Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular Graphics and Modelling 23 (2005) 381–388
Journal of Molecular Graphics and Modelling
www.elsevier.com/locate/JMGM

The function of the amino terminal domain in NMDA receptor modulation

David J. Huggins, Guy H. Grant*

Department of Chemistry, Physical and Theoretical Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QZ, UK

Received 10 May 2004; received in revised form 25 October 2004; accepted 2 November 2004
Available online 16 December 2004

Abstract

N-methyl-D-aspartate (NMDA) receptors are ligand-gated channels important in neurotransmission which are activated by the combined presence of glutamate and glycine. They are comprised of four subunits that form a dimer of dimers. The activity of NMDA receptors is modulated by a variety of endogenous ligands such as zinc ions, phenylethanolamines, polyamines and protons. Findings show that the binding sites for these modulators are found in the amino terminal domain of such receptors, but different modulators appear to affect different subunits. However, despite the enormous efforts expended in mutagenesis and patch clamp experiments on NMDA receptors, the exact assembly of these subunits and the effects of the modulatory species are not well understood.

We have modelled dimers of the amino terminal domains of these receptors based on their homology with the extracellular dimer of a metabotropic glutamate receptor. Conserved cysteine residues, which have been highlighted as important in previous work, are shown to form a disulphide bridge, stabilizing a four-helix bundle between subunits. This establishes a hinge in the receptor. The model also highlights a zinc binding site in the binding crevice of the NR2a subunit of the receptor that stabilizes the open state of the amino terminal domain. The similar effect of ifenprodil is thus explained by its stabilization of the open state of the amino terminal domain (ATD). The presence of three histidine residues in the zinc site is used to explain the pH dependence of zinc inhibition. Previous work has also implicated certain residues in spermine stimulation of such receptors. The homology model shows that this site is found at the inter-subunit boundary of the dimer. This predicts a binding site between subunits, a result not calculable by the homology modelling of single subunits done previously. Finally, these results are drawn together to yield a consistent picture of NMDA receptor activation and desensitization. An understanding of how these receptors work and how they can be modulated is an important step toward rational drug design.

Keywords: Glutamate; Receptors; NMDA; Glycine; Zinc; Spermine; Desensitization; Docking; Homology modelling; Inhibition

1. Introduction

Mammalian ionotropic glutamate receptors (GLRs) are multimeric, ligand-gated channels, comprised of four or five subunits, that function to perceive glutamate during neurotransmission [1]. Binding of glutamate to the ligand binding domain of these receptors results in gating of the trans-membrane channel, allowing cations to enter the cell. Based on their pharmacological properties and structural similarities, three different groups of mammalian ionotropic GLRs (AMPA, NMDA and kainate receptors) have been identified. *N*-methyl-D-aspartate (NMDA) receptors are ionotropic glutamate receptors that are activated by the combined presence of glutamate and glycine. They are found in the brain and are responsible for neuronal communication. Their structure is thought to be a dimer of dimers, with four subunits assembling to make a channel through the membrane. These multimeric structures are composed of assemblies of three different classes of subunits, NR1–NR3 [2], with the combination of subunits determining the specific properties of the receptor. The ligand binding domains are known to bind glycine in the

* Corresponding author. Present address: Unilever Centre for Molecular Informatics, The University Chemical Laboratory, Cambridge University, Lensfield Road, Cambridge CB2 1EW, UK. Tel.: +44 1223 963 981; fax: +44 1223 763 076.
E-mail address: ghg24@cam.ac.uk (G.H. Grant).

1093-3263/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmgm.2004.11.006

case of NR1 and NR3 subunits and glutamate in the case of NR2. The majority of receptors are composed of a combination of NR1 and NR2 subunits and thus require both glutamate and glycine to allow channel opening. However, some receptors are composed of NR1 and NR3 subunits and require only glycine. Each subunit contains three trans-membrane helices, one membrane-embedded helix, one bi-lobed domain that forms the ligand binding site and one bi-lobed amino terminal domain, thought to be homologous to LIVBP [3] (Fig. 1).

The activity of NMDA receptors is modulated by a variety of endogenous ligands such as zinc ions [4], polyamines [5] and protons [6]. It is thought that the binding sites for these modulators are found in the amino terminal domain of such receptors, but it is not known exactly where they are or how they work. There are also a large number of modulatory species that affect the different subunits. However, despite the enormous efforts in mutagenesis and patch clamp experiments on NMDA receptors, the exact assembly of these subunits and the effects of the modulatory species are not well understood.

NMDA receptors are tonically inhibited by protons. At physiological pH they may be inhibited by as much as 50%. The presence of the NR1a subunit seems to be vital for this effect, as receptors containing the NR1b subunit are not affected. This seems to be due to the presence of an extra exon (exon 5) in the gene for the amino terminal domain of the NR1b subunit. This extra segment contains an insert of approximately 50 residues and is thought to relieve pH inhibition [6]. Acidic residues [7] and histidine residues [8] in the amino terminal domain (ATD) have been implicated in the mechanism of proton inhibition.

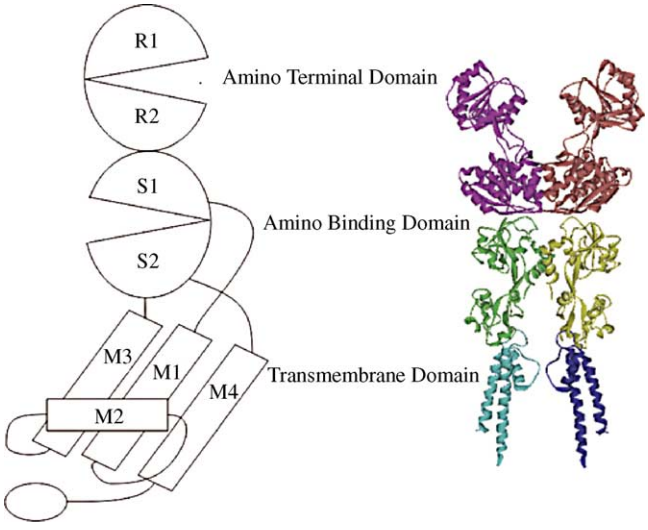

Fig. 1. The proposed topology of NMDA receptors: (a) conceptual format, (b) an amalgamation of the structures of a potassium channel (PDB ID 1BL8), a GLR glutamate binding region (PDB ID 1FTJ) and LIVBP (PDB ID 2LIV). There is a large amino terminal domain (R1R2), a large ligand binding domain (S1S2), three trans-membrane helices (M1, M3, M4) and a re-entrant loop (M2).

Polyamines such as spermine relieve the pH inhibition of NMDA receptors and restore normal function. However, only receptors containing the NR1a subunit are affected. NR1b subunits, containing the extra segment, are unaffected. Polyamines have been predicted to act by mimicking the action of the exon 5 insert [6], which, like spermine, is known to contain many positively charged residues. Mutation of acidic residues in this region shows that the polyamine binding site is in the amino terminal domain of NMDA receptors [7]. Evidence suggests polyamines may have a number of effects on NMDA receptors and that there may be more than one polyamine binding site [6].

Amino-glycoside antibiotics are known to increase the action of NMDA receptors and it has been posited that this occurs by binding to a spermine binding site and thus relieving pH inhibition [9] (amino-glycosides contain a number of amino groups with a similar spacing to those in polyamines). Only receptors containing the NR1a/NR2b combination of subunits are affected by amino-glycosides.

Zinc ions inhibit receptors containing the NR2a or NR2b subunit [4]. It is not known how zinc ions inhibit these receptors. However, for NR2a subunits this inhibition is intensified at low pH. This has led to the idea that zinc ions act by enhancing proton inhibition [8]. Mutagenesis data shows that the zinc binding site is in the amino terminal domain [10].

Phenylethanolamines such as ifenprodil inhibit NMDA receptors in a pH dependent manner leading to the suggestion that they act in a similar way to zinc, by enhancing proton inhibition [11]. However, ifenprodil affects only those receptors containing the NR2b subunit. Mutagenesis data shows that the ifenprodil binding site is in the amino terminal domain [12].

The large number of modulatory ligands acting at NMDA receptors, combined with the lack of understanding in how ligand binding couples to channel opening, means that the mechanism of action of each ligand is difficult to explain. However, it is useful to note that all of these modulatory ligands are thought to act in the amino terminal domain (ATD) of NMDA receptors. The ATD of such receptors has not been studied in as much detail as the downstream glutamate binding region, despite the fact that it is larger and appears to have an interesting biochemistry.

The ATDs of NMDA receptors have not been crystallized but it has been noted that there is homology with other periplasmic binding proteins such as leucine/isoleucine/valine binding protein (LIVBP) [3] and polyamine binding protein (PotD) [13] These proteins are composed of two domains (R1 and R2) and have open and shut conformations (agonist bound and unbound). The movement of these domains produces agonist bound and unbound configurations, facilitating the binding of modulatory ligands in the ATD.

The glutamate binding domain of NMDA receptors is thought to be homologous with periplasmic binding proteins