become a weak partial agonist at AMPA receptors. We report that these CNQX-evoked responses actually desensitize. A kinetic analysis of responses in the presence of CNQX suggests that for AMPA receptors with TARPs, but not AMPA receptors alone, CNQX inhibits channel function in part by desensitizing receptors. These data demonstrate three things, first that internal and external polyamine block, previously thought to occur through a common mechanism, are in fact dissociable processes. Second, that TARPs do not significantly reduce the potency of CNQX as an antagonist and third, that TARPs enable CNQX to inhibit AMPARs by both competitive antagonism and by desensitization. As the effects of TARPs and other auxiliary proteins become known, such re-evaluations of pharmacological tools like polyamines and competitive antagonists will be required, particularly as CP-AMPARs are emerging as therapeutic targets.

2708-Pos

Crystal Structure of KA2-Subtype Ionotropic Glutamate Receptor Amino Terminal Domain

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The glutamate receptor ion channels that mediate excitatory synaptic transmission in the mammalian brain have a unique modular architecture distinct from that for other ligand gated ion channels. It took ten years since publication of the first crystal structure of an isolated ligand-binding domain (S1S2) (1) to solve structures of the amino terminal domain (ATD) of GluR6 (2) and GluR2 (3). These structures give insight into the regulation of subtype specific assembly by ATDs. However, structures of ATDs from other subtypes are still unknown. We have now determined high-resolution crystal structures of KA2 ATD in 2 forms at 1.4 Å & 1.6 Å respectively. KA2ATD has a clamshell-like fold similar to GluR6 and GluR2. However, the dimer assembly is significantly different than that for GluR6 & GluR2 where the R1 & R2 domains of both the protomers contribute equally to dimer formation. In KA2, the R2 domains also co-assemble and form close contacts similar to GluR6 & GluR2 ATDs but the R1 domains are separated. This assembly is interesting because the R1 domain has loops that likely specify subtype specific assembly. These loops make no contacts across the dimer interface in KA2 ATD, consistent with obligate co-assembly of KA2 with GluR5-7 for formation of functional ion channels.

References

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2709-Pos

A Comparative Molecular Dynamics Simulation Study of the Amino Terminal Domain of Ionotropic Glutamate Receptors

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Glutamate receptors account for the vast majority of excitatory neurotransmission in the vertebrate nervous system. The modular architecture of these membrane receptors consists of an extracellular amino terminal domain (ATD) and ligand binding domain (LBD) as well as a transmembrane ion channel. While numerous water soluble constructs of the LBD have been crystallised, high resolution structures of the ATD have been unavailable till very recently. Multiple long (3 x 50 ns) molecular dynamics simulations of five ATD dimer structures - two GluA2 (AMPA) and three GluK2 (kainate) - were performed to evaluate the stability of the structure and the rigidity of the dimer interface. The dimers remained intact throughout the course of all simulations. Overall, these structures appear to undergo very little motion. It is yet unknown whether the ATD is capable of binding a ligand. Simulations of these ligand-free structures suggest two possible regions with increased flexibility that may lead to a potential binding site. Several water molecules are also conserved across the structures as well as the two families. Electrostatic calculations indicate that the "bottom" of the ATD and the "top" of the LBD exposes complementary charge surfaces, which could explain how the two modular regions may interact in the full-length receptor.

2710-Pos

Structurally Variable Regions of the Ligand-Binding Domain of Ionotropic Glutamate Receptors Link Dynamics and Functional Properties

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It has been hypothesized that the behavior of the channel gate is controlled by structural and dynamical events within the bi-lobate ligand binding domain (LBD) of ionotropic glutamate receptors (iGluRs). With the ulterior goal of developing reliable coarse-grained models that would allow probing this hypothesis with μs to ms time-scale simulations, we have characterized the ns time-scale conformational dynamics of the LBD of GluR-2, GluR-6 and NR2A, respectively representing the AMPA, kainate and NMDA receptor class. Each LBD was modeled both using the AMBER SB99 and the GROMOS G43A1 force field. The structural and dynamical properties of the 3 LBDs were obtained from extensive molecular dynamics simulations (at least 3 independents 20 ns run per system and force field). To compare these properties across the three classes of LBD a common structural core was defined based on structural similarity. The results of these computational experiments show that (1) all 3 LBDs possess 2 common rigid body domain motions that modify the degree of twist and hinge bending of the lobes; (2) these motions do not appear to be class specific at this time-scale; instead (3) structural elements outside of the common structural core modulate the conformational space of the core and are responsible for defining class specific dynamics of the LBDs. These structural elements have been implicated previously in the modulation of ligand efficacy and extent of receptor desensitization. Thus, together these observations suggest that these identified structural elements impart class specific dynamics behavior to the LBD of iGluRs and may be responsible for the distinct functional properties of these receptors.

2711-Pos

Theoretical Investigation of Structure and Gating Mechanisms in Glutamate Receptor Ion Channels

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The ionotropic glutamate receptors (iGluRs) are excitatory synaptic transmitters found in the fore-brain. Together with potassium, sodium, and calcium channels they form a tetrameric ligand gated ion channel class of P-loop receptors. The structures of iGluRs are currently not resolved, while functional mechanisms are not well understood. To gain insight into the structure of the iGluR channel region, the KcsA potassium channel pore is used as a working model for its known structural homology with iGluR transmembrane regions. A conserved hydrophobic patch among all P-loop receptors is located along the pore lining M3 helix which spans the lipid-water interface. This patch creates a helical bundle crossing where single residue mutations produce constitutive open channels in mice, suggesting its role in the control of ion conduction. Molecular dynamics simulations and umbrella sampling methods were used to examine the opening of the KcsA M3 bundle crossing starting from the closed KcsA crystal structure (PDB ID: 1k4c). The potential of mean force defining the free energy landscape obtained from the structures of KcsA is then used to abstract the unknown closed and open forms of iGluR transmembrane regions. Assuming the P-loop region comprising the selectivity filter changes little between functional states, energetically defined conformations provide points of refinement for unknown structures of transmembrane regions for proteins such as iGluRs. In order to describe a mechanistic model of iGluR, Ca2+/Mg2+ selectivity is currently under investigation for an NMDA type iGluR. Homology modeling of the transmembrane region based on potassium channels is being used to infer structure; and Ca2+/Mg2+ interactions with organic caging agents are used to parameterize divalent ion selectivity. Understanding how iGluRs control ion conduction together with monovalent versus divalent ion selectivity will aid in describing ion channel structure-function relation.

2712-Pos

Rearrangements at the Heterodimer Interface are Integral to NMDA Receptor Activation

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Upon binding the neurotransmitter glutamate and the obligatory co-agonist glycine, NMDA receptors activate by opening a membrane permeable pore or desensitize by switching into a high-affinity non-conducting conformation. Both activation and desensitization require that the agonist-binding clamshell within each subunit closes to engulf the agonists. It has been hypothesized that this movement strains the contacts between agonist-binding domains of GluN1 and GluN2 and rupture of this interface causes receptor desensitization by disengaging agonist-binding from pore-opening. To investigate the role of inter-subunit contacts in NMDA receptor gating, we cross-linked the dimer interface by introducing cysteine residues at positions predicted to interact across subunits: N521 and L777 of GluN1 and E516 and L780 of GluN2A, respectively. Steady-state single-channel recordings indicated that cross-linked receptors had drastically reduced open probabilities (~200-fold, Po = 0.0032) due to ~5-fold shorter openings and ~100-fold longer closures (means, SEM): MOT = 1.8 ± 0.2 ms, MCT = 792 ± 213 ms (n = 6; 80,028 events). However, the mean duration of closed intervals associated with desensitization remained unaltered (wt, tauD=2700 ms; mut, tauD=3150). Reduction of the disulfide bonds (10 mM DTT) significantly potentiated single channel currents (means, SEM: Po = 0.14 ± 0.02) by restoring the mean duration of openings (11.7 ± 1.6 ms) and significantly shortening mean closed durations (90 ± 19 ms), but had no discernible effects on microscopic desensitization (n = 7; 262,396 events). Based on these data, we propose that flexibility in the heterodimer interface at the level of agonist-binding domains represents an integral part of NMDA receptor activation.

2713-Pos

Activation of Recombinant Rat GluN1/gluN2D NMDA Receptors

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N-methyl-D-aspartate (NMDA) receptors are members of a class of ionotropic glutamate receptors and mediate slow, Ca2+-permeable synaptic transmission. Four separate GluN2 subunit genes (N2A-D) have been identified, which give rise to many of the observed differences in functional properties of the NMDA receptors, including conductance levels, open probability, and deactivation time course upon removal of agonists. A number of macroscopic and single channel properties of NMDA receptors can be grouped according to functional similarities. To study the distinctions between N2A and N2D-containing NMDA receptors, we have conducted single channel voltage-clamp recordings of N1/N2A and N1/N2D receptors to compare channel properties such as open probability and conductance. N1/N2A receptors have a higher channel conductance level (69 + 0.5 pS) and open probability (0.48 + 0.05) than N1/N2D receptors, which have a lower main conductance level and prominent subconductance level (55 + 2.3 pS and 33 + 1.4 pS) and a low open probability (0.015 + 0.004). One explanation of this difference in open probability is that rates constants describing activation steps are slower for NR1/N2D than for N1/N2A receptors, thereby reducing the probability of activating the receptor. However, our whole cell voltage clamp recordings indicate N1/N2D show a surprisingly rapid rise time (6.7 + 0.49 ms), similar to N1/N2A (8.5 + 0.50 ms). To understand how these two receptors with such strikingly different open probabilities can activate at a similar rate, we have fitted models of NMDA receptor activation to our single channel recordings. Preliminary analysis of our data has identified several rate constants describing pre-gating activation steps for N1/N2A that are more than 10-fold faster than in N1/N2D, suggesting that it may be possible to identify pre-gating steps responsible for the distinct characteristics of the N1/N2A and N1/N2D NMDA receptors.

2714-Pos

Effect of *lurcher* Motif Mutations on NMDA Receptor Kinetics

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The juxtamembrane domain, which connects the ligand-binding clamshell to transmembrane helices, is an important transduction element in the gating of glutamate-activated ion channels. In particular, residues in the *lurcher* motif, a highly conserved nine-residue sequence at the end of the M3 helix, have been implicated in controlling channel gating and proton sensitivity. It was reported that a single residue substitution in the GluN1 subunit, A652Y, results in increased gating and decreased proton inhibition. To investigate the mechanism(s) by which these changes in gating and modulation occur, we characterized the stationary gating kinetics of single NMDA receptors composed of GluN1(A652Y) and GluN2A subunits, denoted here A7Y, in cell-attached patches of HEK293 cells, with saturating concentrations of glutamate and glycine, at pH 8.0 and pH 6.5. We found that at pH 8.0, the A7Y substitution caused a 35% increase in P_o. This potentiation was entirely due to ~7-fold increase in open durations: wt, 8.3 ± 1.1 ms (n=6); A7Y 56 ± 12 ms (n=6), which offset a mild (~3-fold) lengthening in closures. At pH 6.5, the A7Y mutation had a more drastic effect, but occurred through a similar mechanism: P_o increased ~140% mostly because of ~16-fold longer openings: wt, 2.2 ± 0.3 ms (n=6); A7Y, 37 ± 3 ms (n=4), which offset a modest ~3-fold increase in closed durations. These results suggest that the A7Y mutation causes receptors to remain open for substantially longer periods of time, regardless of proton concentration. In contrast, the receptor's proton sensitivity was only mildly affected by the mutation; increasing proton concentration from pH 8.0 to pH 6.5 caused a 70% reduction in P_o for A7Y and 80% reduction for wt receptors. Based on these results we suggest that the A7Y mutation affects channel gating by a mechanism that is separate from proton sensitivity.

2715-Pos

Glutamate is a Partial Agonist at GluN2A Containing NMDA Receptors

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NMDA receptors are tetrameric glutamate-activated channels with a dual agonist requirement; gating occurs only after both glutamate and glycine bind within homologous clefts of ligand-binding domains (LBD) of GluN1 and GluN2 subunits, respectively. Glutamate and glycine represent the physiological stimuli for NMDA receptor activation and are considered full agonists. For glutamate-gated channels, it has been proposed that full-agonists stabilize a fully closed conformation of the LBD cleft. To investigate how full-cleft closure correlates with channel activity, we generated receptors with LBDs cross-linked in a closed-cleft conformation by engineering cysteine residues within the GluN1 ($N1^{CC}$) and GluN2A ($2A^{CC}$) subunits. We recorded steady-state single-channel currents from on-cell patches containing only one N1/2A (n=10), $N1^{CC}$/2A (n=11), or N1/$2A^{CC}$ (n=8) receptor. Kinetic analyses of these three data sets indicated that engineered receptors preserved a basic gating mechanism consisting of five closed and two open states, similar to wild-type N1/2A receptors. Further, activity elicited by glutamate alone from $N1^{cc}$/N2A receptors (P_O = 0.57 ± 0.05) was kinetically equivalent to that elicited by glycine and glutamate from N1/2A receptors (P_O = 0.54 ± 0.04, p>0.05). In contrast, glycine alone elicited increased activity from N1/$N2A^{CC}$ receptors, (P_O = 0.69 ± 0.04, p<0.05). This resulted solely from shorter closures (3.4 ± 0.7 vs. 6.0 ± 0.8 ms, p=0.02) with no change in open durations (p=0.5). These results are consistent with structural studies showing that glycine is maximally effective at stabilizing the closed-cleft conformation of the LBD, and can be defined as a true full agonist at the N1 subunit. However, our data show that receptors with cross-linked N2A LBDs are more effective than glutamate at activating the receptor. We suggest that glutamate is a partial agonist at N1/2A receptors, and that it may be feasible to design synthetic agonists with higher efficacy.

2716-Pos

Subunit-Specific Activation of NMDA Receptors

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NMDA receptors are ligand-gated ion channels assembled from two NR1 and two NR2 subunits, and are activated upon simultaneous binding of glycine and glutamate to the NR1 and NR2 subunits, respectively. The different NR2 subunits (NR2A-D) endow the NMDA receptors with markedly different biophysical and pharmacological properties. We have focused on how the conformational changes that are induced by agonist binding and enable channel gating are specific for the NR2 subunit. For this purpose, we have developed a series of N-hydroxypyrazole-5-glycine (NHP5G) compounds that are partial agonists for the glutamate binding site of the NR2 subunit. This structurally related series of partial agonists show a broad range of relative efficacies at NR2 subunits and weakly activate the channel, allowing better identification of the steps associated with channel opening. Propyl-substituted NHP5G shows strong subunit selectivity in that it