movements of loop 2, loop 9, and M2-M3 loop in functional GLIC channels reconstituted into liposomes. The loops undergo significant proton-dependent motion, with loop 2 and loop 9 moving inward toward the channel lumen, and the M2-M3 loop moving at least 5 ångström outward away from the channel lumen. The movements are consistent with the gating motions predicted by comparing the ELIC and GLIC structures, and suggest the ELIC structure represents the resting state of pLGICs.

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The Ring of Glutamates in the Charge-Selectivity Filter Region of the Nicotinic Receptor Forms a System of Unanticipated Complexity
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Of all the rings of ionizable side chains that decorate the permeation pathway of nicotinic-type receptors (AChRs), the ring of glutamates at the intracellular end of M2 (the ''intermediate ring'') is, by far, the one that lowers the energetic cost of cation permeation the most. Although these glutamates have received much attention, several properties of this ring (such as the pH insensitivity) have remained unexplained, especially when compared to the properties of similar rings in CNG, Nav and Cav channels. To gain a more detailed understanding of this ''catalytic'' site, we performed a thorough mutational analysis of the intermediate ring of the muscle AChR (which consists of four glutamates and one glutamine) using single-channel electrophysiology. Our results indicate that the commonly held idea that each glutamate represents a negative charge and that each additional charge makes an incremental contribution to the single-channel conductance is incorrect: for example, we found that (in the wild-type channel) only two, not all four, of the glutamates contribute to the single-channel conductance. And, in mutant muscle AChRs bearing five glutamates in the ring (and thus mimicking the non-muscle type), only three glutamates (not five) contribute to the conductance. Our data point to a model of unanticipated complexity in which the conformational dynamics of the glutamate side chains (rather than their acid-base properties) in the ambivalent environment afforded by the membrane-water interface play a fundamental role in determining the amplitude of unitary currents.

2124-MiniSymp
Contribution of Agonist Binding Sites and Coupling Regions to Activation and Desensitization of Homomeric Cys-Loop Receptors
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Homomeric receptors are the simplest structural class of receptors of the Cys-loop superfamily and are therefore invaluable models for probing fundamental relationships between structure and function. To determine how many of the five agonist binding sites are required to be occupied by the agonist for maximal open channel stability we applied an electrical fingerprinting strategy in the homomeric receptor model, α7-5HT$_{3A}$. To vary the number of functional agonist binding sites we installed mutations that prevent agonist binding, and to report the presence of the mutant subunit, we installed mutations that alter single-channel conductance. We find that receptors can be activated by occupancy of only one agonist binding site but open channel lifetime is brief, and occupancy of three non-consecutive sites is required for maximal open channel lifetime. The conformational changes initiated at the binding site are propagated to the gate through the extracellular-transmembrane interface, known as coupling region. We show that structural differences in the coupling region of homomeric α7 and 5-HT$_{3A}$ receptors account for the large differences in open channel lifetime and rate of desensitization between these homomeric members of the superfamily. By applying the electrical fingerprinting strategy, we determine that each coupling region in the pentamer contributes an equal increment to the stability of the open channel. We also determine minimal requirements for channel opening regardless of stability of the open state, and find that receptors can open with one functional binding site and two contiguous and functional coupling regions, or with five functional binding sites and only one functional coupling region. The overall findings show that whereas the agonist binding sites contribute inter-dependently and asymmetrically to open channel stability, the coupling regions contribute independently and symmetrically.

2125-MiniSymp
Structural Dissection of NMDA Receptor Pharmacology
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N-methyl-D-aspartate receptors (NMDARs) belong to the family of ionotropic glutamate receptors and mediate excitatory synaptic transmission in the mammalian brain. NMDARs have been intensely studied over the past thirty years because of their critical involvement in brain development and physiology including learning and memory formation. Despite great importance, structure-function studies of NMDARs have been slow to develop compared to those of non-NMDARs. Diverse functions of NMDARs stems from the presence of pharmacological subtypes defined mainly by a combination of GluN1 subunit and four different subunits of GluN2s (GluN2A through D), which are expressed in different neuronal circuits at different developmental stages. Importantly, subtype-specific regulation of NMDAR activity is considered an effective strategy to treat neurological disorders and diseases that are caused by dysfunctional NMDARs including Alzheimer's disease, Parkinson's disease, schizophrenia, depression, and stroke. A subtype-specific compound, ifenprodil, binds to the amino terminal domain (ATD) of the GluN1/GluN2B receptor subtype and allosterically inhibits the ion channel activity. Ifenprodil and its analogues have been intensively studied over the past two decades because of their therapeutic potentials for depression, neuropathic pains, and Alzheimer's disease. However, there have been limited studies showing the mechanism of ifenprodil binding and ifenprodil-mediated allosteric inhibition. Here we show by a combination of x-ray crystallography and electrophysiology that the binding site of ifenprodil is located at the GluN1-GluN2B dimer interface and that ATD-mediated allosteric inhibition requires a conformational freedom in the GluN2B ATD. The structural and functional insights provided in this study pave the way to improve the design of therapeutic compounds.

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Mechanism of Activation in NMDA Receptors
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The N-methyl D-aspartate receptors belong to the family of ionotropic glutamate receptors. They are obligate heteromeric receptors with two glycine binding subunits (GluN1) and two glutamate binding (GluN2A-D) subunits arranged as a dimer of dimers. We have used luminescence resonance energy transfer measurements to determine the specific arrangement of the GluN1 and GluN2 subunits within the tetramer. Based on this arrangement we have designed sites in the agonist binding domain that can probe changes in distances within the subunit and not across the subunits. By tagging these sites with donor and acceptor fluorophores we have investigated the conformational changes in the agonist binding domain of the GluN1 and GluN2A subunits using luminescence resonance energy transfer. The LRET based distances indicate a cleft closure conformational change at the GluN1 subunit upon binding agonists, however, no significant changes in the cleft closure are observed between partial and full agonists. Single molecule FRET investigations are being performed to determine if the partial agonist bound state exhibits more flexibility relative to the full agonist bound state, accounting for the lower activation. The LRET-based distances for the glutamate binding GluN2A subunit, on the other hand, shows a graded cleft closure upon binding agonists with varying efficacy, with the extent of cleft closure being proportional to the extent of activation. This graded cleft closure indicates that the mechanism of activation in this subunit is similar to that of the glutamate binding α-amino-5-methyl-3-hydroxy-4-isoxazole propionate and kainate subtypes of the ionotropic glutamate receptors.