A new putative cholesterol-recognition motif in transmembrane proteins

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Eukaryotic-Prokaryotic Chimeras for Structure-Function Studies of the Intracellular Domain of Cys-Loop Receptors

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Receptors belonging to the Cys-loop gene superfamily of neurotransmitter-gated ion channels (e.g. acetylcholine, serotonin, GABA, and glycine receptors) mediate fast synaptic transmission. These receptors are targeted by a number of clinically used drugs (e.g. antiepileptics, antipsychotics, anesthetics). Cys-loop receptors contain three domains: extracellular (ECD), transmembrane (TMD), intracellular (ICD). The ECD and TMD have been studied in great detail and their 3-D structures have been determined. The recent identification of bacterial Cys-loop receptor homologues has propelled the structural knowledge to atomic resolution. However, all prokaryotic members lack the ICD. The ICD of metazoan Cys-loop receptors is the most diverse domain with respect to both length and amino-acid composition. The ICD therefore represents an attractive target for developing subtype-selective drugs with the promise of fewer side effects than drugs that target the highly-conserved ECD and TMD. We have engineered functional chimeras with ICDs from different eukaryotic receptors in the two-domain prokaryotic homologue GLIC (one chimera set published, Goyal, Salahudeen, Jansen, JBC 2011). Based on structure predictions others have hypothesized that the ICD is mainly unstructured, but contains an α-helical segment pre-TM4. We hypothesize based on computational results that cationic and anionic receptors have different ICD secondary structures, i.e., only cationic receptors contain an α-helical portion pre-TM4. For each eukaryotic ICD we generated 12 chimeras that differ by the linkers both N- and C-terminal to the inserted ICD. Interestingly, the number of functional chimeras in each set varies drastically between different ICDs. We are testing the functional consequences of inserting various ICDs using electrophysiological studies, and investigating the structure of the ICDs tethered to the TMD and at the lipid bilayer with spectroscopic and biochemical methods.

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Function of nAChRs Containing Alpha5 Subunits

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Neuronal nicotinic acetylcholine receptors (nAChRs) are abundantly expressed in the central nervous system. Neurons in several brain regions co-express alpha5, alpha4 and beta2 subunits. Changes in the receptor composition and stoichiometry of alpha4 and beta2 containing nAChRs are pertinent to disease states. Several genome-wide association and candidate gene studies have identified polymorphisms in the gene for the alpha5 subunit that are linked to an increased risk for nicotine addiction, alcohol addiction and lung cancer. However more information is required about the functional effects of incorporating a normal or mutated alpha5 subunit into the alpha4beta2 receptor pentamer. Here we examine differences in I-V relationships and dose-response relations between alpha4beta2 receptors and alpha4beta2 receptors including alpha5 subunits containing various mutations to the M2 domain expressed in *Xenopus* oocytes.

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Probing Allosteric Relationships Between the Neurotransmitter-Binding Site and the Binding Pocket of the Anthelmintic Drug Ivermectin in Glutamate-Gated Chloride Channels

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Ivermectin (IVM) is a broad-spectrum anthelmintic drug used to treat human parasitic diseases like onchocerciasis (river blindness) and elephantiasis (lymphatic filariasis). IVM acts on invertebrate Cys-loop receptors (GluClRs) that naturally bind the neurotransmitter glutamate (Glu) to open an intrinsic chloride-selective channel. Native heteropentameric GluClα/βRs are irreversibly stabilized in an open-channel conformation by IVM. Hence, IVM causes sustained inflow of chloride ions and hyperpolarization that suppress nervous impulses in nematodes. Consequently, vital physiological processes in the nematode are abolished. Upon expression in Xenopus oocytes, homomeric GluClαRs are activated (being opened) by IVM but not by Glu, whereas homomeric GluClβRs are activated by Glu but not by IVM (Cully et al., Nature, 1994). A heteromeric GluClα/βR carrying a mutation in the β-subunit that inhibits Glu binding is readily activated by IVM (Li et al., FEBS Lett, 2002). These differential responses suggest that the drug and the neurotransmitter binding sites are independent of each other. Yet, a recent X-ray crystal structure shows that IVM-bound homomeric GluClαR can accommodate Glu (Hibbs and Gouaux, Nature, 2011), in line with a study showing that robust activation of homomeric GluClαRs by IVM enables further (slight) activation by Glu (Etter et al., JBC, 1996). Here, we explored whether the structurally separated IVM and Glu binding sites are functionally coupled in heteromeric GluClα/βRs. Mutations introduced between the Glu and IVM binding pockets or inside the IVM binding pocket were examined for their influence on agonistic efficacies. We found mutations that improve IVM efficacy without changing Glu efficacy, and mutations that either reduce or increase the agonistic efficacies of IVM and Glu concomitantly. Our results strongly indicate that the IVM and Glu binding sites in heteromeric GluClRs are functionally coupled.

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A New View on Allosteric Modulation of Alpha 7 Nicotinic Acetylcholin Receptors

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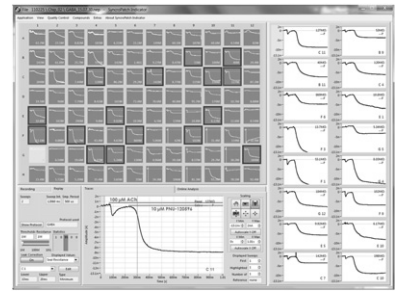
Nicotinic acetylcholine receptors (nAChR) are ligand gated cation channels that are opened by agonists like Acetylcholine and Nicotine. They are widely distributed in neurons all over central nervous system, where they are mainly found at postsynaptic locations of cholinergic nerve endings.

The most abundant homomeric nicotinic acetylcholine receptors in the mammalian brain are the pentameric alpha7 nAChRs which consist of five alpha7 subunits. Each subunit provides an orthosteric low affinity binding site for its endogenous ligand, acetylcholine. Several lines of evidence suggest a link between the alpha7 neuronal nicotinic acetylcholine receptor and brain disorders including schizophrenia, Alzheimer's disease, and traumatic brain injury. Alpha 7 receptors are also a target for non-CNS indications like diabetes and asthma.

Here we show the results of the screen of allosteric modulators on alpha 7 receptors. High parallelization without a compromise in data quality combined with fast and accurate solution exchange allows characterization of many compounds. A second analysis at physiological temperatures showed a pronounced effect on the allosteric modulation.



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A New Putative Cholesterol-Recognition Motif in Transmembrane Proteins

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We report the finding of a new putative cholesterol-recognition motif in the transmembrane region of membrane proteins like the nicotinic acetylcholine receptor (AChR) and other members of the Cys-loop superfamily of neurotransmitters involved in rapid synaptic transmission, as well as in members of the important superfamily of G protein coupled receptors (GPCRs) involved in Alzheimer's disease. The motif is an inverted CRAC domain (cholesterol recognition/interaction amino acid consensus) which we have named CARC and is present in the transmembrane regions of the receptor subunits having extensive contact with the surrounding lipid, predominantly in the first (TM1) segment in the case of the AChR, and optimally suited to convey cholesterol-mediated signaling. Three cholesterol molecules could be docked on the transmembrane regions of the five human AChR subunits, rendering a total of 15 cholesterol molecules per AChR molecule, adding up to a total of about 200 kJ.mol-1 per receptor molecule, i.e. ~40% of the total lipid solvation free energies for the whole AChR molecule. The novel motif is remarkably conserved among Cys-loop receptors along the phylogenetic scale, from prokaryotes to humans, its high degree of conservation suggesting that it could be responsible for some of the structural/functional properties of these transduction macromolecules.

Reference: Baier, C.J., Fantini, J. and Barrantes, F.J. (2011). Sci. Rep. 1:00069.