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1729-Pos Board B459

Functional Chimeras of the Human α7 Acetylcholine Receptor Provide Insights into Allosteric Modulation

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The human α7 neuronal acetylcholine receptor (α7nAChR) is a promising drug target for treatment of psychiatric and neurological disorders. A group of positive allosteric modulators (PAMs) specific to α7nAChR are thought to act through the transmembrane domain (TMD), but the structural basis of functional modulation remains unclear. In this study, we constructed multiple chimeras between the TMD of human α7nAChR and the extracellular domain (ECD) of a bacterial homolog, ELIC, for which a high-resolution structure has been determined. Functions of the chimeras were evaluated in Xenopus oocytes by two-electrode voltage clamp electrophysiology. Functional ELIC-α7nAChR chimeras were obtained when their ECD-TMD interfaces were modified to resemble either the ELIC or α7nAChR interface, but only the latter chimera with the entirely native α7nAChR TMD retained the unique pharmacology of α7nAChR evoked by the modulators, including insensitivity to the anesthetic propofol, potentiation by the PAMs ivermectin, PNU-120596, and TQS, as well as activation by 4BP-TQS. None of the ELIC-α7nAChR chimeras exhibited the fast desensitization characteristic of α7nAChR. Functional analysis of a series of chimeras indicated that efficient coupling at loop 7, in addition to efficient coupling at loop 2 or loop 9, was essential for a channel to be functional. The most efficient coupling at the ECD-TMD interface required optimization of all three loops. Altogether, the study suggests the interdependent of the TMD on the ECD-TMD interface for channel gating, desensitization, and allosteric modulation. (Funded in part by grants from the NIH: R01GM069766, R01GM057257, and R37GM049202)

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Direct Interaction of RIC-3 with the Intracellular Domain of Eukaryotic Cationic Pentameric Ligand-Gated Ion Channels

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Eukaryotic pentameric ligand-gated ion channels (pLGIC) represent targets for a wide variety of drugs such as skeletal muscle relaxants, anti-psychotics, anti-epileptics and drugs against Parkinson's and Alzheimer's diseases. Each subunit consists of an extracellular domain (ECD), a transmembrane domain (TMD) with 4 α-helical segments, and an intracellular domain (ICD) that is 4-14 amino acids long in prokaryotes and ~50-270 amino acids in eukaryotes. The ICD of eukaryotes has been implicated in modulating single channel conductance and kinetic properties of the channel. Additionally, it interacts with cytosolic proteins such as the resistance to inhibitors of cholinesterase protein, RIC-3, that affects plasma membrane-expression of some pLGIC. We created chimeras by introducing the ICD of cationic (5-HT_{3A} , nACh α7) as well as anionic (Glycine α1, $GABA_A$ ρ1) eukaryotic pLGICs into the *Gloeobacter violaceus* pLGIC, GLIC, a well-studied prokaryotic homologue, that only contains ECD and TMD. Electrophysiological experiments after *X. laevis* oocyte expression (EC_{50} and IC_{50}) demonstrate that for each of the four chimera sets, we have constructs that act as functional proton-gated ion channels, similar to the parent GLIC. Co-expression of cationic chimeras as well as wild-type pLGIC together with RIC-3 showed significant changes in current amplitudes, whereas RIC-3 did not affect anionic receptor chimeras or wild-type channels. We established that cationic chimeras and RIC-3, both overexpressed and purified to homogeneity from *E. coli*, bind to each other. Currently, we are using the chimeras to identify interacting proteins in native brain lysates. Our results clearly demonstrate a direct interaction between the ICD of cationic pLGIC and RIC-3. Specific interaction points will be identified in further studies.

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The Prokaryote Ligand-Gated Ion Channel Elic Captured in a Pore Blocker-Bound Conformation by the Alzheimer's Disease Drug Memantine

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Pentameric ligand-gated ion channels (pLGICs) catalyze the selective transfer of ions across the cell membrane in response to a specific neurotransmitter. A variety of chemically diverse molecules block ion conduction by plugging the channel pore. Understanding the structural basis of pore block is important, as some of these molecules, including the antipsychotic chlorpromazine, the local anaesthetic lidocaine and the Alzheimer's disease drug memantine exert their therapeutic effects by specifically blocking these and other receptors. In this study, we report X-ray crystal structures of a prokaryote model ion channel ELIC containing a pore mutation F16'S, which is inhibited by pore blockers with affinities similar to human pLGICs. Structures in complex with memantine and its brominated derivative, Br-memantine, reveal that these pore blockers bind at the extracellular entryway of the channel pore, and cause a structural change, stabilizing the pore-lining helices in a conformation that is more closed than in the unbound structure. In addition, we observe that memantine binds at the agonist binding site, where it can act as a competitive antagonist. We further investigated the conformational dynamics of the pore domain by using voltage clamp fluorometry, which demonstrated that the pore helix orientation changes in opposite directions during pore block and channel activation, consistent with the crystal structures. These data are the first to reveal a conformational change in the pore initiated by a channel blocker, and have important implications for the action of drugs such as memantine which bind to channel pores.

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Probing Pentameric Ligand-Gated Ion Channels with Bromoform Reveals Many Interconnected Anesthetic Binding Sites

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Sauguet et al. recently solved the structure of the pentameric ligand-gated ion channel GLIC in complex with the general anesthetic bromoform [1]. This structure highlights three binding sites in an intra-subunit cavity close to the propofol and desflurane binding sites previously described by Nury et al. [2]. Additionally, a F238A (F14'A) mutant reveals a supplementary inter-subunit binding site.

In this work we characterize these and additional sites by computational methods. We combine several approaches to address three key questions: (i) are the crystal binding sites spontaneously accessible? (ii) can bromoform travel from one site to another? (iii) what is the bromoform affinity for each binding site? Molecular dynamics (MD) simulations of flooding the receptor with bromoform recover most of the experimentally observed sites, with a modulated occupancy between the open and the locally closed conformations. Hundreds of short MD simulations were carried out to extensively explore the binding pockets, providing data on possible routes connecting them. These simulations furthermore highlight residues that may play key roles in controlling the interaction between anesthetic and receptor molecules. Free energy of binding calculations indicate significant affinity for all crystallographic binding sites in open and locally closed conformations, in some cases modulated by pH.

[1] Sauguet et al. 2013, Nature communications. 4:1697.
[2] Nury et al. 2011, Nature. 469:428-31.

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Molecular Insights into the Gating Mechanism of GLIC, a Prokaryotic Ligand-Gated Ion Channel

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Ligand-gated ion channels (LGICs) mediate intercellular signaling by allowing the selective passage of ions upon neurotransmitter binding. The passage of ions depends on a delicate conformational equilibrium between open (active) and closed (resting) states of these proteins. Here we study the closing mechanism of a prokaryotic homologue of the nicotinic acetylcholine receptor, called GLIC, which is activated at low pH. Because the only available structures of GLIC to date are in an open conformation, we simulated a pH jump to 7, in order to induce channel closure. By starting from different conformational sub-states, which differed in channel hydration levels and local conformations of pore-lining residues, we found that multiple conformations, both open and closed, were stable on the microsecond timescale, even at pH 7, suggesting channel closure may be a slower process than expected.