

binding to the amino-terminal domain of GluN2 subunits. Interestingly, zinc inhibits GluN2A-containing receptors at lower concentrations than GluN2B-containing channels. Luminescence resonance energy transfer (LRET) was used to study GluN1 and GluN2A subunits of the NMDA receptor. Donor and acceptor tags were attached through maleimide linkages to non-disulphide bonded cysteines introduced at key sites to allow the probing of distances and conformational changes. The amino-terminal domain tetramer organization was also studied using this LRET technique. The LRET based distances between sites on the amino-terminal domain of GluN1 and GluN2A in the full length receptor are similar to the distances in the tetrameric structure of the isolated amino-terminal domains of GluN1- GluN2B and are dissimilar to the structure of full length AMPA receptor. These results indicate that the full length receptor has a similar organization as the structure of the isolated amino-terminal domain of GluN1-GluN2B with the dimers being closely packed. Additionally, the distances between subunits at the N-terminus of the amino-terminal domain of GluN2A did not change upon addition of the allosteric inhibitor zinc or upon binding of the agonists glutamate and glycine to the ligand-binding domain. The lack of changes at the amino terminus suggests that the allosteric modulator-induced conformational changes are propagated towards the agonist binding domain and in turn to the channel segments.

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Glycine Gating of NR1/NR2A NMDA Receptors

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N-Methyl-D-Aspartate receptors (NMDARs) are ligand-gated ion channels that mediate excitatory neurotransmission in the mammalian central nervous system. They are required for normal neuronal function and are a factor in several neuropathies including Alzheimer's disease and schizophrenia. Classical NMDARs require both glycine (NR1) and glutamate (NR2) bound for receptor activation. Reaction mechanisms for NMDA receptors have been developed for several isoforms, however these models assume saturation of glycine sites and a quantitative understanding of glycine association and dissociation kinetics is currently inadequate. We measured current responses with fast application of glycine (in 1 mM glutamate) onto outside-out patches containing several NR1/NR2A receptors and observed relatively slow kinetics, with a rise time of 4.9 ± 0.6 ms and deactivation of 105 ± 8 ms. Anticipating a slow dissociation rate for glycine, we first developed models for two low-affinity glycine-site full-agonists: L-serine (95% efficacy; $EC_{50}=0.21$ mM) and 3,3,3-trifluoro-DL-alanine (132% efficacy; $EC_{50}=2.2$ mM). We used these data to select best fitting multi-state kinetic models of several arrangements and further validated these with cycles of simulations and experimental measurements which included macroscopic responses to several stimulation patterns. These models will be used to estimate glycine association and dissociation rate constants. Work is in progress to validate these results with macroscopic measurements. Knowledge about how glycine gates NMDA receptors will offer insight to glycine-dependent NR1/NR2A kinetic mechanisms and contribute to a more comprehensive understanding of the activation of these physiologically important receptors.

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Inhibitory Effects of 2-Naphthoic Acid on the Gating of NMDA Receptors

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N-methyl-D-aspartate (NMDA) receptors mediate excitatory synaptic transmission in central nervous system and play important roles in development and synaptic plasticity, but have also been found to mediate neurotoxicity. Recently, 2-naphthoic acid (NPA) and its derivatives have been identified as allosteric, noncompetitive NMDA receptor inhibitors. The inhibitory selectivity of NPA derivatives among NMDA receptor subtypes was mapped to the ligand-binding domain (LBD), and the binding site of NPA is proposed to be located in the LBD dimer interface. However, the mechanism by which NPA exerts inhibitory effect is still unclear. We examined how NPA affects the gating reaction of NMDA receptors. Whole-cell patch clamp on HEK 293 cells expressing recombinant NR1-1a/NR2A showed that NPA has 90% maximum inhibition with IC_{50} of 2.1 mM. Furthermore, by recording single-channel currents from NR1-1a/NR2A receptors with 4 mM NPA, we found a 62% decrease in open probability (P_o), due to a 2.5-fold decrease in mean open time (MOT) and a 2-fold increase in mean closed time (MCT). Kinetic modeling suggests NPA increases energy barrier of gating and destabilizes the open state, thus making NRs accumulate in the closed states along the activation pathway. These results provide insight into the inhibitory mechanism of NPA, and help anticipate its effects on both physiological and pathological conditions.

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Novel Ligand Binding Mechanisms in AvGluR1

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Glutamate receptors ion channels (iGluRs) mediate excitatory transmission at synapses in the vertebrate CNS, but have homologs in bacteria and plants. We present a structural and functional characterization of AvGluR1, a recently discovered iGluR from the primitive eukaryote *Adineta vaga* that has been suggested as an evolutionary intermediate between bacterial and animal iGluRs. Structure based alignments reveal that AvGluR1 clusters with bacterial receptors, lacks a domain 1 loop and a conserved alpha-helix found in animal iGluR ligand binding domains (LBDs), but contains a disulfide bond found only in eukaryotic iGluRs. Although AvGluR1 crystallizes as a dimer that superposes well on the LBD of the intact rat GluA2 receptor structure, the isolated LBD does not measurably interact even at protein concentrations as high as 135μ M. Competitive $[3H]$ -glutamate displacement assays for AvGluR1 LBD as well as electrophysiological experiments on the intact receptor, demonstrate that in addition to Glu, Asp and Ser, the hydrophobic amino acids Ala, Met and Phe can also bind to and activate AvGluR1, producing moderately rapid and fully desensitizing responses. Crystal structures of AvGluR1 LBD complexes with Glu, Asp, Ser and Ala reveal a novel binding mechanism involving two domain 2 Arg residues that directly coordinate the gamma-carboxyl group of Glu/Asp. Binding of neutral amino acids like Ser/Ala also utilizes a unique mechanism in which a Cl⁻ ion substitutes for gamma carboxyl group of Glu, acting as a countercharge for domain 2 Arg residues. $[3H]$ -glutamate binding experiments establish that the absence of Cl⁻ drastically lowers the receptor's affinity for Ser/Ala but not Glu/Asp. Thus the present work illustrates a novel scheme for the binding of multiple dissimilar ligands that may be utilized by the distantly related odorant/chemosensory receptors, while emphasizing the need for additional structure-based studies on iGluR-ligand interactions.

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Mechanism of Inhibition of the GluA2 AMPA Receptor Channel Opening by 2,3-Benzodiazepine Derivatives

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2,3-Benzodiazepine (2,3-BDZ) compounds are a group of AMPA receptor inhibitors and are drug candidates for treating neurological diseases involving excessive AMPA receptor activity. We investigated the mechanism by which GluA2_{Q_{flip}} receptor channel opening is inhibited by two 2,3-BDZ derivatives, i.e. (2,3-BDZ-11-2) and its 1-(4-amino-3-chlorophenyl) analogue (2,3-BDZ-11-4). Both compounds have a 7,8-ethylenedioxy moiety instead of the 7,8-methylenedioxy feature in the structure of GYKI 52466, the prototypic 2,3-BDZ compound. Using a laser-pulse photolysis approach with a time resolution of $\sim 60 \mu$ s and a rapid solution flow technique, we characterized the effect of the two compounds on the channel-opening process of the homomeric GluA2_{Q_{flip}} receptor. Both 2,3-BDZ-11-2 and 2,3-BDZ-11-4 inhibit the receptor noncompetitively with specificity for the closed-channel conformation of the GluA2_{Q_{flip}} receptor. 2,3-BDZ-11-4 is ~ 10 -fold stronger, defined by its inhibition constant for the closed-channel conformation (i.e., $K_I = 2 \mu$ M), than 2,3-BDZ-11-2. From double-inhibitor experiments, we find that both compounds bind to the same site, but this site is different from the two other non-competitive sites we reported earlier (Ritz et al., 2008, Ritz et al., 2011). In other words, 2,3-BDZ-11-2 and 2,3-BDZ-11-4 do not bind to either the site where GYKI 52466 binds or the site where 2,3-BDZ-2 binds (see their structures below). Our results provide mechanistic clues to better understand AMPA receptor regulation and establish a structure-activity relationship for designing more potent 2,3-BDZ compounds with predictable properties for this new noncompetitive site.

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Biophysical Coupling Mechanisms in NMDA Receptor Gating

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NMDA receptors (NMDARs) are heterotetrameric glutamate-gated ion channels, typically composed of 2 GluN1 and 2 GluN2 subunits. The central function of NMDARs is gating - the process of converting agonist binding energy into pore opening by repositioning M3, the major pore-lining transmembrane helix. For glutamate receptors, it is largely assumed, though lacking direct evidence, that gating occurs through tension: ligand-binding domain (LBD) closure around an agonist generates tension in the linkers connecting the LBD to M3 (the M3-S2 linker), shifting M3 away from the central pore axis.