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

Katie M. Vance, Kasper B. Hansen, Kevin K. Ogden, Stephen F. Traynelis.
Department of Pharmacology, Emory University School of Medicine, Atlanta, GA, USA.

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

Swetha Murthy, Gabriela K. Popescu.
University at Buffalo, SUNY, Buffalo, NY, USA.

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

Cassandra Kussius, Gabriela K. Popescu.
University at Buffalo, Buffalo, NY, USA.

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

Kasper B. Hansen[1], Pieter Burger[2], Katie M. Vance[1], James P. Snyder[2], Rasmus P. Clausen[3], Stephen F. Traynelis[1].
[1]Emory University School of Medicine, Dept. of Pharmacology, Atlanta, GA, USA, [2]Emory University, Dept. of Chemistry, Atlanta, GA, USA, [3]University of Copenhagen, Dept. of Medicinal Chemistry, Copenhagen, Denmark.

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

activates NR1/NR2D (37%), but does not appear to activate NR1/NR2A (~0%).
Single-channel recordings for NR1/NR2D expressed in HEK293 cells using partial agonists (NH5PG, ethyl-, and propyl-NH5PG) and the full agonist glutamate enable us to determine which states in the process of channel activation can be modulated in an agonist-specific manner. These data show that glutamate, NHP5G, ethyl-, and propyl-NH5PG have strikingly different mean open times for NR1/NR2D (0.747, 0.432, 0.319, and 0.181 ms, respectively). We are currently comparing the rate constants for activation in models fitted to data from different partial agonists. We have also performed molecular dynamics (MD) simulations of the agonist binding domains for NR1/NR2A and NR1/NR2D bound with glutamate or propyl-NHP5G to predict how the agonists interact differentially with receptor subtypes. The synthesis of these lines of investigation will be used to identify structural elements that can be modified using mutagenesis to test working hypotheses on the structural basis for subunit-specific activation of NMDA receptors.

2717-Pos
Gating Effects of a Single-Residue Substitution in the Pore of NMDA Receptors
Stacy A. Amico-Ruvio, Thomas P. Smith, Gabriela K. Popescu.
University at Buffalo, Buffalo, NY, USA.
NMDA receptors are glutamate-gated ion-channels with high Ca2+-permeability, voltage-dependent block by extracellular Mg2+, and slow gating kinetics. These intrinsic attributes are critical to coincidence detection and synaptic plasticity at excitatory synapses in brain. The GluN1/GluN2A isoform (N1/N2A) predominates in adult brain; it has strong voltage-dependent Mg2+ block and the fastest kinetics of all isoforms. In macroscopic measurements, Mg2+ block generates a region of steep negative slope in the current-voltage (I/V) relationship at membrane potentials negative to −50 mV. In single-channel current records, the binding of one Mg2+ ion precludes conduction by other cations and results in a discrete, resolvable gap. In the presence of strong metal chelators (EDTA) and for receptors that carry a single-residue substitution (N596G) in the N2A subunit (N1/N2AN+1G), the macroscopic I/V plot reverts to its normal linear shape and the Mg2+-dependent gap is absent from single-channel traces. To investigate whether the N+1G substitution in the pore impinges on the receptor's gating kinetics, we recorded single-channel current traces from cell-attached patches of HEK-cells containing only one N1/N2AN+1G receptor and compared these with those recorded from wild-type receptors under similar conditions. Measurements done in the absence of extracellular Mg2+ (1 mM EDTA in the recording pipette) revealed that the N+1G substitution caused a ~2-fold decrease in activity (Po, 0.31 $\pm$ 0.04, n = 10 vs. 0.65 $\pm$ 0.04, n = 12 for wild-type, $p < 0.001$) due to ~2-fold shorter openings and ~3-fold longer closures. Our results indicate that a perturbation in the pore that was intended to render the channels less sensitive to block by extracellular divalent cations also has a significant effect on the gating of NMDA receptors.

2718-Pos
Development of AMPA Receptor Aptamers
Zhen Huang, Jae Seon Park, Weimin Pei, Yan Han, Sabarinath Jayaseelan, Li Niu.
State University of New York at Albany, Albany, NY, USA.
The α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtype of glutamate ion channel receptors plays an essential role in the mammalian brain activities such as memory and learning, whereas the excessive receptor activation has been implicated in neurological diseases such as stroke, epilepsy, and amyotrophic lateral sclerosis. Inhibitors against AMPA glutamate receptors are drug candidates for a potential treatment of these neurological diseases. Using systematic evolution of ligands by exponential enrichment (SELEX), we have successfully identified three different classes of aptamers with nanomolar affinity against the GluR2Qflip receptor, a key AMPA receptor subunit that controls the calcium permeability and mediates excitotoxicity. One class is a group of competitive aptamers, which we selected by using NBQX, a classic competitive inhibitor. The highest potency or IC50 value for one of the aptamers in this class reached 30 nM, rivaling any exiting AMPA receptor inhibitors. We have also identified two other classes of aptamers that are differentially selective to different conformations of GluR2Qflip: one class uniquely inhibits the open-channel conformation whereas the other inhibits the closed-channel conformation. As an initial proof-of-principle experiment, our results suggest the possibility of developing aptamers that are nanomolar affinity, water-soluble and highly selective to both an AMPA receptor subunit and a unique receptor conformation. These aptamers are therefore excellent water-soluble templates for design of better inhibitors and better drug candidates against AMPA receptors.

2719-Pos
A Functional Probe of Ligand Binding and Agonist Efficacy in Ionotropic Glutamate Receptors
Margaret W. Thompson, Kathryn A. McMenimen, Henry A. Lester, Dennis A. Dougherty.
California Institute of Technology, Pasadena, CA, USA.
Members of the ionotropic glutamate receptor (iGluR) family (NMDA, AMPA, and kainate receptors) are allosteric ligand-gated ion channels that mediate synaptic plasticity in the mammalian nervous system. Crystal structures of the two-lobed binding domains of these receptors suggest that ligand binding causes the lobes to move toward one another like a clamshell closing. It has also been proposed that the degree of closure is proportional to the degree of receptor activation. Using nonsense suppression methodology to incorporate unnatural amino acids, we have created a functional probe of ligand binding and the subsequent clamshell closure. By altering the electronic (e.g., converting glutamate to nitrohomoalanine) and steric (e.g., converting tyrosine to homotyrosine) properties of binding site residues of the NMDA and AMPA receptors, we have been able to define the requirements for both full and partial agonist binding and to confirm that agonist efficacy is related to the degree of clamshell closure.

2720-Pos
Modal Behavior of IGluR3 AMPA-Receptor-Channels in Cell Attached Recordings
Kinning Poon.
Cornell University, Ithaca, NY, USA.
Ionotropic glutamate receptors (iGluR's) are ligand gated ion channels that mediate most of the fast excitatory neurotransmission in the CNS. Aberrant function of glutamate neurotransmission can lead to epilepsy and other neurodegenerative disorders. The extracellular ligand binding domain is a bilobal structure that binds an agonist and induces channel activation. Cell attached patch recordings were performed with both full and partial agonists on HEK 293 cells stably expressing homomeric GluR3-flip receptor channels. Single channel data were analyzed using QuB software to detect channel conductance states and to determine a simple model of agonist dependent channel activity and underlying modal behavior. Amplitude analysis uncovered three conductance states, 15 pS, 27 pS, and 40 pS, in the presence of the full agonist, glutamate, as well as the partial agonists, fluorowillardiine, chlorowillardiine and nitrowillardiine. Different modes of activation ranging from low to high open probability exist for this channel. The dwell times for the high mode are longer compared to the low mode. In the presence of the full agonist, glutamate, during a high mode of activation, the channel prefers to open to the intermediate and large conductance states. In the presence of the willardiine partial agonists, the channel opens more frequently to the smallest and intermediate conductance states. Kinetic modeling using maximum interval likelihood rate optimization revealed two time constants in each open state and at least three in the closed state for the full and partial agonists. These data suggests that the mechanisms of channel activation are similar for both full and partial agonists but the transition rates between states differ. Supported by NIH NS049223 and NS063518.