Structural Dynamics of the Glycine-binding Domain of the N-Methyl-d-Aspartate Receptor*

Received for publication, August 15, 2014, and in revised form, November 12, 2014. Published, JBC Papers in Press, November 17, 2014, DOI 10.1074/jbc.M114.605436

Drew M. Dolino†, David Cooper§, Swarna Ramaswamy†, Henriette Jaurich§, Christy F. Landes§¶, and Vasanthi Jayaraman†‡

From the †Center for Membrane Biology, Department of Biochemistry and Molecular Biology, Graduate School of Biomedical Sciences, University of Texas Health Science Center, Houston, Texas 77030 and the Departments of §Chemistry and ¶Electrical and Computer Engineering, Rice University, Houston, Texas 77251

Background: The agonist glycine binds to a cleft in the bilobed extracellular domain of NMDA receptors.

Results: The full agonist-bound forms of the agonist-binding domain populate more of the higher efficiency closed-cleft states of the receptor.

Conclusion: Cleft closure dynamics differ for the full and partial agonist-bound forms.

Significance: Dynamics and the extent of cleft closure control agonist efficacy.

N-Methyl-d-aspartate receptors mediate the slow component of excitatory neurotransmission in the central nervous system. These receptors are obligate heteromers containing glycine- and glutamate-binding subunits. The ligands bind to a bilobed agonist-binding domain of the receptor. Previous x-ray structures of the glycine-binding domain of NMDA receptors showed no significant changes between the partial and full agonist-bound structures. Here we have used single molecule fluorescence resonance energy transfer (smFRET) to investigate the cleft closure conformational states that the glycine-binding domain of the receptor adopts in the presence of the antagonist 5,7-dichlorokynurenic acid (DCKA), the partial agonists 1-amino-1-cyclobutanecarboxylic acid (ACBC) and L-alanine, and full agonists glycine and D-serine. For these studies, we have incorporated the unnatural amino acid p-acetyl-L-phenylalanine for specific labeling of the protein with hydrazide derivatives of fluorophores. The single molecule fluorescence resonance energy transfer data show that the agonist-binding domain can adopt a wide range of cleft closure states with significant overlap in the states occupied by ligands of varying efficacy. The difference lies in the fraction of the protein in a more closed-cleft form, with full agonists having a larger fraction in the closed-cleft form, suggesting that the ability of ligands to select for these states could dictate the extent of activation.

NMDA receptors, a subtype of the ionotropic glutamate receptors (iGluRs),3 are cation channels that contribute to the slow component of the excitatory neurotransmission in the mammalian central nervous system (1, 2). The NMDA receptors play an important role in many physiological and pathological processes, such as synaptic plasticity, learning and memory, neuropathic pain, Parkinson disease, and Alzheimer disease (2, 3). One of the central questions in this family of proteins is how agonist binding leads to channel activation. Insight into iGluR structure and function has been dominated by studies on the AMPA subtype of this family. Such studies have illustrated the overall structure of iGluRs as tetramers (4). Each individual subunit is structured in a modular fashion, containing the extracellular amino-terminal domain and agonist-binding domain, a transmembrane pore domain, and an intracellular carboxyl-terminal domain (4). The agonist-binding domain folds into a clamshell-like shape that can close upon its ligands, inducing a conformational change that results in channel opening. Crystal structures show that the extent of this cleft closure correlates with the efficacy of the ligand, with weak agonists inducing partial closure, and full agonists inducing full cleft closure and full channel activation (5–10). Such a relationship between agonist-binding domain cleft closure and ion channel activation provides an elegant means of explaining the link between conformational changes at the agonist-binding site and opening of the channel pore (11). Single molecule fluorescence resonance energy transfer (smFRET) experiments as well as NMR show that, in addition to the inherent ability of a ligand to induce cleft closure, the dynamics also play an important role in dictating activation (12–17).

The overall architecture of the AMPA receptors has been shown to correlate well with the NMDA receptor (4, 18, 19), albeit with several significant differences. One striking difference between the two receptors is that the NMDA receptor arranges as obligate heteromers that require both a glycine-
binding subunit and a glutamate-binding subunit. More importantly, ensemble luminescence resonance energy transfer measurements show that although at the glutamate-binding site of the NMDA receptor the extent of cleft closure at the agonist binding-domain does appear to correlate to activation, such a graded cleft closure was not observed in the crystal structures or ensemble luminescence resonance energy transfer measurements for the glycine-binding domain (20–23). Based on the crystal structures, it has been suggested that the glycine-binding GluN1 subunit could follow a mechanism of conformational selection wherein the apo state probes both the closed-cleft and the open-cleft conformations. In this mechanism, agonist efficacy is governed by the stabilization of the closed conformation rather than the formation of intermediate states. Consistent with this hypothesis and with the distinction from AMPA receptors, theoretical investigations of the apo state of GluN1 reveal a narrowly distributed closed-cleft population in addition to an expected broad open-cleft population (24). Although the theoretical studies shed light on the apo- and glycine-bound states of the NMDA receptor, the mechanism of partial agonism at the agonist-binding domain is still largely unknown.

To address this question, here we used smFRET, which allows us to examine the conformational landscape that the isolated glycine-binding GluN1 agonist-binding domain (GluN1 S1S2) probes in the presence of full agonists, partial agonists, or an antagonist. Additionally, to specifically label the protein at the desired sites, we incorporated the unnatural amino acid p-acetyl-L-phenylalanine into the protein (25). The unique ketone group of p-acetyl-L-phenylalanine can be coupled to hydrazide-conjugated fluorescent dyes for the smFRET studies. Although smFRET on glutamate receptors has been done before through the labeling of cysteine residues (16, 17), this new procedure allows us to investigate the protein without concern of labeling or mutating out inherent cysteine residues. We find that there is significant overlap between the different liganded states with the antagonist-bound protein samples exhibiting both the closed-cleft and the open-cleft conformations, similar to what is predicted with the molecular dynamic simulations. Interestingly, we also find that the 1-amino-1-cyclobutane carboxylic acid (ACBC) partial agonist-bound proteins show greater rigidity in its population distribution, which is different from what has previously been observed in the AMPA receptors (17).

EXPERIMENTAL PROCEDURES

Generation of Site-directed Mutants—A pET22B vector encoding the rat GluN1 isolated agonist-binding domain was provided by Eric Gouaux (Oregon Health and Science University, Portland, OR) (21). Sites were chosen based on accessibility and distance across the cleft. Ser-507 and Thr-701 (Fig. 1a) were mutated to TAG using standard site-directed mutagenesis with Pfu Turbo (Agilent) so that they would encode the amber stop codon UAG upon transcription. The original stop codon of this construct was also mutated from the amber stop codon to encode an opal stop codon (UAA) to allow for successful translation termination.

Protein Expression—Mutant plasmid was co-transformed into Origami B (DE3) cells (Novagen) along with the pEVOL plasmid containing the genes for the suppressor tRNA and aminooacyl-tRNA synthetase needed to incorporate p-acetyl-L-phenylalanine (pEVOL plasmid was provided by Peter Schulz,
Scripps Research Institute, La Jolla, CA (25). 1-liter liquid cultures were grown in LB broth, Miller (Fisher Scientific) supplemented with 50 μg/ml ampicillin (Sigma), 15 μg/ml kanamycin (Fisher Scientific), 12.5 μg/ml tetracycline (Calbiochem), and 50 μg/ml chloramphenicol (Acros Organics) until they reached an A_{\text{600}} of 0.8. Then, protein expression was induced by adding isopropyl-β-D-galactopyranoside (Fisher Scientific) to a final concentration of 0.5 mM. Simultaneously, the unnatural amino acid machinery was induced by adding 0.02% arabinose (Sigma) and 1 mM p-acetyl-L-phenylalanine (AcF) (RSP Amino Acids). Induction was carried out at 20 °C for 20–24 h. The cultures were then pelleted down, and the pellets were stored at −80 °C until use.

**Protein Purification**—Pellets were thawed and lysed with a cell disruption vessel (Parr Instruments). Then, cell debris was pelleted by spinning at 40,000 rpm for 1 h at 4 °C. The supernatant was collected and purified by binding with 1 ml of nickel-nitrilotriacetic acid-agarose resin (Qiagen). Protein was then eluted with 200 mM imidazole (Sigma), concentrated down, and then brought to 500 μl in PBS, pH 7, supplemented with 1 mM glycine (Fisher Scientific). Western blots of the purified mutant protein confirm expression of the full 35-kDa His-tagged agonist-binding domain only upon induction of the unnatural amino acid machinery, showing successful incorporation and utilization of the unnatural amino acid (Fig. 2a).

**Labeling**—Fluorescent dyes were added to the above protein sample. Alexa Fluor 555 hydrazide and Alexa Fluor 647 hydrazide (Invitrogen) were used as the donor and acceptor, respectively. For cysteine labeling, the maleimide derivatives were used. To label the unnatural amino acids, we used ketone-reactive, hydrazide-conjugated fluorescent dyes (Fig. 1b). Donor and acceptor dyes were premixed and then added such that dye:protein molar ratios were 1:1 for donor and 4:1 for acceptor, to minimize proteins labeled with only donor fluorophores. Protein was labeled overnight at 4 °C. The following day, excess dye was removed from the protein by dialysis in 2 liters of PBS for 6 h, changing the dialysis buffer every 2 h. Glycine was added to the dialysis buffer up to 1 mM for the glycine samples. For samples liganded to D-serine (Acros Organics), L-alanine (Acros Organics), ACBC (Aldrich), or 5,7-dichlorokynurenic acid (DCKA) (Abcam), the dialysis buffer contained no ligand, but the appropriate ligand was added to the dialysis sample before and after each exchange (1, 15, 10 and 1 mM, respectively, based on differential affinities for each ligand). This type of ligand substitution was also performed for glycine. No significant changes were noted, and the glycine data were pooled for final analysis. For smFRET experiments, 1 μg of biotin-conjugated anti-His epitope antibody (Rockland Immunocchemicals) was added to a 500-μl sample.

**Isothermal Calorimetry**—The functionality of the GluN1 agonist-binding domain protein with unnatural amino acids tagged with fluorophores was determined using isothermal calorimetry (Fig. 2b). For these experiments, the protein was extensively dialyzed to the apo state in buffer containing 20 mM HEPES (pH 7), 150 mM NaCl, 1 mM EDTA, and 10% glycerol. Calorimetric titrations were performed with VP-ITC (MicroCal) using 1 μM protein with 20 10–μl injections of 15 μM (E)-4,6-dichloro-3-(2-phenyl-2-carboxyethyl)indole-2-carboxylic acid (MDL 105,519) (Sigma) at 23 °C. Data analysis was performed using Origin (OriginLab).

**smFRET Sample Preparation**—For all single molecule measurements in this study, plasma-cleaned 22 × 22-mm micro glass coverslips (VWR International) were immersed in a VECTABOND-acetone solution (1% w/v, Vector Laboratories, Burlingame, CA) for 5 min, rinsed with molecular biology-grade water, dried with nitrogen, and stored under vacuum to prevent contamination (16). A silicon template was placed on the VECTABOND-functionalized slide to allow filling of the future chamber area with PEG solution (5-kDa biotin-terminated PEG (2.5% w/w in molecular biology grade water, NOF Corp.) and sodium bicarbonate (Sigma)), and the filled slide was allowed to dry in the dark for 4–6 h. Excess PEG was washed off with 10–12 ml of MB water, and after nitrogen-drying the slide, a custom HybriWell chamber (Grace Bio-Labs) fitted with an inlet and outlet port (press-fit tubing connectors, Grace Bio-Labs) was arranged precisely over the PEGylated area. After filling the chamber with PBS buffer, a control image was taken to ensure a clean sample, followed by insertion of 0.2 mg/ml streptavidin (Invitrogen) in PBS buffer. Biotin-streptavidin binding was allowed to progress for 10 min. The protein of interest, with the biotin-conjugated anti-His antibody for streptavidin association,
was added to the chamber at an approximate concentration of 20 nM and incubated for 20 min. Unbound protein was washed out by flushing the chamber with an excess of PBS buffer.

Measurements—The sample chamber was secured to a closed-loop x-y-z piezo stage (P-517.3CL; Physik Instrumente) with 100 × 100 × 20-μm travel range and 1 nm specificity (SPM 1000, RHK Technology) to allow for precise movement of the sample area. To extend the lifetimes of the fluorophores, an oxygen-scavenging buffer solution of 33% w/w β-D-(+)-glucose (Sigma), 1% w/w glucose oxidase, 0.1% v/v catalase immersion microscope objective lens (Carl Zeiss, GmbH) to a 315M-100 SL) focused through a FLUAR 100 1.3 NA oil objective and was passed through the chamber using a syringe pump flow system at a rate of 1 μl/min (26).

Additionally, the above concentrations of the specific agonist-binding domain ligand, depending on the experimental conditions, was included in the buffer solution. The custom-built confocal microscope (Zeiss Axiovert 200 M) described previously was used for all smFRET measurements (27, 28). A 532-nm diode-pumped solid-state laser (Coherent, Compass 315M-100 SL) focused through a FLUAR 100 × 1.3 NA oil immersion microscope objective lens (Carl Zeiss, GmbH) to a power density of 50 watts/cm² at the sample was used to excite the sample. Emitted light was collected back through the same objective and was passed through a notch filter (zet532nf, Chroma Technology) and toward the detector box. The fluorescence emission light was separated by a 640-nm high-pass dichroic mirror (Chroma 640 DCXR) and collected by two corresponding avalanche photodiode detectors (SPCM AQR-15, PerkinElmer) set to 570 and 670 nm using band-pass filters (NHPF-532.0, Kaiser Optical and ET585, Chroma Technology) for donor and acceptor signal collection. An area of 10 × 10 μm was raster-scanned to locate individual molecules. After a single molecule was chosen for observation, the stage was moved to focus the laser on the particular molecule, and then the donor and acceptor fluorescence signals were collected until photobleaching of the fluorophores occurred.

Data Analysis—A 1-ms time resolution was used to record the emission intensity trajectories and then binned up to 10-ms frames during data processing to improve the signal-to-noise ratio. The data analysis was performed by an in-house script using MATLAB (R2009b, MathWorks), which processed the signals via the wavelet denoising technique (29, 30). The denoised signal was then used to calculate the FRET efficiency at each time point, using the following equation,

$$E_D = \frac{I_A}{I_A + I_D}$$

(Eq. 1)

where $E_D$ is the apparent FRET efficiency, $I_A$ is the background-corrected acceptor fluorescence intensity, and $I_D$ is the background-corrected donor fluorescence intensity (16, 17). From this FRET efficiency, the distance was determined through the Förster equation,

$$E = \left(1 + \left(\frac{R}{R_0}\right)^6\right)^{-1}$$

(Eq. 2)

where $R$ is the distance between the dyes, and $R_0$ is the Förster radius. The Förster radius, the distance at which the efficiency of energy transfer is half-maximal, is 51 Å for the Alexa Fluor 555-Alexa Fluor 647 fluorophore pair used for these experiments. Error in FRET efficiencies was set at 0.03 based on measurements under the same conditions performed with a rigid DNA double strand. The S.E. for the fraction of proteins with FRET efficiencies higher than 0.96 was calculated using the above error in FRET efficiency and determining the fractions at the two extremes in error and by dividing by the square root of the number of molecules studied for each ligand-bound state. After processing the data, the traces were further filtered for single molecule verification and excluded if they showed criteria of multistep bleaching or exceptionally high background adapted from a normal distribution.

RESULTS

Cysteine Labeling versus Unnatural Amino Acid Labeling—Previous protocols to study single molecule dynamics typically make use of cysteine residues to enable site-specific labeling (16, 17). An important first step for such approaches is to remove endogenous cysteines that may be undesirably labeled. In the GluN1 agonist binding domain, there is one non-disulfide-bonded cysteine at position 459 (Fig. 1). The C459S mutant S152 protein could not be expressed well in Escherichia coli. Further, smFRET investigations of wild type protein labeled with thiol-reactive dyes shows signal at the donor emission frequency but not at the acceptor frequency (FRET signal) relative to the blank slide studied under the same conditions (Fig. 3, a and b). This result indicates that the single cysteine is accessible under these labeling conditions, although the disulfide-bonded cysteines are not labeled. This is further confirmed by the double cysteine mutant at positions Ser-507 and Thr-701, which shows signal in both the donor and acceptor frequencies (Fig. 3c).

These considerations made it difficult to use cysteines for analyzing dynamic data. To address this issue, we introduced the unnatural amino acid AcF by mutating to an amber stop codon at site 507 in domain 1 and at site 701 in domain 2 (Fig. 1). In contrast to the experiments with the thiol-reactive fluorophores, the wild type protein shows no signal even after overnight treatment with ketone-reactive dyes, similar to the blank control (Fig. 3, d and e), indicating that none of the natural amino acids are reactive to the ketone-reactive dyes. Additionally, the mutant protein with AcF at positions 507 and 701 showed signal at both the donor and acceptor frequencies, showing that the dyes specifically label only the introduced AcF (Fig. 3f).

smFRET Investigations of the GluN1 Agonist-binding Domain with Full Agonist, with Partial Agonist, and with Antagonist—The GluN1 agonist-binding domain was examined by smFRET in the presence of the full agonists glycine and D-serine, the partial agonists L-alanine and ACBC, and the antagonist DCKA (Fig. 4, a–e). Donor and acceptor photon counts of excited proteins were measured with millisecond resolution, collected into 10-ms bins for efficiency determination, denoised using wavelet decomposition, and then plotted as separate histograms as described previously (16, 17, 28).

The smFRET histograms of the different ligand-bound states show FRET efficiencies that range from 1 to 0.5 with a peak at 0.95. The FRET efficiency of 0.95 corresponds to a distance of 31 Å. This distance is similar to the 34 Å measured in the crystal
structure between the Cα of residue 507 and the Cα of residue 701, which is in good agreement with the FRET data, given that the FRET distances are measures between the fluorophores (21). A smaller peak appears around an efficiency of 0.72, corresponding to a distance of 44 Å. This distance is comparable with the 41 Å distance measured in the apo crystal structure. The fact that the different liganded states show occupancy covering this entire range suggests that the protein probes both the closed- and open-cleft-conformational states (24). The small fraction of occupancies (less than 10%) at efficiencies below 0.6 reflect hyperextended open-cleft conformations that are most likely accessible due to the isolated nature of the agonist-bind- 

A shift toward lower efficiency states is seen when comparing the smFRET data of full agonists to that of the antagonist DCKA (Fig. 5a). Specifically, the DCKA-bound form of the protein has a reduced number of occurrences at 0.96 or higher efficiencies and a much larger fraction at 0.88 efficiency (37 Å) relative to the forms bound to the full agonists glycine or D-serine. This trend to lower efficiencies is again consistent with the crystal structures, which show an open-cleft conformation for the antagonist-bound protein (21). Interestingly, the common 0.95 peak underlies the significant overlap in the smFRET data between the antagonist- and agonist-bound forms of the GluN1 agonist-binding domain. These data are consistent with theoretical calculations of the apo state of the glycine-binding domains of the NMDA receptor, which show that the protein probes both open-cleft and closed-cleft conformations (24). The smFRET data, however, do not show a clear appearance of a new, distinct population between the two liganded forms, but only a shift in the population from a more closed-cleft conformation in the full agonist-bound state to a more open-cleft conformation in the antagonist-bound state (Fig. 5a).

The histograms for the GluN1 agonist-binding domain when bound to the partial agonists ACBC or L-alanine also show a peak FRET efficiency at 0.95, which corresponds to a distance of 31 Å (Fig. 4). This distance is in agreement with the distance of 32 Å measured in the crystal structure of the ACBC-bound agonist-binding domain between the Cα of residue 507 and the Cα of residue 701 (22). The agonist-binding domain in complex with L-alanine has not been crystallized. The peak efficiency seen in the partial agonist-bound forms of the agonist-binding domain is similar to that found in the glycine-bound state of the protein, again consistent with the crystal structures that show a similar closed-cleft conformation with all activating agonists. Crucially, both of the partial agonist-bound forms of the agonist-binding domain probe smaller ranges of conformations and are thus more rigid when compared with the glycine-bound state of the protein (Fig. 5b). Moreover, between the two partial agonists, the ACBC-bound form seems even more rigid than L-alanine, in line with ACBC being a less effective partial agonist than the latter (22, 31). This finding seems to be in direct contrast to the AMPA receptor, which shows a broader range of closed-cleft conformations when comparing partial agonist-bound forms with full agonist-bound forms (17).

DISCUSSION

Here, we show that the mechanisms of agonist action at GluN1 are different from those of AMPA receptors due to the fact that the protein tends to occupy a much narrower spread of states in the full agonist-, partial agonist-, and antagonist-
bound forms. The differences are more evident in the partial agonist- and antagonist-bound forms, as a decrease in agonism for the AMPA receptors is reflected by a large increase in the spread of cleft closure states. In the GluN1 agonist-binding domain, the shifts are much less dramatic. The decreased spread in the cleft closure states probed by the GluN1 agonist-binding domain, the shifts are much less dramatic. The decreased spread in the cleft closure states probed by the GluN1 agonist-binding
domain could be one of the reasons that no significant changes were observed in the extent of cleft closure in the crystal structures between the partial agonist- and full agonist-bound forms.

Although the two receptors show differences in terms of dynamics of the agonist-binding domain between the various ligand-bound states, there is still a linear dependence for the GluN1 agonist-binding domain between activation and the fraction of protein exhibiting FRET efficiencies greater than 0.96 (Fig. 6). This result is similar to what was observed in the AMPA receptors where a similar linear dependence between the fraction of receptors in high-efficiency states versus activation was observed (17). Thus, although the dynamics are different for the two subtypes, the underlying mechanism wherein the extent of cleft closure controls the extent of receptor activation still seems to be preserved.

Apart from the characterization of the GluN1 agonist-binding domain dynamics, the studies performed here show that unnatural amino acids can be used as a means to label proteins for smFRET. The ability to introduce amino acids with a unique ketone functional group allows for labeling of proteins with high specificity. Avoiding the conventional thiol-maleimide chemistry allows investigators to disregard any problems with cysteines native to the protein, as well as alleviating worries about the formation of disulfide bridges with the introduced cysteines. The commercial availability of p-acetyl-L-phenylalanine, as well as the commercial availability of various hydrazide-conjugated fluorescent labels, allows for the use of a wide variety of FRET fluorophore pairs with various distance ranges.

In conclusion, we have demonstrated here the use of ketone-containing unnatural amino acids for smFRET measurements to analyze the conformational dynamics of the GluN1 agonist-binding domain in complex with the full agonists glycine and D-serine, the partial agonists L-alanine and ACBC, and the antagonist DCKA. The use of unnatural amino acids allows for the specific labeling of proteins and has the flexibility of being used with a wide variety of fluorophores. The smFRET histograms of the GluN1 agonist-binding domain show a common high-efficiency peak, corresponding to a closed-cleft conformation accessible to all examined liganded forms of the protein. These data are consistent with previous theoretical results where the closed conformation was seen in both apo-bound and glycine-bound forms of GluN1 (24). Additionally, the difference in efficacy appears to be correlated with the ability of the ligand to select specifically for the closed conformation.

REFERENCES

1. Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashev, N., Sakmann, B., and Seeburg, P. H. (1992) Heteromeric NMDA receptors: molecular and functional distinction of subtypes. Science 256, 1217–1221

2. Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., Hansen, K. B., Yuan, H., Myers, S. J., and Dingledine, R. (2010) Glutamate receptor ion channels: structure, regulation, and function. Pharmacol. Rev. 62, 405–496

3. Johnson, K. A., Conn, P. J., and Niswender, C. M. (2009) Glutamate receptors as therapeutic targets for Parkinson’s disease. CNS Neurol. Disord. Drug Targets 8, 475–491

4. Sobolevsky, A. I., Rosconi, M. P., and Gouaux, E. (2009) X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. Nature 462, 745–756

5. Armstrong, N., and Gouaux, E. (2000) Mechanisms for activation and antagonism of an AMPA-sensitive glutamate receptor: crystal structures of the GluR2 ligand binding core. Neuron 28, 165–181

6. Jin, R., Banke, T. G., Mayer, M. L., Traynelis, S. F., and Gouaux, E. (2003) Structural basis for partial agonist action on ionotropic glutamate receptors. Nat. Neurosci. 6, 803–810

7. Hogner, A., Kastrup, J. S., Jin, R., Liljefors, T., Mayer, M. L., Egebjer, J., Larsen, I. K., and Gouaux, E. (2002) Structural basis for AMPA receptor activation and ligand selectivity: crystal structures of five agonist complexes with the GluR2 ligand-binding core. J. Mol. Biol. 322, 93–109

8. Jin, R., Hornig, M., Mayer, M. L., and Gouaux, E. (2002) Mechanism of activation and selectivity in a ligand-gated ion channel: structural and functional studies of GluR2 and quisqualate. Biochemistry 41, 15635–15643

9. Ramanoudjame, G., Du, M., Mankiewicz, K. A., and Jayaraman, V. (2006) Allosteric mechanism in AMPA receptors: a FRET-based investigation of conformational changes. Proc. Natl. Acad. Sci. U.S.A. 103, 10473–10478

10. Mankiewicz, K. A., Ramschardan, A., Wathen, L., and Jayaraman, V. (2008) Chemical interplay in the mechanism of partial agonist activation in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. Biochemistry 47, 398–404

11. Gouaux, E. (2004) Structure and function of AMPA receptors. J. Physiol. 554, 249–253

12. Fenwick, M. K., and Oswald, R. E. (2008) NMR spectroscopy of the ligand-binding core of ionotropic glutamate receptor 2 bound to 5-substituted willardiine partial agonists. J. Mol. Biol. 378, 673–685

13. Maltsev, A. S., Ahmed, A. H., Fenwick, M. K., Jane, D. E., and Oswald, R. E. (2008) Mechanism of partial agonism at the GluR2 AMPA receptor: measurements of lobe orientation in solution. Biochemistry 47, 10600–10610

14. Ahmed, A. H., Thompson, M. D., Fenwick, M. K., Romero, B., Loh, A. P., Jane, D. E., Sondermann, H., and Oswald, R. E. (2009) Mechanisms of antagonism of the GluR2 AMPA receptor: structure and dynamics of the complex of two willardiine antagonists with the glutamate binding domain. Biochemistry 48, 3984–3903

15. Ahmed, A. H., Prak, C. P., Fenwick, M. K., Hsieh, C. L., Weiland, G. A., and Oswald, R. E. (2013) Dynamics of cleft closure of the GluA2 ligand-binding domain in the presence of full and partial agonists revealed by hydrogen-deuterium exchange. J. Biol. Chem. 288, 27658–27666

16. Landes, C. F., Ramschardan, A., Taylor, J. N., Salatan, F., and Jayaraman, V. (2011) Structural landscape of isolated agonist-binding domains from single AMPA receptors. Nat. Chem. Biol. 7, 168–173

17. Ramaswamy, S., Cooper, D., Podnar, N., MacLean, D. M., Ramschardan, A., Taylor, J. N., Uhri, H., Landes, C. F., and Jayaraman, V. (2012) Role of...
conformational dynamics in α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor partial agonism. J. Biol. Chem. 287, 43557–43564
18. Lee, C.-H., Lu, W., Michel, J. C., Goehring, A., Du, J., Song, X., and Gouaux, E. (2014) NMDA receptor structures reveal subunit arrangement and pore architecture. Nature 511, 191–197
19. Karakas, E., and Furukawa, H. (2014) Crystal structure of a heterotetrameric NMDA receptor ion channel. Science 344, 992–997
20. Rambhadran, A., Gonzalez, J., and Jayaraman, V. (2011) Conformational changes at the agonist binding domain of the N-methyl-D-aspartic acid receptor. J. Biol. Chem. 286, 16953–16957
21. Furukawa, H., and Gouaux, E. (2003) Mechanisms of activation, inhibition and specificity: crystal structures of the NMDA receptor NR1 ligand-binding core. EMBO J. 22, 2873–2885
22. Inanobe, A., Furukawa, H., and Gouaux, E. (2005) Mechanism of partial agonist action at the NR1 subunit of NMDA receptors. Neuron 47, 71–84
23. Hansen, K. B., Tajima, N., Risgaard, R., Perszyk, R. E., Jørgensen, L., Vance, K. M., Ogden, K. K., Clausen, R. P., Furukawa, H., and Traynelis, S. F. (2013) Structural determinants of agonist efficacy at the glutamate binding site of N-methyl-D-aspartate Receptors. Mol. Pharmacol. 84, 114–127
24. Yao, Y., Belcher, J., Berger, A. J., Mayer, M. L., and Lau, A. Y. (2013) Conformational analysis of NMDA receptor GluN1, GluN2, and GluN3 ligand-binding domains reveals subtype-specific characteristics. Structure 21, 1788–1799
25. Young, T. S., Ahmad, I., Yin, J. A., and Schultz, P. G. (2010) An enhanced system for unnatural amino acid mutagenesis in E. coli. J. Mol. Biol. 395, 361–374
26. Cooper, D., Uhlm, H., Tausin, L. J., Poddar, N., and Landes, C. F. (2013) Photobleaching lifetimes of cyanine fluorophores used for single-molecule Forster resonance energy transfer in the presence of various photo-protection systems. ChemBiochem 14, 1075–1080
27. Darugar, Q., Kim, H., Gorelick, R. J., and Landes, C. (2008) Human T-cell lymphotropic virus type 1 nucleocapsid protein-induced structural changes in transactivation response DNA hairpin measured by single-molecule fluorescence resonance energy transfer. J. Virol. 82, 12164–12171
28. Nick Taylor, J., Darugar, Q., Kourentzi, K., Willson, R. C., and Landes, C. F. (2008) Dynamics of an anti-VEGF DNA aptamer: a single-molecule study. Biochem. Biophys. Res. Commun. 373, 213–218
29. Taylor, J. N., and Landes, C. F. (2011) Improved resolution of complex single-molecule FRET systems via wavelet shrinkage. J. Phys. Chem. B 115, 1105–1114
30. Taylor, J. N., Makarov, D. E., and Landes, C. F. (2010) Denoising single-molecule FRET trajectories with wavelets and Bayesian inference. Biophys. J. 98, 164–173
31. Chen, P. E., Geballe, M. T., Katz, E., Erreger, K., Livesey, M. R., O’Toole, K. K., Le, P., Lee, C. J., Snyder, J. P., Traynelis, S. F., and Wyllie, D. J. (2008) Modulation of glycine potency in rat recombinant NMDA receptors containing chimeric NR2A/2D subunits expressed in Xenopus laevis oocytes. J. Physiol. 586, 227–245