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The function of the amino terminal domain in NMDA receptor modulation

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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

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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.

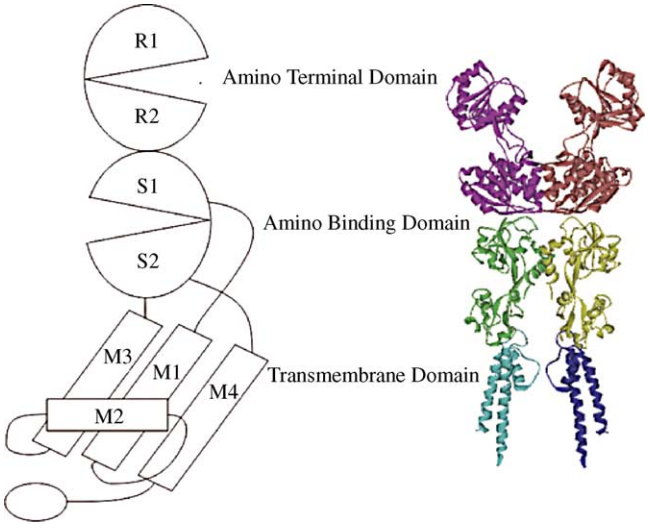

Fig. 1. The proposed topology of NMDA receptors: (a) conceptual format, (b) an amalgamation of the structures of a potassium channel (PDB ID 1BL8), a GLR glutamate binding region (PDB ID 1FTJ) and LIVBP (PDB ID 2LIV). There is a large amino terminal domain (R1R2), a large ligand binding domain (S1S2), 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

such as lysine/arginine/ornithine binding protein (LAOBP) and glutamine binding protein (QBP) [14]. These proteins are also composed of two domains, an upper (S1) and a lower (S2). Results from the recent crystal structure studies of the glutamate binding regions of the NMDA NR1a subunit [15] and a similar protein, the glutamate receptor GluR2, strongly support this theory [16,17].

Recently, the assembly of two of the GluR2 glutamate binding regions of has been elucidated by X-ray diffraction [18]. Each subunit is composed of an upper (S1) and lower (S2) portion. In the closed state, two subunits associate to form a dimer, with two helices from the S1 domain of each subunit contributing to form a four-helix bundle (Fig. 1). Results also determine two other accessible states, an open state and a desensitized state. Opening of the channel involves the closure of the S1S2 cleft to bind glutamate with the retention of this interface: the S2 lobes of the subunits swing out and away from one another, pulling apart the helices that block the channel, producing an open state. Desensitization involves closure of the S1S2 cleft to bind glutamate and breaking of the S1–S1 domain interaction. This leaves the gate closed but with glutamate bound to the receptor.

2. Results and discussion

2.1. Homology with a metabotropic glutamate receptor (mGluR1)

Crystallisation of the glutamate binding region of the NR1a subunit did not yield a dimer and this could suggest that this domain does not participate in the dimer interface. However, it has been shown that it is the ATD that is vital in functional assembly of AMPA and kainate receptors [19] and that protein–protein interactions in both the S1S2 and ATD domains are important for subunit assembly [20]. Thus, the ATD seems vital for dimerisation of NMDA receptor subunits. In fact, the model of desensitization proposed by Sun et al. [18] states that the S1 lobes are in close proximity in the closed state. Thus, the R2 lobes, which are linked to the S1 lobes, would also be in close proximity for the closed state.

Sequence analysis highlights two regions of the ATD that correspond to two helices in mGluR1 and ANP-C that form a four-helix bundle between two subunits (Fig. 2). Residues in these regions show very high sequence similarity between NR2a and NR2b but these are very different from NR1a. This means that complementary subunits would have to combine to produce a functioning receptor, as is found in nature. The model predicts that an NMDA dimer has a structure in which there are protein–protein interactions linking both the S1 and R1 domains. The ATDs would thus be linked together in an analogous way to the S1S2 glutamate binding region. Further evidence comes from mutagenesis data on cysteine residues [21]. A cysteine residue in the ATD has been implicated in zinc inhibition but the results show that it modulates the zinc effect without binding to zinc. For an NMDA–ATD dimer, these cysteine residues are located on helices that form the four-helix bundle and the equivalent residues for the mGluR1 dimer are in close proximity (See Fig. 2). This strongly suggests that a cysteine bridge links the two subunits at this point. Sequence analysis reveals that this residue is conserved across all NMDA receptor subunits and also across the AMPA and kainate receptors studied (data not shown). The two subunits of the mGluR1 dimer are also connected by a cysteine bridge, but at slightly different points in the equivalent domains.

To elucidate the function of the ATD of these receptors, a homology model was created. Recently, ifenprodil binding has been explored by modelling of the NR2b subunit based on the structure of LIVBP [12]. This work highlights homology not only with LIVBP, but also with the extracellular domain of the metabotropic glutamate receptor mGluR1 and a hormone binding protein, ANP-C. Both proteins occur naturally as dimers and the extracellular domain of mGluR1 [22] and ANP-C [23] have been crystallized as dimers. Models for the open and closed forms of the NR1a/NR2a and NR1a/NR2b dimers were constructed, exploiting their homology with the mGluR1 dimer (Fig. 3). The insertions found in mGluR1 with respect to LIVBP were removed, as they are not believed to be present in NMDA receptors.

2.2. A zinc site in the binding crevice

The residues implicated in zinc binding [10] are found in the binding crevice of the NR2a subunit. Mutagenesis data implicates residues from both the R1 and R2 domains. This implies that zinc binds to the closed state of the ATD. However, in the closed state Lysine 233 in the R2 domain (highlighted in blue in Fig. 2), which is implicated in zinc binding [24], appears to interact with five residues from the R1 domain that are also involved in zinc binding (histidine 42, histidine 44, aspartate 102, aspartate 105 and histidine 128) (highlighted in blue in Fig. 2 and shown in Fig. 4a and b). It seems likely, therefore, that domain opening would remove the Lysine from this site and allow zinc to bind in its place. Zinc and ammonium ions are of a similar size (88 and 125 pm) and both would bind readily to this site: zinc due entirely to an electrostatic interaction and ammonium due to a combination of electrostatic interaction and hydrogen bonding. Thus, a desensitized state is likely to involve an open configuration of the ATD with a zinc ion bound to the R1 domain. Analysis of the energy minimised structure shows that in the open state this Lysine residue forms an interaction with the Glutamate 266 residue from the R2 domain. This glutamate is the final residue implicated in zinc binding [24], completing the model (highlighted in blue in Fig. 2 and shown in Fig. 4c and d).

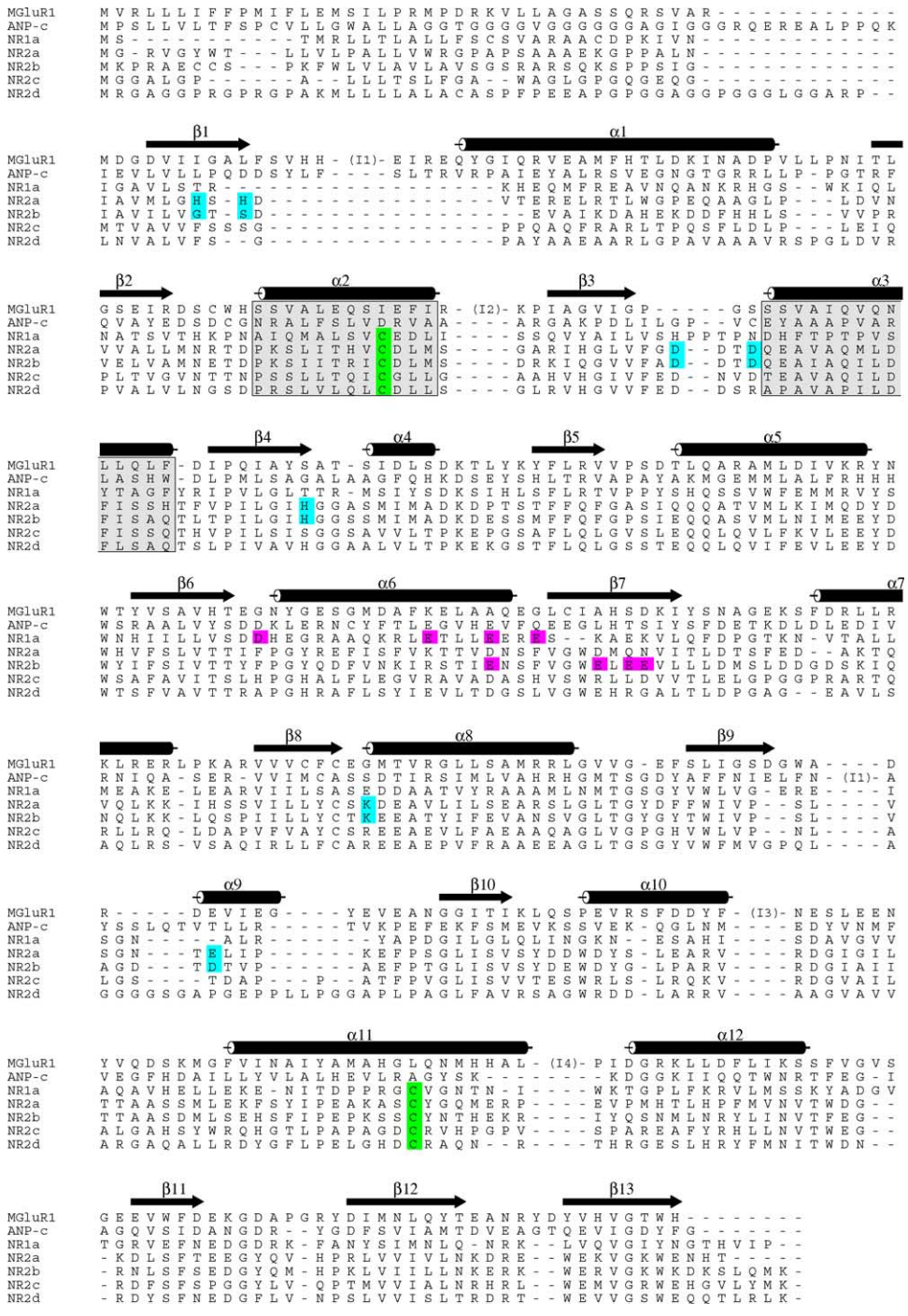

Fig. 2. Multiple sequence analysis of the ATD of NMDA receptor subunits and mGluR1 and ANP-C. The regions that are known to form a four-helix bundle between adjacent subunits are highlighted in the grey box. Residues implicated in zinc binding are highlighted in blue. Cysteine residues implicated in zinc binding are highlighted in green. Residues implicated in spermine binding are highlighted in lilac.

2.3. Binding of ifenprodil

The residues implicated in ifenprodil binding [12] are found inside the binding crevice of the NR2b subunit. Residues from both the R1 and R2 domains seem to be important for binding. This suggests that ifenprodil would bind to the closed state of the ATD. However, given the similar effects of zinc and ifenprodil, it seems highly likely that ifenprodil in fact binds to the open state of the ATD and thus stabilizes the desensitized state. Again, there is a binding site for a lysine residue (lysine 234) in the R1 domain and the equivalent residues are implicated in this interaction. However, two of the histidine residues have been replaced by an aspartate residue in the case of the NR2b subunit and the propensity of zinc to bind with N-donors might explain why zinc does not bind as readily in this case.

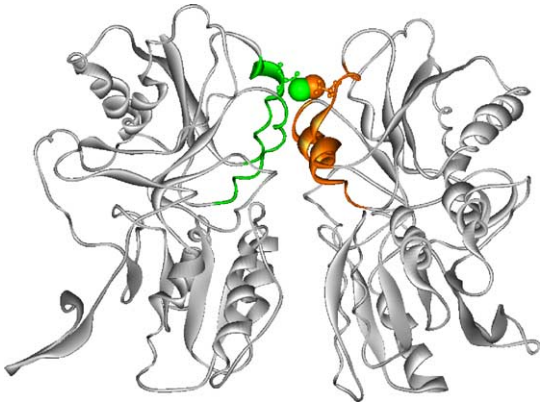

Fig. 3. Proposed structure of the extracellular dimer of an NMDA receptor highlighting the positions of the four-helix bundles (green and orange) and the disulphide bridge (space filling) that may help to stabilize this structure.

The relatively long time (in comparison to zinc) taken for ifenprodil to exert its influence after release begins, and to stop acting after release ends [12], is attributable to its large size and thus its relative inability to enter and leave the binding site. Residues in the NR1 subunit have also been implicated in ifenprodil binding [7]. However, they are all near the NR1–NR2 interface and proximal to the NR2 binding crevice and thus this does not conflict with the model.

2.4. A spermine binding site between two subunits

The residues from NR1a that have been implicated in spermine binding [7] are found not in the binding crevice but on a helix in the R2 lobe on the side of the subunit. The position of the exon 5 insertion is also found in this region.

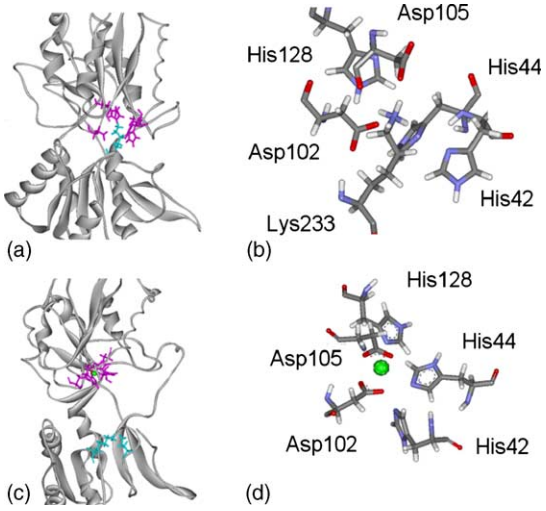

Fig. 4. Homology model of the proposed zinc binding site found in the ATD of an NR2a subunit. (a) In the closed state, the interaction between the lysine 233 in blue and the binding site in pink holds the domains together. (b) In the closed state, lysine 233 in the R2 domain binds to histidine 42, histidine 44, aspartate 102, aspartate 105 and histidine 128 in the R1 domain. (c) In the open state, lysine 233 interacts with glutamate 266 (both in blue) on the opposite side of the cleft, keeping the domains apart. (d) In the open state, the zinc ion is bound to the same five residues in the R1 domain.

Sequence analysis highlights a large number of acidic residues at the interface between the subunits (Fig. 2). These negatively charged residues on both sides would give rise to electrostatic repulsion and destabilize the dimer interface. Spermine could bind to these residues between the R2 lobes of NR1 and NR2 subunits, neutralising the interface between them and thus stabilizing the open and closed states of the receptor relative to the desensitized state (in which the interface has been moved due to rearrangement). However, if residues in the NR2 subunit were important in spermine binding then mutagenesis data should highlight this. Little mutagenesis of acidic residues has been done for NR2 subunits but it has been shown that mutation of E191, E198 and E200 and E201 in the NR2b subunit affect the action of spermine [25]. These residues can be found at the inter-subunit boundary of the dimer, in close proximity to the

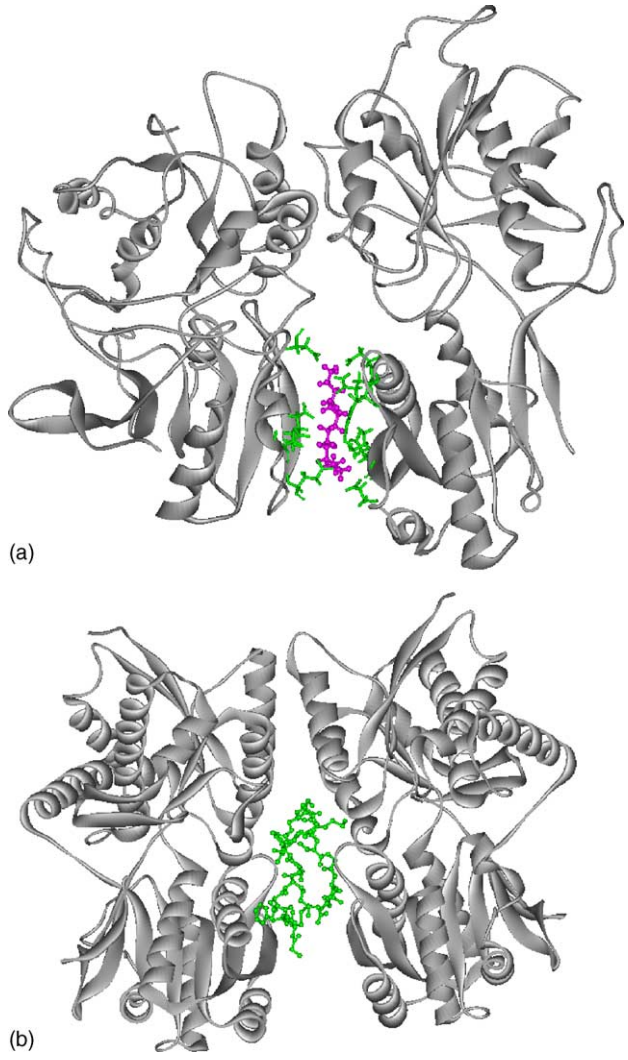

Fig. 5. Homology model of the proposed spermine binding site found at the interface between the NR1a and NR2b subunits. (a) Residues D169, E181, E185 and E192 in the NR1a subunit and residues Y174, Q180, D181, E200, V202 and D211 in the NR2b subunit are displayed in green and the spermine is displayed in pink. (b) The crystal structure of ANP-C in green with the hormone bound between subunits in an analogous fashion.

helix controlling spermine action in the NR1a subunit. It is thus likely that the spermine binding site is found between the subunits (see Fig. 5a). Further mutagenesis studies on residues in this area would help to confirm or reject this idea.

The presence of a ligand-binding site between subunits is also found in the hormone binding protein ANP-C, which has homology with NMDA receptors (see Fig. 5b). Interestingly, the implicated residues from the NR2b subunit are not present in the NR2a subunit. However, it has been suggested that glycine independent spermine stimulation occurs only for receptors containing the NR2b subunit [5]. Such stimulation increases the magnitude of the current passing through the membrane. The second type of spermine stimulation, glycine dependent, must occur at a different site. This stimulation increases the affinity of the receptor for glycine only, suggesting that the binding site is located entirely on the NR1a subunit.

2.5. Opening, closing and desensitizing dimers

Any movement following glutamate binding must transmit upstream to the ATD. If it is assumed that the ATD has a bi-lobed structure like LIVBP and is approximately the same size (S1–S2 is composed of approximately 320 residues and R1–R2 of approximately 350) then a simple mechanism suggests itself. The cysteine linked four-helix bundle between the R1 domain can act as a hinge for the ATDs, allowing the R2 lobe to open and close whilst the R1 lobes remain in the same position. The ATD is in the closed conformation for the open and closed states of the receptor (see Fig. 6). Spermine stabilizes these states by binding at the interface between subunits, which is otherwise destabilized by electrostatic repulsion. Desensitization leads to the S1 lobe swinging out and away from the body of the receptor and this can be transmitted upstream if this pulls the R2 domain yielding an open configuration for the ATD. Zinc or ifenprodil bind to the R1 domain of the open ATD and thus stabilize the desensitized state of the receptor (see Figs. 4 and 6). The pH dependence of zinc inhibition of NR2a containing receptors is known to be affected by mutations at the three histidine residues (histidine 42, histidine 44 and histidine 128) [8]. The mechanism described above, combined with this data, would mean that protonation of one or more of these residues increases either the thermodynamic stability of the desensitized state or increases the rate of its formation. The first suggestion seems unlikely. A positive charge on one of these histidines seems more likely to destabilize a zinc bound state due to electrostatic repulsion. The second suggestion has merit. The main barrier to R1R2 domain opening would appear to be the charge separation between the positive lysine 233 residue and the negative Asp102 and Asp105 residues.

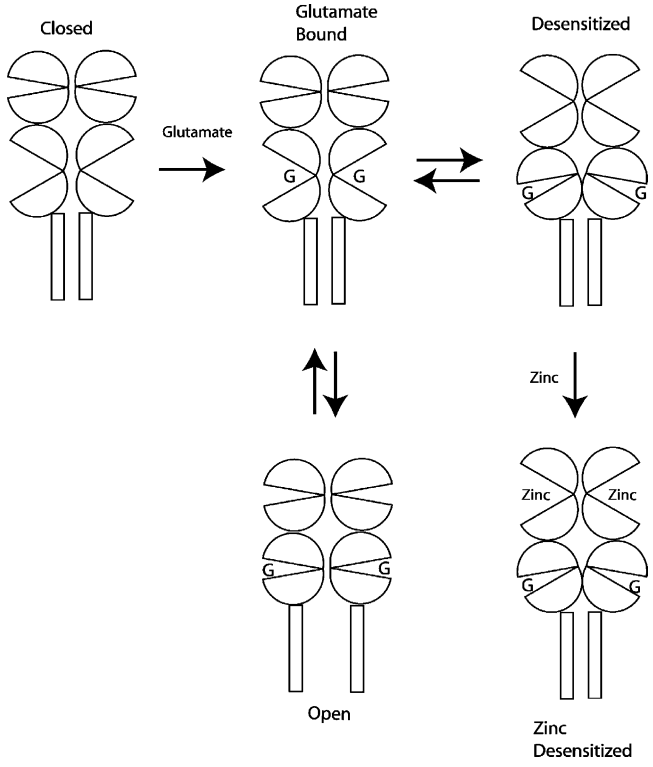

Fig. 6. Proposed states of the NMDA receptor showing the ATD (upper oval), ligand binding domain (lower ovals) and the trans-membrane channel (rectangles). glutamate and/or glycine (G) binding yields a receptor that can transform into two energy minima: an open state in which the ligand binding domain closes (open channel) and a desensitized state, in which the ligand binding domain closes, opening the ATD (closed channel). This second step is proton catalysed. The final step involves zinc binding to the ATD, stabilizing the desensitized state.

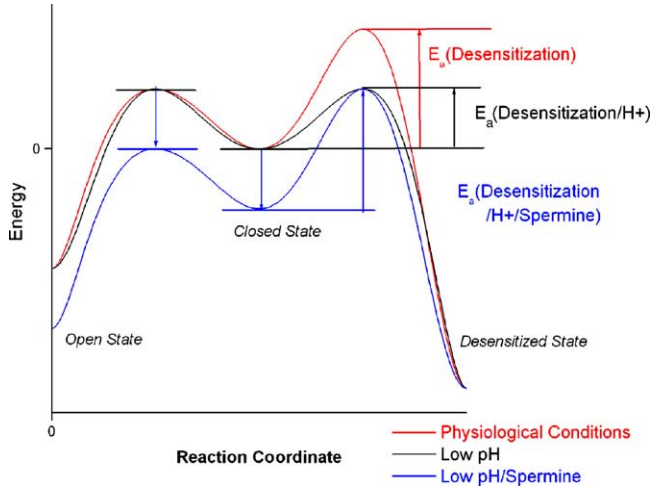

Fig. 7. Hypothetical energy level diagrams of the NMDA receptors. The open and desensitized states lie at lower energies than the closed state but the energy barrier to the desensitized state is greater leading to an initial spike in the open probability of the receptors. Protons lower the activation barrier for transition to the desensitized state and thus inhibit channel opening. Spermine stabilizes the open and closed states of the receptors and thus raises the activation barrier for transition to the desensitized state. This returns normal function to inhibited receptors.

However, if one of the histidines were protonated, the barrier to domain opening would be decreased and the rate would thus increase. This would explain the pH dependence of receptor desensitization. The next step, zinc binding, would then occur by exchange with a bound proton. Effectively, the proton is acting as a catalyst for R1R2 domain opening and lowers the activation barrier for desensitization (see Figs. 6 and 7).

Spermine can then counter this effect by stabilizing the closed state, thus effectively returning the activation barrier to its original value.

3. Summary and conclusions

The elucidation of this method of action has interesting implications. If ifenprodil can bind to the open state of the NR2b subunit, it should be possible to design a ligand to bind in an analogous fashion to the NR2a subunit and perhaps the NR1a, NR2c and NR2d subunits. This work also highlights the importance of examining the complete structure of these receptors and not simply the individual subunits. The spermine binding site at the monomer–monomer interface could not have been found by examining the monomer alone. Indeed, it seems highly likely that another spermine binding site can be found at the dimmer–dimer interface around residues E339 and E342 of NR1a. These residues have been highlighted by mutagenesis as being important for glycine-independent spermine stimulation and are positioned perfectly to interact with another subunit of the tetramer. The structure of this tetramer may also explain the importance of a further pair of conserved cysteine residues (cysteine 308 in NR1a and cysteine 320 in NR2a) which have been implicated in desensitization but are not thought to bind zinc [21]. These residues are found on the surface of the subunits at the apex of the ATD and may combine to form a disulphide bridge linking the two dimers together to yield a functioning tetramer. The stability of the ATD could also be exploited by proteolytically severing the tetrameric ATD head of these receptors, allowing crystallization and analysis of the structure.

4. Computational methods

The crystal structures of the metabotropic glutamate receptor (mGluR1) were obtained from the Protein Data Bank (PDB ID 1EWT for the apo (open) state and 1EWK for the (closed) glutamate-bound state).

The mGluR1 monomer sequence, the ANP-C monomer sequence and the human NMDA sequences were initially aligned using ClustalW [26]. The homology package of Insight II was then used to assign coordinates to the amino acid sequences of an NR1a/NR2a and an NR1a/NR2b dimer, based on the open and closed forms of the mGluR1 structure, yielding four structures (an open and a closed form for both subunit pairs). The models obtained were then refined by CHARMM to optimise the three-dimensional structure [27]. A CHARMM27 forcefield was employed and the minimization was run for 600 steps with all but hydrogens fixed, 600 steps with the protein backbone fixed and 6000 steps with all atoms free.

The relevant ligands were then docked to these structure using the multi-scale docking algorithm [28]. In each case, the ligand–protein complex was refined by CHARMM to optimise the structure after docking was complete [29].

References

[1] S. Nakanishi, Science 258 (1992) 597–603.
[2] S. Das, Y.F. Sasaki, T. Rothe, L.S. Premkumar, M. Takasu, J.E. Crandall, P. Dikkes, D.A. Conner, P.V. Rayudu, W. Cheung, H.S. Chen, S.A. Lipton, N. Nakanishi, Nature 393 (1998) 377–381.
[3] M.J. Sutcliffe, Z.G. Wo, R.E. Oswald, Biophys. J. 70 (1996) MP397–MP397.
[4] N.S. Chen, A. Moshaver, L.A. Raymond, Mol. Pharmacol. 51 (1997) 1015–1023.
[5] K. Williams, A.M. Zappia, D.B. Pritchett, Y.M. Shen, P.B. Molinoff, Mol. Pharmacol. 45 (1994) 803–809.
[6] S.F. Traynelis, M. Hartley, S.F. Heinemann, Science 268 (1995) 873–876.
[7] T. Masuko, K. Kashiwagi, T. Kuno, N.D. Nguyen, A.J. Pahk, J. Fukuchi, K. Igarashi, K. Williams, Mol. Pharmacol. 55 (1999) 957–969.
[8] C.M. Low, F. Zheng, P. Lyuboslavsky, S.F. Traynelis, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 11062–11067.
[9] T. Masuko, T. Kuno, K. Kashiwagi, T. Kusama, K. Williams, K. Igarashi, J. Pharmacol. Exp. Ther. 290 (1999) 1026–1033.
[10] P. Paoletti, F. Perin-Dureau, A. Fayyazuddin, A. Le Goff, I. Callebaut, J. Neyton, Neuron 28 (2000) 911–925.

[11] D.D. Mott, J.J. Doherty, S.N. Zhang, M.S. Washburn, M.J. Fendley, P. Lyuboslavsky, S.F. Traynelis, R. Dingledine, Nat. Neurosci. 1 (1998) 659–667.
[12] F. Perin-Dureau, J. Rachline, J. Neyton, P. Paoletti, J. Neurosci. 22 (2002) 5955–5965.
[13] K. Williams, K. Kashiwagi, J. Fukuchi, K. Igarashi, Mol. Pharmacol. 48 (1995) 1087–1098.
[14] Y. Sternbach, B. Bettler, M. Hartley, P.O. Sheppard, P.J. Ohara, S.F. Heinemann, Neuron 13 (1994) 1345–1357.
[15] H. Furukawa, E. Gouaux, EMBO J. 22 (2003) 2873–2885.
[16] N. Armstrong, E. Gouaux, Neuron 28 (2000) 165–181.
[17] N. Armstrong, Y. Sun, G.Q. Chen, E. Gouaux, Nature 395 (1998) 913–917.
[18] Y. Sun, R. Olson, M. Horning, N. Armstrong, M. Mayer, E. Gouaux, Nature 417 (2002) 245–253.
[19] W.D. Leuschner, W. Hoch, J. Biol. Chem. 274 (1999) 16907–16916.
[20] G. Ayalon, Y. Stern-Bach, Neuron 31 (2001) 103–113.
[21] Y.B. Choi, H.S.V. Chen, S.A. Lipton, J. Neurosci. 21 (2001) 392–400.
[22] N. Kunishima, Y. Shimada, Y. Tsuji, T. Sato, M. Yamamoto, T. Kumasaka, S. Nakanishi, H. Jingami, K. Morikawa, Nature 407 (2000) 971–977.
[23] X.L. He, D.C. Chow, M.M. Martick, K.C. Garcia, Science 293 (2001) 1657–1662.
[24] A. Fayyazuddin, A. Villarroel, A. Le Goff, J. Lerma, J. Neyton, Neuron 25 (2000) 683–694.
[25] M.J. Gallagher, H. Huang, E.R. Grant, D.R. Lynch, J. Biol. Chem. 272 (1997) 24971–24979.
[26] J.D. Thompson, D.G. Higgins, T.J. Gibson, Nucleic Acids Res. 22 (1994) 4673–4680.
[27] B.R. Brooks, R.E. Bruccoleri, B.D. Olafson, D.J. States, S. Swaminathan, M. Karplus, J. Comput. Chem. 4 (1983) 187–217.
[28] M. Glick, G.H. Grant, W.G. Richards, J. Med. Chem. 45 (2002) 4639–4646.
[29] B.R. Brooks, R.E. Bruccoleri, B.D. Olafson, D.J. States, S. Swaminathan, M. Karplus, J. Comput. Chem. 4 (1983) 187–217.