Biochemical and Biophysical Research Communications 424 (2012) 687–690

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

On mechanism of allosteric modulation of NMDA receptor via amino-terminal domains

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ARTICLE INFO

Article history:
Received 2 July 2012
Available online 10 July 2012

Keywords:
Ionotropic glutamate receptor
N-methyl-D-aspartate receptor
NMDA receptor
Allosteric modulation
Ifenprodil
Molecular dynamics

ABSTRACT

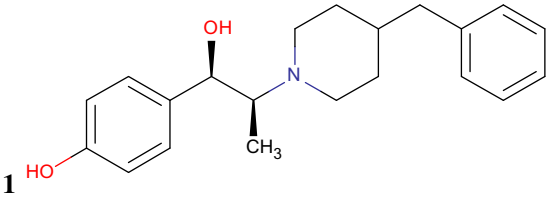
A possible mechanism of action of the allosteric modulators of NMDA (*N*-methyl-D-aspartate) receptors is proposed that involves the stabilization of the twisted closed-clamshell configuration of the amino-terminal domains of GluN1 and GluN2B subunits by negative modulators while positive modulators stabilize a roughly parallel tight arrangement of these domains. These respective motions may play an important role in the transition between the open-channel and closed-channel states of the receptor. In addition, some features of the negative modulator binding site found by means of the molecular dynamics study and pocket analysis can be used in the rational design of the allosteric NMDA receptor modulators.

1. Introduction

NMDA receptors are integral membrane proteins that act as cation channels in central nervous system selectively activated by *N*-methyl-D-aspartate. Together with kainate and amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors, they form a large family of glutamate-gated ion channels [1]. One of the driving forces of research in this area is the involvement of the NMDA receptor in acute neuronal death induced by its hyperactivation by excessive glutamate release during brain injuries [2]. Some data reveals NMDA receptor involvement in the development of neuropathic pain [3]. In addition, it can be used as a target for the treatment of major depression [4], Alzheimer's [5] and Parkinson's [6] diseases. They also play an important role in processes of learning and memory regulation.

These receptors occur in the organism as heterotetramers formed by two GluN1 (NR1) subunits and two GluN2 or GluN3 (NR2 or NR3) subunits. Four subtypes of the GluN2 subunit (GluN2A–GluN2D) differ in their localization and physiology [7,8]. Each subunit has a modular structure, consisting of four separate domains: amino-terminal domain (ATD), ligand-binding domain, participating in interactions with naturally occurring agonists, transmembrane domain forming an ion channel, and intracellular C-terminal domain [9]. Activation of the receptor and the cation current through the membrane are caused by simultaneous binding of glutamate and glycine in the ligand-binding core. The influx of positively charged particles induces membrane depolarization and signal transduction. At the same time, increase of calcium concentration in neurons triggers various signaling pathways.

A number of sites are known in the receptor where binding of small molecules can influence the opening of the ion channel. Among them are the direct channel blocking site [10], competitive antagonist binding sites [11,12], amino-terminal domain modulatory sites [13], and a newly discovered noncompetitive allosteric antagonist binding site formed by the loops connecting transmembrane and ligand binding domains [14]. In this paper our attention is focused on the ligands of the amino-terminal domains. One of their interesting features is a strong selectivity of the negative modulators such as ifenprodil (**1**) for the GluN2B subunit type that can minimize the side effects of their clinical use [15,16]. On the other hand, amino-terminal domains of GluN2A and GluN2B are sensitive to polyamine compounds such as spermidine (**2**) and spermine (**3**).



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Abbreviations: NMDA, *N*-methyl-D-aspartate; AMPA, amino-3-hydroxy-5-methyl-4-isoxazolepropionate; ATD, amino-terminal domain.

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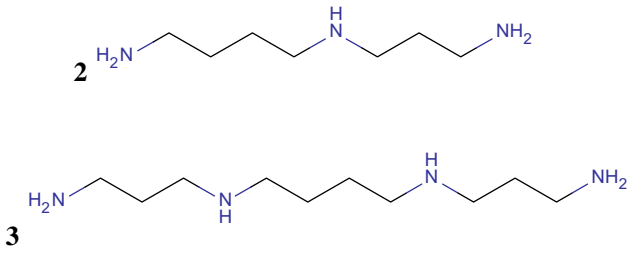
http://dx.doi.org/10.1016/j.bbrc.2012.07.009



Great efforts have been made in order to find out the mechanism of binding of these allosteric modulators. Mutagenesis studies revealed some crucial residues responsible for ligands binding [17]. On the other hand, relatively high sequence similarity with ligand binding domain of metabotropic glutamate receptor $mGluR_1$ and soluble bacterial periplasmic proteins was observed, and a number of homology modeling studies were performed [18,19]. Unfortunately, when the crystal structure of the GluN1/GluN2B complex with ifenprodil was obtained, significant differences from the homology models were found [20]. In the work [21] a general organization of the polyamine bound state was proposed. In the present paper we aim to find out the nature of binding of the amino-terminal domain modulators and possible mechanism of their action using the molecular dynamics study of GluN1/GluN2B complex with ifenprodil and homology modeling of polyamine binding site.

2. Materials and methods

Three dimensional structure of GluN1/GluN2B complex was taken from Protein Data Bank (PDB ID: 3QEL) [20]. All molecular dynamics (MD) simulations were performed using Amber 11 suite [22]. Force field parameter set for protein and ligand atoms was derived from FF10 and GAFF. Ifenprodil was used in the protonated state, charges on ligand atoms were calculated using the semi-empirical AM1BCC method. Two systems were subjected to simulation: the ligand-bound and unbound forms of the GluN1/GluN2B complex. All structures were solvated then placed into truncated octahedron and surrounded by a 12 Å water layer. Sodium atoms were added to achieve charge neutrality. The simulation protocol included the following steps: (1) 500 steepest descent minimization steps and 500 conjugate gradient steps were applied to each system; (2) 50 ps of constant volume simulation were performed to increase temperature to 300 K; (3) 50 ps of constant pressure simulation were performed in order to drive the system density to the equilibrium state. Harmonical restraints were applied to protein and ligand atoms with 10 kcal/mol value during the last two stages; (4) after removal of the restraints, a 25 ns MD simulation was performed. The constant temperature was maintained using the Langevin thermostat. The integration step value of 2 fs was chosen in combination with SHAKE algorithm.

Visualization was provided by the VMD software [23] as well as by SYBYL-X 1.3 program package [24]. Binding pocket characteristics were evaluated with MDpocket [25] using a standard parameter set for the cavity detection. Hydrogen bond analysis was provided by Hbonanza [26], which facilitates its visualization in VMD.

Homology modeling was carried out with Modeller 9.8 [27] based on the alignment built using ClustalX [28]. X-ray structure of the dimeric amino-terminal domain of the AMPA receptor (PDB ID: 3KG2) [9] was used as a template. Fifty models were built by a standard automodel protocol, and the best one was selected for further analysis.

3. Results and discussion

Molecular dynamics simulations of the GluN1/GluN2B amino-terminal domain complex revealed certain differences in the behavior of its ligand-bound and unbound forms. In an unbound form the two lobes of the GluN2B domain are rotated with respect to each other. This rotation can be tracked using a dihedral angle between four residues from different lobes (Asn184, Leu229, Gly125, Thr119). As can be seen from Fig. 1, by 25 ns of the simulation the movement of the GluN2B part in unbound state comes to stability with the dihedral angle about 82°, while in the complex with negative modulator ifenprodil the angle changes only slightly around 68°. Thus, the negative modulator binding involves significant inter-lobe movement.

Taking into account the experimental evidence that zinc and ifenprodil can compete with each other [29], we also analyzed possible influence of ifenprodil on the zinc binding site. Distance between α-carbon atoms of His127 (GluN2B) and Glu284 (GluN2B), which are known to be involved in zinc binding, was tracked over the trajectory. The binding of ifenprodil increases the average distance between these residues from 8.7 to 9.9 Å. In the crystal structure of zinc-receptor complex (PDB ID: 3JPY) this distance is 7.0 Å

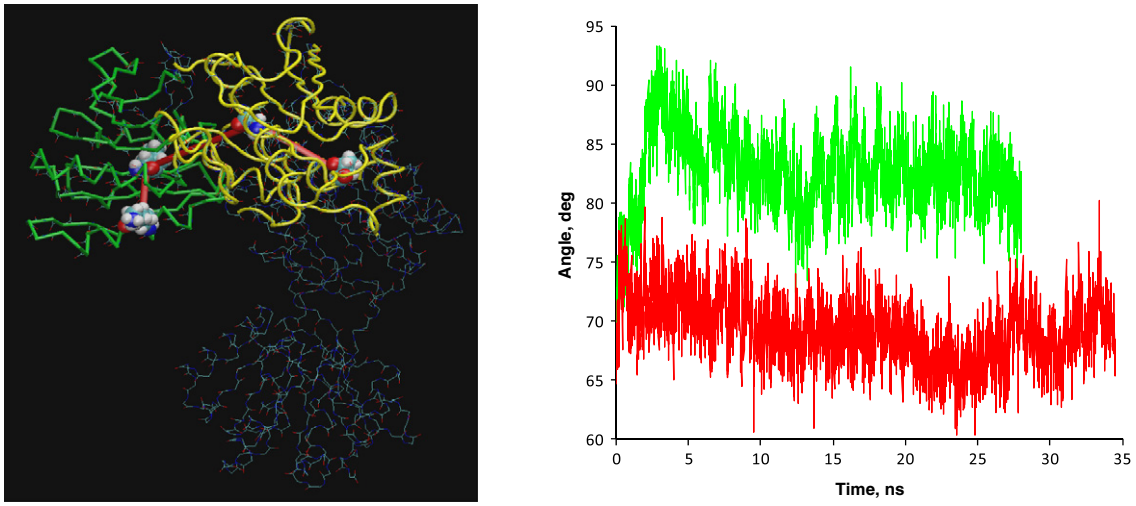


Fig. 1. (Left) Dihedral angle formed by the GluN2B residues (left to right) Asn184 (1), Leu229 (2), Gly125 (3), Thr119 (4). (Right) Evolution of the dihedral angle value over the simulation trajectory for unbound (green, black in print version) and ligand-bound (red, grey in print version) receptor forms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

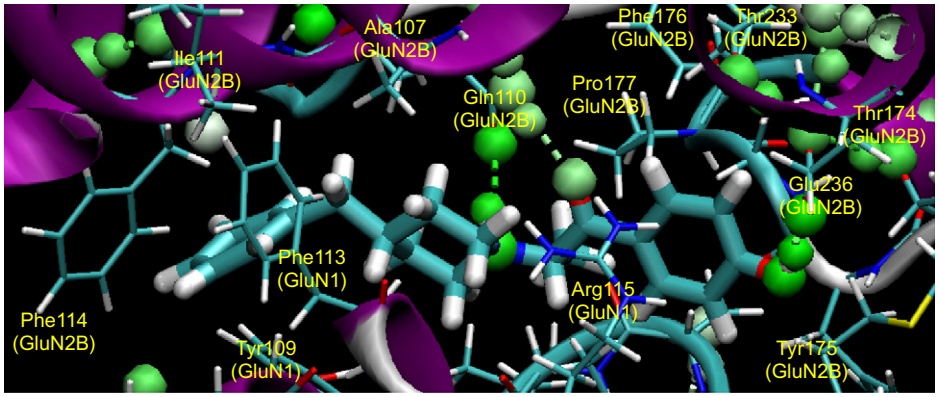


Fig. 2. The scheme of ifenprodil binding site with hydrogen bonds detected from the MD trajectory. The intensity of hydrogen bonds coloring reflects the frequency of their appearance in the trajectory.

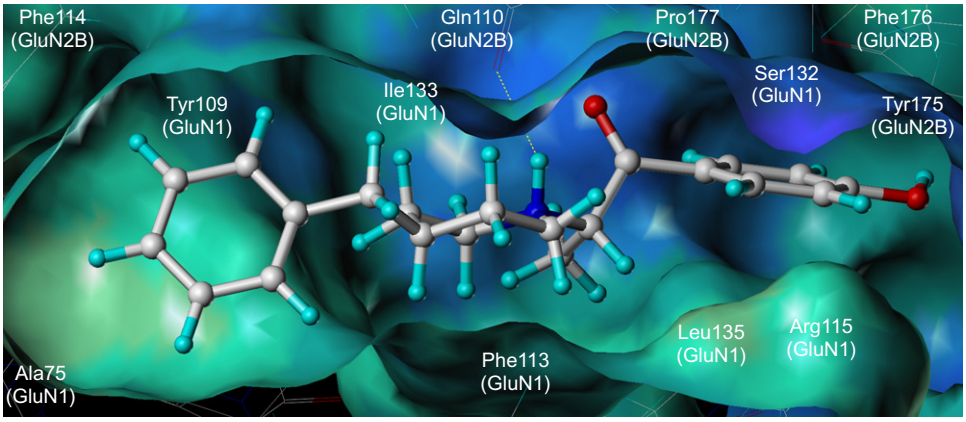


Fig. 3. Position of ifenprodil molecule in the binding cavity between GluN1 and GluN2B subunits. The Connolly surface of the protein is colored by electrostatic potential.

[30]. This fact indicates that ifenprodil binding and ATD internal rotation can affect the zinc binding site.

Analysis of the hydrogen bonds behavior (Fig. 2) shows that a bond between protonated (positively charged) ifenprodil nitrogen and Gln110 (GluN2B) carbonyl oxygen exists over the whole trajectory. In addition, phenolic hydroxyl group of ifenprodil binds to the side-chain carboxyl group of Glu236 (GluN2B). This residue along with Thr174 (GluN2B) and Thr233 (GluN2B) serve as key parts of a hydrogen bonding grid that links the elements of the secondary structure. In an unbound receptor state this grid is broken, leading to the formation of a salt bridge between Glu236 (GluN2B) and Arg115 (GluN1).

Analysis of the cavities present during the simulation reveals a hydrophobic pocket formed by the residues Phe114 (GluN2B), Tyr109 (GluN1) and Ala75 (GluN1) that is partially filled by the distal phenyl group of ifenprodil (Fig. 3). Indeed, ifenprodil analogues with longer linker between phenyl ring and a positively charged moiety show similar activity [31]. Two other interesting pockets are close to the piperidine and phenolic moieties.

The model of the polyamine binding site constructed by homology with the amino-terminal domains of AMPA receptor indicates that the residues known to participate in binding are indeed positioned in close proximity and can act as a binding site. The majority of the hydrophobic surface residues are localized in the dimer interface. The distance between α-carbon atoms of residues Glu181 (GluN1) and Glu201 (GluN2B) was found to be 8.8 Å, which is consistent with the experimental data [20]. It should be noted that the structures of the amino-terminal domains within a dimer differ from the crystal structures of the free GluN1 and GluN2B domains deposited in PDB. As we have shown above, the inter- and intra-subunit rotation may occur under certain conditions. This structure can be used as a rough model of polyamine-stabilized state of amino-terminal domains.

In both ligand-bound and unbound forms of the amino-terminal domains, the contact surface between the subunits is rather hydrophobic and unlikely to be exposed to water medium. However, sliding and twisting motions of the subdomains are possible that can achieve a conformational "induced fit". As shown earlier [20], the positive ATD modulators such as polyamines stabilize the roughly parallel tight arrangement of the GluN1 and GluN2B subunits by shielding the negative charges in the interface. Based on the results presented here, we believe that negative modulators such as ifenprodil act in the opposite way, by stabilizing the twisted closed-clamshell configuration of the ATD subunits. These respective motions may play an important role in the transition between open and closed states of the receptor. In addition, some features of the negative modulator binding site found by means of the molecular dynamics study and pocket analysis can be used in the rational design of the allosteric NMDA receptor modulators.

Acknowledgment

This work is supported by the grant from the Government of Russian Federation (Resolution No. 220 dated 09.04.2010).

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