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REVIEW

Pharmacological insights obtained from structure–function studies of ionotropic glutamate receptors

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Ionotropic glutamate receptors mediate the vast majority of fast excitatory synaptic transmission in the CNS. Elucidating the structure of these proteins is central to understanding their overall function and in the last few years a tremendous amount of knowledge has been gained from the crystal structures of the ligand-binding domains of the receptor protein. These efforts have enabled us to unravel the possible mechanisms of partial agonism, agonist selectivity and desensitization. This review summarizes recent data obtained from structural studies of the binding pockets of the GluR2, GluR5/6, NR1 and NR2A subunits and discusses these studies together with homology modelling and molecular dynamics simulations that have suggested possible binding modes for full and partial agonists as well as antagonists within the binding pocket of various ionotropic glutamate receptor subunits. Comparison of the ligand-binding pockets suggests that the ligand-binding mechanisms may be conserved throughout the glutamate receptor family, although agonist selectivity may be explained by a number of features inherent to the AMPA, kainate and NMDA receptor-binding pockets such as steric occlusion, cavity size and the contribution of water-bridged interactions.
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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CTZ, cyclothiazide; GluR, glutamate receptor; NMDA, *N*-methyl-D-aspartate; NR, *N*-methyl-D-aspartate receptor

Introduction

Elucidating the structure of ionotropic glutamate receptors is fundamental to our understanding of their function and although this is not as well developed as for other ionotropic receptors (e.g. nicotinic acetylcholine receptors; Unwin, 2003; Colquhoun & Sivilotti, 2004), a major breakthrough was achieved with the first crystal structure of the GluR2 subunit glutamate-binding pocket and the subsequent structures of the NMDA NR1 and NR2A ligand-binding regions by Gouaux and co-workers (Armstrong *et al*., 1998; Armstrong & Gouaux, 2000; Furukawa & Gouaux, 2003; Furukawa *et al*., 2005). Not only have these studies given us a visual scheme of how these receptors may operate but in some cases have provided insights into how agonist binding may lead to receptor gating. Structural studies have triggered a flurry of activity in an attempt to relate how the initial binding of the agonist is transduced into the opening of the ion pore and we are now able to suggest how full and partial agonists may act and furthermore a structural basis for ligand selectivity at glutamate receptor subtypes can be proposed.

Excitatory synaptic transmission in the mammalian CNS is mainly mediated by the amino acid, L-glutamate acting on three classes of ionotropic glutamate receptors (iGluRs) – α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate and *N*-methyl-D-aspartate (NMDA) receptor-channels. AMPA receptors can exist as either homomeric or heteromeric assemblies of GluR1–4 receptor subunits, whereas kainate receptors are homomeric or heteromeric assemblies of GluR5–7 and KA1 and KA2 subunits – these latter two subunits are incapable of forming channels (Herb *et al*., 1992) and need to assemble with GluR5–7 to form a functional receptor-channel. The NMDA receptor is unique among the glutamate receptors in that it requires the binding of both glutamate and the co-agonist glycine to the receptor for the channel to open. Glycine binds to NR1 subunits while glutamate binds to NR2A-D subunits. NMDA receptors can also contain NR3A or NR3B subunits that modulate channel function (Perez-Otano *et al*., 2001). The NMDA receptor has been intensively studied not only as it mediates the slow component of the glutamatergic excitatory postsynaptic current (EPSC) but also because it is proposed to have several roles in other physiological and pathophysiological processes in the CNS (Dingledine *et al*., 1999; Hardingham & Bading, 2003; Collingridge *et al*., 2004; Erreger *et al*., 2004).

Ionotropic glutamate receptor topology

Ionotropic glutamate receptors are thought to be tetrameric (see below) and the individual subunits making up the macromolecular complex are comprised of distinct functional regions (Figure 1a). An amino terminal domain (ATD) is the site of action for a number of molecules that modulate glutamate receptor function (Herin & Aizenman, 2004) and structure–function studies have shown that this region is also

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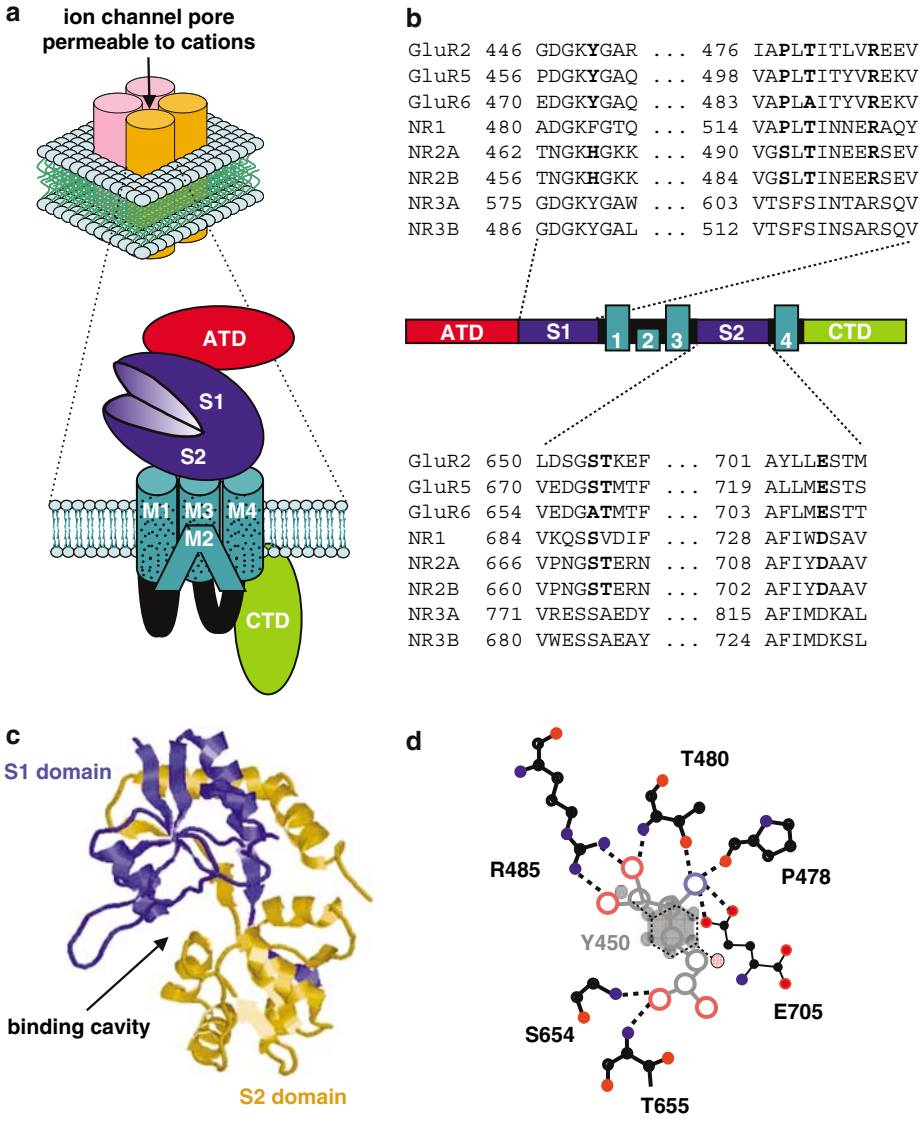

Figure 1 (a) Proposed iGluR structure and topology. iGluRs are thought to exhibit a tetrameric stoichiometry in a 'dimer of dimers' configuration. NMDARs consist of two NR1 and NR2 subunits (though some may contain NR3 subunits), while AMPARs and kainate receptors can exist in either homomeric or heteromeric configurations. The proposed topological structure of an iGluR subunit is shown in greater detail in the cartoon below. The subunit is composed of a number of functional domains: an extracellular amino terminal domain (ATD), a ligand-binding region (S1 and S2), three transmembrane domains (M1, 3 and 4) and a re-entrant loop (M2) and a carboxyl terminal domain (CTD). (b) Partial amino-acid alignments highlighting the location of known ligand-binding residues (bold) identified by the crystal structures of GluR2, GluR5, GluR6 and NR1 S1S2 domains. Analogous residues in the NR2A and NR2B subunits are included for comparison. The partial sequence alignments for the NR3A and 3B subunits are also included; in both subunits, both essential threonine residues in the S1- and S2-binding domains are absent. Numbering is according to the mature polypeptide (Monyer *et al*., 1992). For GluR5 and GluR6 numbering is according to Mayer (2005a). (c) Structure of the S1S2 ligand-binding pocket for GluR2 in complex with kainate. The S1 (blue)- and S2 (gold)-binding domains form a hinged clamshell-like structure with the ligand-binding cavity nested between both regions. GluR2 coordinates were obtained from Armstrong *et al*., 1998 (PDB accession code-1gr2) and visualized using RasMol software. (d) A schematic representation of the contact residues within the GluR2 S1S2-binding pocket in direct contact with glutamate (open circles). Hydrogen bonds formed between binding pocket side chains and glutamate are shown as dashed lines. The residues Tyr450 and Glu705 appear 'above' and 'below' the complexed ligand within the binding site as if one was looking into the pocket from the top. In the GluR6 binding scheme, the Thr480 and Ser654 residues are replaced by two alanines (Ala 487 and Ala 658) reducing the stability of the GluR6–glutamate complex. Carbon atoms are black, oxygen, red and nitrogen, blue. Key for standard abbreviations of amino acids: A, Ala; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Iso; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp and Y, Tyr. Panels (c) and (d) reprinted with permission from Erreger *et al*. (2004) (*Critical Reviews in Neurobiology* **16**, 187–224. © 2004 Begell House Inc.).

involved in assembly of subunits within the receptor complex (Ayalon & Stern-Bach, 2001). The ligand-binding domain consists of two regions termed S1 (N-terminal of M1 transmembrane spanning domain) and S2 (between regions M3 and M4) (Gouaux, 2004; Mayer & Armstrong, 2004; Mayer, 2005b). The membrane-associated region consists of

Table 1 Amino-acid residues according to total and mature protein numbering conventions

Total protein (including signal peptide)				*Mature protein (excluding signal peptide)*			
GluR2	*NR1*	*NR2A*	*NR2B*	*GluR2*	*NR1*	*NR2A*	*NR2B*
Y471	F484	H485	H486	Y450	F466	H466	H460
P499	P516	S511	S512	P478	P498	S492	S486
T501	T518	T513	T514	T480	T500	T494	T488
R506	R523	R518	R519	R485	R505	R499	R493
S675	S688	S689	S690	S654	S670	S670	S664
T676	V689	T690	T691	T655	V671	T671	T665
L725	W731	Y730	Y731	L704	W713	Y711	Y705
E726	D732	D731	D732	E705	D714	D712	D706
M729	V735	V734	V735	M715	V717	V715	V709
Y753	F758	Y761	Y762	Y732	Y740	Y742	Y736

Total and mature peptide numbering schemes for the equivalent GluR2, NR1, NR2A and NR2B amino-acid residues described in this review.

three transmembrane spanning domains (M1, 3 and 4) and a re-entrant loop (M2; analogous to the P-loop found in voltage-gated potassium channels) and residues from this M2 region line the lumen of the ion channel pore and control ion selectivity and permeability (Wollmuth & Sobolevsky, 2004). An intracellular carboxy terminal domain (CTD) contains a number of structural motifs that allow the interaction with numerous signal transduction and scaffolding proteins (Kim & Sheng, 2004) and is important for correct regulation, trafficking and localisation of the receptor protein (Song & Huganir, 2002; Wenthold *et al*., 2003; Pérez-Otaño & Ehlers, 2005).

Many of the residues in the S1 and S2 ligand-binding domains are conserved across ionotropic glutamate receptor subunits. However, confusion can arise in the numbering of such residues, depending on whether they are numbered with respect to the total protein (i.e. including signal peptide) or the mature protein. Table 1 lists the main residues involved in ligand binding in GluR2, NR1, NR2A and NR2B receptor subunits using both numbering conventions. In this review, we refer to residues with the numbering system that was used in the original papers and, where appropriate, indicate the equivalent residues in the alternative system.

Structural features of the glutamate-binding pocket – GluR2

As glutamate receptors and bacterial amino-acid-binding proteins share some weak sequence homology (Nakanishi *et al*., 1990), this led to the suggestion that the binding site for glutamate was located in two extracellular domains, defined as S1 and S2 (see above), present in individual receptor subunits. The role these regions play in forming the glutamate-binding pocket and in determining agonist selectivity was later confirmed by structure–function studies (Stern-Bach *et al*., 1994). Furthermore, soluble S1S2 proteins, joined together by a short, flexible peptide linker, were able to bind ligands with 'affinities' and selectivities similar to those observed with intact AMPA receptors (Kuusinen *et al*., 1995; Tygesen *et al*., 1995; Arvola & Keinanen, 1996). Across iGluR subunits, these regions show a high degree of sequence homology (Figure 1b). By analogy with the previously obtained crystal structures of the bacterial amino-acid-binding proteins (O'Hara *et al*., 1993; Oh *et al*., 1993; 1994; Felder *et al*., 1999), it was predicted that the binding pocket would exhibit a bilobar structure of both S1 and S2 domains similar in shape to a 'Venus fly trap' or 'clamshell' (Figure 1c). Gouaux and co-workers were able to confirm this when they obtained the first crystal structures of the agonist-bound GluR2S1S2 fusion protein (Armstrong *et al*., 1998). Furthermore, crystallization of the ligand-bound protein allowed them to identify, unequivocally, amino-acid residues that interact with the agonists kainate, glutamate, AMPA and the competitive antagonist, 5,6-dinitroquinoxalinedione (DNQX) (Armstrong *et al*., 1998; Armstrong & Gouaux, 2000).

GluR2S1S2 (denoted as S1S2I or J depending on the length of the interdomain peptide linker) crystals were found to exist as dimerized subunits, implying that the tetrameric AMPA receptor complex may consist of a dimer of dimers configuration (Armstrong & Gouaux, 2000). This type of configuration is supported by electron microscopic images of the full GluR2 channel as the purified recombinant protein exhibits two-fold symmetry (Safferling *et al*., 2001; Tichelaar *et al*., 2004). Structural studies suggest that this oligomeric architecture may be present in both GluR5 and GluR6 S1S2 pockets, although the dimer interface for GluR5 subunits is remarkably different from either GluR2 or GluR6 (Nanao *et al*., 2005; Naur *et al*., 2005). In accord with the dimeric arrangement of non-NMDA ligand-binding pockets, the NR1-NR2A S1S2 co-crystal structures have revealed that the NR1 and NR2 subunits probably assemble as heterodimers *via* the hinge regions of their ligand-binding cores (Furukawa *et al*., 2005). Indeed, functional evidence supports this dimer of dimers arrangement for both AMPA and NMDA receptor complexes (Ayalon & Stern-Bach, 2001; Robert *et al*., 2001; Bowie & Lange, 2002; Sun *et al*., 2002; Schorge & Colquhoun, 2003; Sobolevsky *et al*., 2004).

X-ray crystallography has revealed that the ligand resides in a cavity formed between both domains (see Figure 1c) further supporting the notion that a number of residues, identified in structure–function studies, from both domains, interact with the ligand. Three residues from the S1 domain (Pro478, Thr480 and Arg485) and from the S2 domain (Ser654, Thr655 and Glu705) make direct hydrogen bonds with glutamate. The aromatic side chain of Tyr450, while not directly hydrogen bonding with glutamate, forms an electron-dense ring structure above the ligand-binding pocket (Figure 1d) and mutations of this tyrosine residue alter agonist potency and desensitization kinetics in GluR2-containing receptors (Holm *et al*., 2005b). Within the binding cavity, four water molecules

form a network that establishes indirect hydrogen bonds between the ligand and side-chains of amino acids within the pocket. Indeed, this arrangement is significant in shaping ligand specificity between AMPARs with different subunit compositions (Banke *et al*., 2001; Pentikainen *et al*., 2003; Frandsen *et al*., 2005). A tetrahedral-like structure is formed between the α-amino group of the ligands (glutamate, AMPA, or kainate) and the residues Pro478, Thr480 and Glu705, while the α-carboxyl group is anchored by the charged Arg485 with additional support from interactions with a Ser654 and Thr480 residues. For glutamate, AMPA and kainate, the interactions between their α-carboxyl and α-amino groups and the residues within the pocket (Arg485, Glu705, Thr480 and Pro478) are conserved (Figure 1d). However, distinct binding patterns occur with the γ-carboxyl groups of these agonists (Figure 2). Thus, while the γ-carboxyl groups of glutamate and kainate interact with the same subsites (hydroxyl and backbone-NH groups of Ser654 and Thr655 and two water molecules), the isoxazole group of AMPA interacts differently, leaving one subsite empty (normally occupied by the γ-carboxyl group of glutamate or kainate). In the case of AMPA, a water molecule is recruited to this unoccupied subsite thus enabling AMPA to behave like a bioisosteric mimic of glutamate (Armstrong & Gouaux, 2000).

The competitive antagonist, DNQX, has a slightly different pharmacophoric pattern from glutamate. The two carbonyl groups of DNQX mimic the α-carboxyl group of glutamate interacting with both Thr480 and Arg485 residues. DNQX is further stabilised by additional hydrogen bonds formed between Pro478 and *via* a water molecule to Tyr405. Finally, stabilising π-interactions are provided by the aromatic residue (Tyr450) which is oriented parallel to the quinoxalinedione ring of DNQX (Armstrong & Gouaux, 2000).

Mechanisms of partial agonism at AMPA receptors

When a ligand is in complex with the GluR2S1S2 protein, an increase in the angle of domain closure compared to the unliganded (apo) conformation was observed between the two binding lobes (Figure 3a). For competitive antagonists such as DNQX, a slight movement of the lobes was observed ($<3^{\circ}$), but probably insufficient to induce the conformational changes necessary for receptor gating. Interestingly, ATPO ((*S*)-2-amino-3-[5-*tert*-butyl-3-(phosphonomethoxy)-4-isoxazolyl]-propionic acid), another AMPAR selective antagonist also only induces a minimal amount of domain closure but is structurally very different from DNQX (Hogner *et al*., 2003). Both prevent further domain closure by different mechanisms. For example, the phosphonate and *tert*-butyl groups of ATPO increase the stability of the open conformation by steric

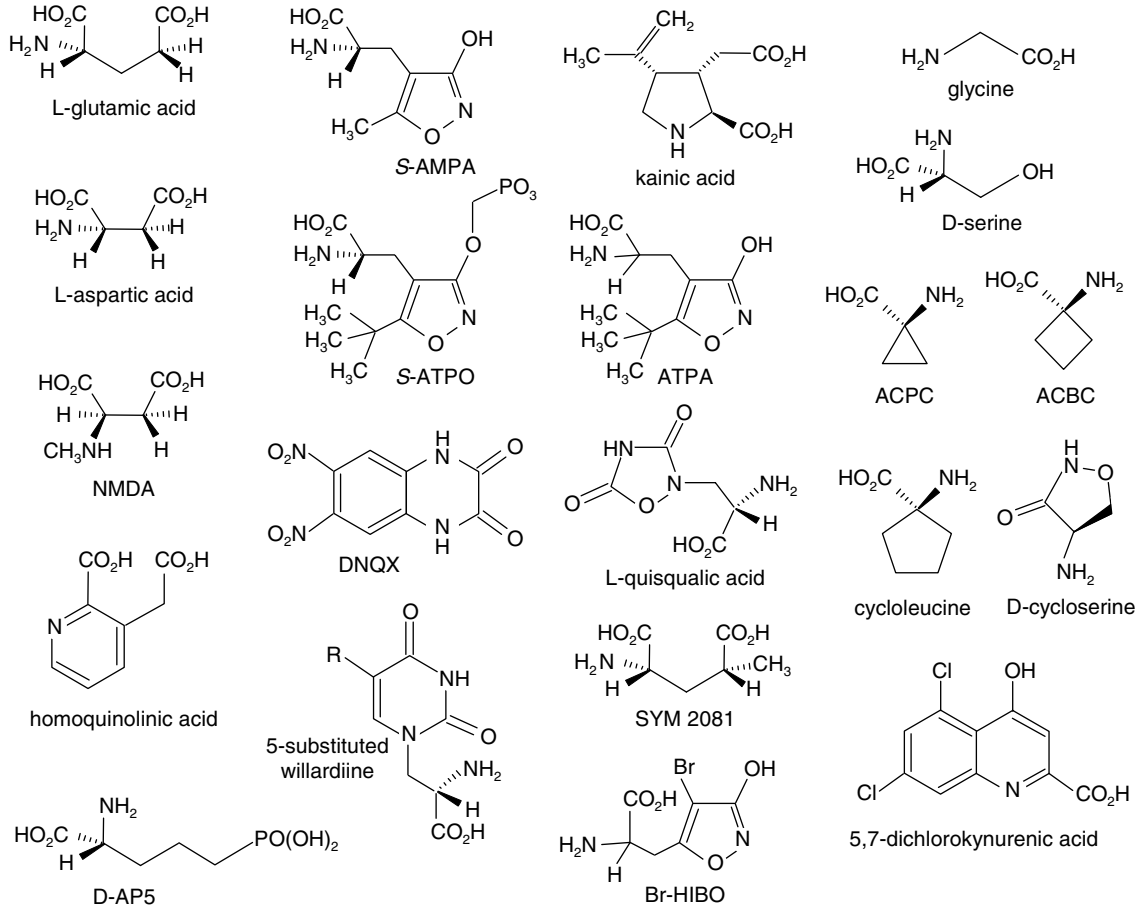

Figure 2 Chemical structures of the various agonists and antagonists that interact with the glutamate-binding site, in NMDA, AMPA and kainate receptor subunits and the glycine-binding site of the NR1 NMDA receptor subunit.

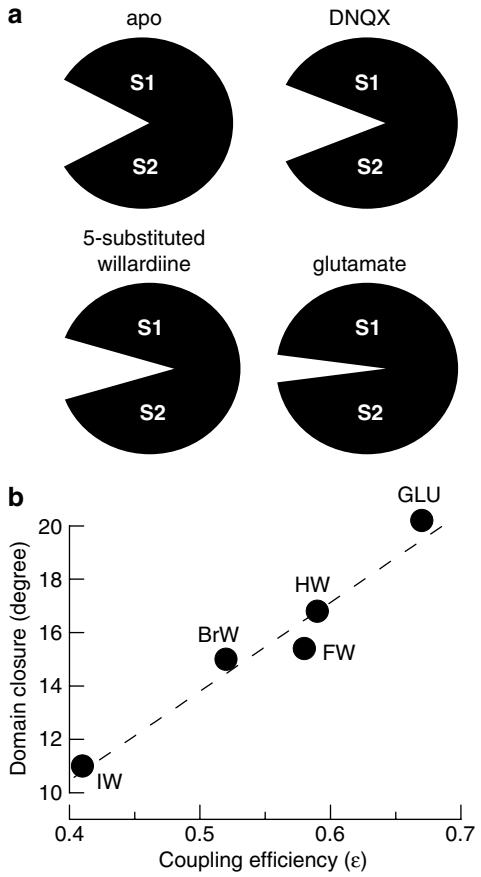

Figure 3 (a) Cartoon to illustrate the fact that different ligands cause difference amounts of domain closure of GluR2 receptor subunits. In the unliganded (apo) state the separation between the S1 and S2 domains is at its greatest. Antagonists such as DNQX cause only small changes in the degree of domain closure. Partial agonists such as the 5-substituted willardiine cause greater domain closure than is seen with antagonists but less than that seen with full agonists such as glutamate. (b) The extent of domain closure is correlated to the efficiency with which agonists open AMPARs containing the GluR2 receptor subunit. Key to abbreviations: GLU = glutamate; HW = willardiine; FW = fluorowillardiine; BrW = bromowillardiine; IW = iodowillardiine. Panel (b) adapted and reprinted, with permission, from Jin *et al.* (2003) (*Nature Neuroscience* **6**, 803–810. © 2003 Nature Publishing Group (www.nature.com/)).

hindrance, whereas the 6-nitro group of DNQX prevents the formation of interdomain contacts within the pocket (see chemical structures in Figure 2). In contrast to antagonists, a far greater increase in domain closure of the clamshell was observed for agonists and this correlates with agonist efficacy (partial agonists inducing a degree of domain closure in between those induced by full agonists or antagonists) (Armstrong & Gouaux, 2000; Hogner *et al.*, 2002; Jin *et al.*, 2002; 2003; Armstrong *et al.*, 2003). This is best exemplified by a series of 5-substituted willardiines that differ in the size of a halide substituent, for which an increase in substituent size sterically hindered domain closure in a graded fashion (Jin *et al.*, 2003). The degree of domain closure was found to correlate with increased efficacy of channel activation (Figure 3b). In agreement with the evidence that partial agonists induce less domain closure than full agonists, molecular dynamics simulations have shown that partial agonists such as kainate are held less rigidly within the GluR2 pocket than full agonists (glutamate) and, as a consequence of this, water mobility at specific subsites within the pocket is agonist dependent (Arinaminpathy *et al.*, 2006). It has been hypothesized that domain closure induces considerable strain on the rest of the protein leading to sufficient conformational rearrangements that trigger gating or desensitization of the receptor (see below).

Desensitzation of AMPA receptors

The individual ligand-binding cores form dimers and the stability of these interdimer interactions determines the extent of desensitization in AMPA receptors. Allosteric modulators (such as cyclothiazide) and point mutations (such as L483Y) within the interdimer region that abolish desensitization each have been shown to increase the stability of the dimer complex (Stern-Bach *et al.*, 1998; Sun *et al.*, 2002). Moreover, point mutations that disrupt the salt bridge, hydrogen bond network and van der Waals interactions between neighbouring ligand-binding cores greatly accelerate desensitization (Horning & Mayer, 2004). It has been proposed that during desensitization the rearrangement of the dimer interface triggers the disengagement of the conformational alterations induced by agonist-binding cleft closure from the activation of channel gating (Sun *et al.*, 2002). The propagation of the desensitization signal from the agonist-binding domain to the channel gate may, in part, be mediated by electrostatic interactions in regions outside the ligand-binding domains (e.g. between S2 and M3 regions) (Yelshansky *et al.*, 2004). Certain partial agonists such as (*RS*)-Br-HIBO that accelerate desensitization may exert their effects by twisting the S1 and S2 domains by 6° compared to the apo-form and changing the conformation of another leucine residue (Leu650; Holm *et al.*, 2005a). Stability of cleft closure is also important as mutations that destabilize the closed cleft conformation accelerate recovery from desensitization (Robert *et al.*, 2005).

AMPAR potentiators, such as aniracetam and CX614, that slow deactivation kinetics more than desensitization (in contrast to CTZ), bind within a crevice formed by the dimer interface of two GluR2S1S2 proteins. Disruption of the interactions between aniracetam or CX614 and this hinge region impair potentiator action (Jin *et al.*, 2005). These modulators may exert their influence on deactivation kinetics by stabilizing the binding pocket in the closed-cleft, agonist-bound configuration as well as reducing desensitization by stabilizing the dimer complex. Although it is difficult to separate the modulation of deactivation and desensitization processes *via* the action of aniracetam and CX614, these findings suggest that deactivation and desensitization may occur through distinct yet closely related molecular mechanisms in relation to the conformation of the ligand-binding core within the receptor complex.

Structural features of the glutamate-binding pocket in GluR5/6 receptor subunits

The agonist-binding pockets of the kainate receptor subunits GluR5 and GluR6 share similar features to those from the GluR2 subunit (Mayer, 2005a; Nanao *et al.*, 2005; Naur *et al.*,

2005). The interaction of the α-carboxyl, α-amino and γ-carboxyl groups of glutamate within the binding pocket involve similar contact residues as those described for the GluR2–glutamate complex. These include the two conserved charged arginine (Arg508, Arg492) and glutamic acid (Glu723, Glu507) residues and an essential threonine residue (Thr675, Thr659) that is present in all glutamate-binding subunits (Figure 1b). The higher sensitivity of glutamate for GluR5 compared to GluR6 may be explained by the substitution of two contact residues (Thr503 and Ser674) with alanine (Ala487 and Ala658) (Figure 1b). The stability of the GluR6–glutamate complex is reduced by the subsequent loss of hydrogen bonding with the α-amino group of glutamate and other water-mediated interactions with another contact residue (Glu723).

Two other characteristics distinguish agonist specificity in the GluR5/6-binding pockets from that of GluR2. Firstly, the GluR5-binding pocket is 40% larger than its AMPA receptor counterpart enabling the trapping of more water molecules not normally present in the GluR2-binding cavity (GluR2 contains four water molecules, whereas GluR5 contains six water molecules and GluR6 has five water molecules) and GluR5 selective agonists are able to exploit this ordered network of water molecules. Secondly, steric occlusion plays a major part and is dictated by three smaller pocket lining residues within domain 2, compared to larger equivalents in GluR6 (GluR5: Ser706, Leu720 and Ser726; GluR6: Asp690, Phe704 and Thr710). For instance, it has been previously demonstrated that the Ser706 and Asp690 residues are known to play a role in specifying AMPA selectivity between GluR5- and GluR6-containing receptors (Swanson *et al*., 1997; 1998). GluR5 selective agonists such as the 5-*tert*-butyl derivative of AMPA, 2-amino-3-(5-*tert*-butyl-3-hydroxy-4-isoxazolyl) propionic acid (ATPA) and 5-iodowillardiine (Figure 2) are sterically hindered by the side chain from Asp690 when docked into the GluR6 pocket and this is relieved by the presence of the smaller Ser706 at the equivalent position in GluR5. This confirms mutagenesis work suggesting that ATPA selectivity in GluR5 may be specified by residues that interact with the bulky 5-*tert*-butyl group (Nielsen *et al*., 2003). Steric obstruction may also explain the reduced selectivity of ATPA for AMPARs as larger residues than serine impede stable docking of the 5-*tert*-butyl group within the GluR2 pocket (Lunn *et al*., 2003).

Molecular dynamics simulations suggest that docking AMPA into the GluR6 pocket positions the isoxazole ring at an inappropriate angle for stable interactions with Asp690 and sites normally used by the γ-carboxyl group of glutamate (Mayer, 2005a). In GluR5, this hindrance is relieved, partly, by the replacement of the asparagine with a smaller serine residue but this is still not enough for optimal binding. In contrast, a structurally related ligand such as quisqualate is able to mimic the stereochemistry of glutamate and the projection of the heterocyclic ring is able to establish similar contacts to those made by the γ-carboxyl group of glutamate within the pocket.

Steric occlusion partly explains the higher selectivity of the agonist 2*S*, 4*R*-4-methylglutamate (SYM 2081) for kainate receptors over AMPA receptors. SYM 2081 makes additional interactions *via* the 4-methyl group with Tyr457 and Val654 residues of GluR6 (Mayer, 2005a). In GluR2, the valine residue is replaced by a bulkier leucine residue and in combination with hydrogen bonds formed between residues in domain 2 and a nearby GluR2 specific water molecule reduce the likelihood of a more stable interaction with SYM 2081. This hindrance may also restrict the greater domain closure observed in GluR6 for SYM 2081 and glutamate (26.4° and 26.6°) compared to GluR2 (20°) pockets.

The higher potency of the partial agonist, kainate, for GluR6-containing receptors than GluR2 is due to a greater degree of domain closure within the GluR6–kainate complex (23.3°) compared to that seen in the GluR2–kainate complex (12°), although this is still less than that observed with the full agonist glutamate (26.6°) (Mayer, 2005a). A reduction in steric hindrance between the two lobes (compared to GluR2) allows the formation of multiple interdomain contacts within GluR6, leading to a larger degree of domain closure compared to GluR2. Intriguingly the side chain of the GluR6 partial agonist, domoic acid, only forms hydrogen bonds with residues in domain 1 (Nanao *et al*., 2005).

The correlation between agonist 'efficacy' with degree of domain closure is conserved among AMPA and kainate glutamate receptor subtypes. Partial agonists such as kainate and domoic acid cause less of a degree of domain closure in the GluR5/6 pockets compared to full agonists such as glutamate, quisqualate, AMPA or 2*S*,4*R*-4-methylglutamate (Mayer, 2005a; Nanao *et al*., 2005).

The glycine co-agonist-binding site in the NR1 NMDA receptor subunit

The crystal structure of the NR1 NMDA receptor subunit glycine-binding domain (NR1S1S2) revealed that it too retains a general bilobar clamshell-like architecture reminiscent of the other AMPA and kainate subunit-binding pockets (Furukawa & Gouaux, 2003). However, there are slight differences in the loop orientations between NR1 and GluR2; for example in NR1, the extended loop structures causes domain 1 to be slightly wider than its equivalent in GluR2. Corresponding to the observations in GluR2, glycine makes direct and indirect interactions (*via* five water molecules) with a number of residues within the S1 and S2 domains. An essential contact is made between the guanidinium group of Arg523 and the α-carboxyl group of glycine. This feature is conserved among all glutamate receptor ligand-binding subunits as the equivalent arginine residues (Arg485 in GluR2, Arg508 in GluR5, Arg492 in GluR6 and Arg499 in NR2A) interact or are predicted to interact with the α-carboxyl of glutamate (see GluR2, GluR5/6 and NR2A sections). The α-amino group of glycine makes contacts with Pro516, Thr518 and Asp732 analogous to those found for equivalent residues in the GluR2 subunit (Pro478, Thr480 and Glu705 in GluR2 – see Figure 1d). The main distinction between glycine and glutamate binding in the NR1 and GluR2 pockets is centred around the specific interactions at the γ-carboxyl group of either ligand. Firstly in NR1, the Thr655 residue found in GluR2 (which is present in all glutamate-binding subunits – Figure 1b) is replaced by a valine (Val689), preventing the formation of hydrogen bonds between the OH group of threonine and the γ-carboxyl group of glutamate. Secondly, the indole ring from the tryptophan residue (Trp731) in NR1 would sterically clash with the γ-carboxyl group of glutamate. In GluR2, the smaller leucine side chain (Leu704) adopts an orientation that creates a more favourable environment for the γ-carboxyl group of

glutamate. Therefore, both of these factors determine glutamate and glycine specificity at either the GluR2- or NR1-binding pockets.

As is the case with GluR2 S1S2, agonist binding to the NR1 S1S2 causes domain closure (Furukawa & Gouaux, 2003; Inanobe *et al*., 2005). However, the relative degree of domain closure for both partial (D-cycloserine, 1-aminocyclopropane-1-carboxylic acid, ACPC and 1-aminocyclobutane-1-carboxylic acid, ACBC) and full agonists (glycine and D-serine) only varies by 0.5° or less which is in contrast to the findings that the degree of domain closure in GluR2 is related to agonist 'efficacy' (Jin *et al*., 2003). Even with partial agonists possessing incrementally larger carbocyclic rings such as ACPC and ACBC, the same degree of domain closure was observed in the NR1 S1S2 (Inanobe *et al*., 2005). Therefore, partial agonists may exert their actions at the NR1-glycine site *via* molecular mechanisms (which remain to be elucidated) distinct from AMPA/kainate receptors. In agreement with observations of antagonist binding for DNQX at the GluR2 S1S2, the competitive glycine site antagonists, 5,7-dichlorokynurenic acid and cycloleucine, stabilize the open-cleft conformation of the NR1S1S2 pocket. 5,7-Dichlorokynurenic acid, like DNQX, appears to interact with residues mainly within the S1 domain, depriving glycine of perhaps its initial contact sites with the open cleft of the binding pocket. Cycloleucine being bulkier than both ACPC and ACBC causes an expansion of the binding cleft allowing the recruitment of two additional water molecules and alters the hydrophobic interdomain contact between the side chains of Phe484 and the indole group of Trp731. Therefore, a large enough expansion of the binding cavity will stabilize the open cleft configuration and prevent the NR1 S1S2 pocket from achieving enough domain closure to possibility induce the conformational changes necessary for successful receptor gating.

The glutamate-binding site in the NR2A NMDA receptor subunit

The crystal structure of the NR2A ligand-binding pocket shares similar molecular elements involved in ligand interactions previously observed in other glutamate-binding cores (GluR2, GluR5 and GluR6). Residues Arg518, Ser511, Thr513, Ser689 and Thr690 share identical binding modes with the other glutamate-binding pocket structures (Figure 4a). However, there are a number of subtle differences that are due in part to the location of water molecules within the pocket (Furukawa *et al*., 2005). Firstly, in contrast to the role played by the equivalent negatively charged glutamic acid residue in GluR2 (Glu705), the Asp731 (NR2A-Asp712/NR2B-Asp706 in the mature protein) residue does not directly interact with the α-amino group of glutamate but instead forms interdomain contacts with Tyr761. The α-amino group of glutamate is anchored by Ser511 and Thr513 residues. Additional bonds are provided by water-mediated interactions (water molecule, W2) with Glu413 and Tyr761 residues. Secondly, the γ-carboxylate group is not only secured by the Ser689 and Thr690 residues from the S2 domain but also forms contacts with the aromatic Tyr730 residue. This tyrosine residue is supported by interdomain interactions with Glu413 (Figure 4b).

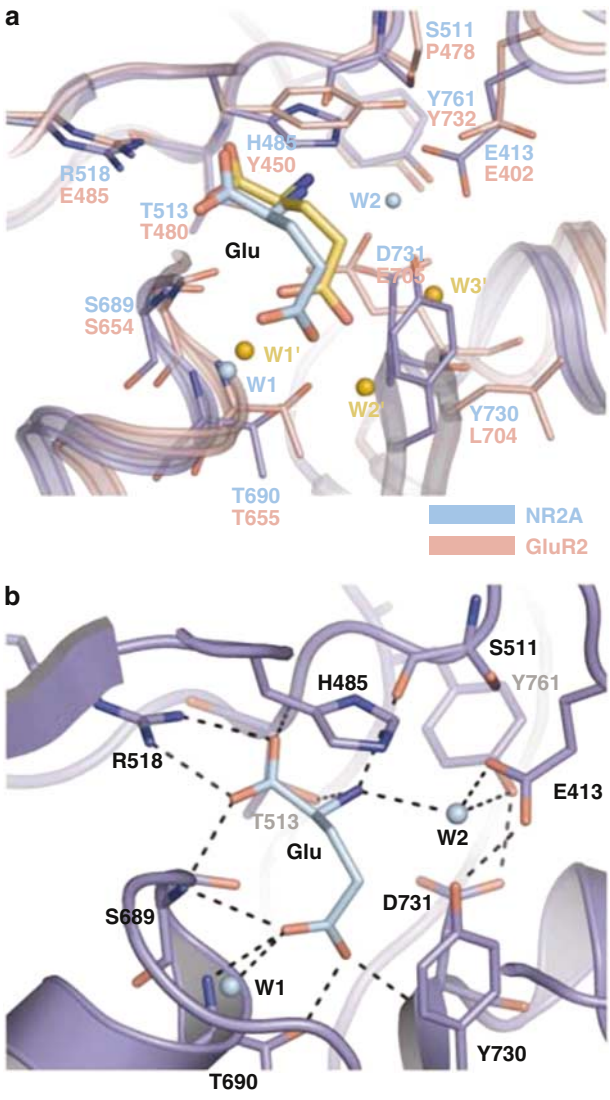

Figure 4 (a) Superimposition of structures showing glutamate docked in the ligand-binding site of NR2A NMDA receptor subunits (grey) and GluR2 AMPA receptor subunits (pink). (b) Illustration showing the residues directly and indirectly interacting with glutamate in the crystal structure of the NR2AS1S2-binding pocket. Dashed lines represent hydrogen bonds or salt bridges and the water-mediated network is formed by two water molecules (W1 and W2). In contrast from previous NR2 homology models, the Asp731 residue does not directly interact with the α-amino group of glutamate, while the Tyr730 residue interacts directly with the γ-carboxyl group of glutamate. Reprinted with permission from Furukawa *et al*. (2005) (*Nature* **438**, 185–192. © 2005 Nature Publishing Group (www.nature.com/)).

Before the emergence of the NR2A-glutamate crystal structure, structure–function studies predicted that a number of these residues would be involved in agonist binding. The agreement between structural and functional studies is not surprising, as there is a great deal of sequence homology between the NMDA and non-NMDA receptor subunits in the agonist-binding region. The residues that lie in direct contact with the ligand are largely conserved among the glutamate-binding sites for AMPA and NR2 subunits (see Figure 1b), the exceptions being the serine in the S1 region of the NR2 subunits (NR2A-Ser492/NR2B-Ser486), which is replaced by

Table 2 Critical residues for ligand binding in NMDA receptors

GluR2 S1S2	*NR1 S1S2*	*NR2AS1S2*	*NR1a/NR2A*	*NR1a/NR2B*
Y450[a]		*H485*[b]	H466A (140)[c]	H460A (469[d], 325[e])
P478[a]	P516[f]	S511[b]	S492A (42)[g]	S486A (43[h], 33[e])
T480[a]	T518[f]	T513[b]	T494A (138)[g]	T488A (549[d], 200[e])
R485[a]	R523[f]	R518[b]	R499K (NF)[g]	R493K (NF)[e,h]
S654[a]	S688[f]	S689[b]	S670G (120)[g]	S664G (118)[h]
T655[a]		T690[b]	T671A (848)[c]	T665A (495[d], 525[e])
E705[a]	D732[f]	*D731*[b]	D712E (NF)[g,i,j]	D706E (NF)[d,e,i,j]
		Y730[b]		Y705A (421)[d]

Residues directly involved in agonist binding and the fold changes in glutamate potency compared to wild type (parentheses) found in recombinant NR1/NR2 receptors containing point mutations within the S1/S2 regions. Equivalent contact residues identified from GluR2, NR1 and NR2A S1S2 structural data are included for comparison. NF indicates nonfunctional. Two exceptions are included which do not directly interact with glutamate but when mutated in NR2 subunits produce large reductions in glutamate potency (italics), (i) the aromatic residues GluR2-Tyr450 and NR2A-His485, provide electron-dense side chains to seal off the pocket from hydrophobic residues and (ii), the Asp731 residue in NR2A, makes interdomain contacts with Tyr761. The equivalently charged GluR2–Glu705 residue makes direct contact with glutamate.
[a]Armstrong *et al*. (1998) and Armstrong & Gouaux (2000).
[b]Furukawa *et al*. (2005).
[c]Anson *et al*. (1998).
[d]Laube *et al*. (2004).
[e]Hansen *et al*. (2005). Residues numbered according to total protein sequence.
[f]Furukawa & Gouaux (2003).
[g]Chen *et al*. (2005).
[h]Laube *et al*. (1997).
[i]Williams *et al*. (1996).
[j]Residues numbered as NR2A(D731) and NR2B(D732) in Williams *et al*. (1996).

a proline in GluR2 (Pro478). There is also shared homology with the five contact residues in the glycine-binding site of the NR1 S1S2 structure (see Table 2). Therefore, the basic building blocks for the binding site are in place throughout the ionotropic glutamate receptor family. Generally, these studies have been accurate in predicting the molecular interactions within the NR2 pocket; however, some differences have emerged due to the precise positioning of water-mediated interactions.

Most of the present ideas about glutamate binding to NR2 have been inferred from structure–function studies and site-directed mutagenesis in the NR2A/B S1/S2 regions. Several mutations that cause large reductions in glutamate potency in recombinant NR1/NR2 NMDA receptors with little or no change in glycine potency suggest that these residues may be important in forming the binding pocket or participate directly in hydrogen bonding. Although reductions in agonist potency induced by a point mutation may also be associated with alterations in efficacy rather than binding alone (Colquhoun, 1998), ligand-binding residues identified by homology modelling from previous crystal structures and ligand docking using molecular dynamics have largely supported the functional data. A number of residues have been predicted to make direct contact with the ligand have been identified by structure–function studies, including NR2A-Arg499/NR2B-Arg493 (Arg485 in GluR2), NR2A-Ser492/NR2B-Ser486 (Pro478 in GluR2), NR2A-Thr494 (Thr480 in GluR2), NR2A-Ser670/NR2B-Ser664 (Ser664 in GluR2), NR2A-Thr671/NR2B-Thr665/NR2D-Thr692 (Thr655 in GluR2) (Williams *et al*., 1996; Laube *et al*., 1997; Anson *et al*., 1998; 2000; Chen *et al*., 2004; Kalbaugh *et al*., 2004; Laube *et al*., 2004; Chen *et al*., 2005; Hansen *et al*., 2005, see Table 2). In addition, mutation of NR2A-His466/NR2B-His460 (Tyr450 in GluR2) also causes significant reductions in glutamate potency. Crystal structures have confirmed the important role played by the charged arginine residue within the pocket. Mutation of the charged arginine residue within the binding pocket of NR2 subunits, NR2A-Arg499/NR2B-Arg493 to lysine, also renders the channel nonfunctional (Williams *et al*., 1996; Laube *et al*., 1997; Chen *et al*., 2005; Hansen *et al*., 2005) and is consistent with other structure–function studies on AMPA receptors (Kawamoto *et al*., 1997; Lampinen *et al*., 2002). Intriguingly, replacement of the NR2B-Arg493 residue with a nonpolar alanine retains channel functionality albeit with a far reduced glutamate potency (831-fold) and may be explained by rearrangement of the water-interaction network within the pocket (Hansen *et al*., 2005). Homology modelling of the NR2A-glutamate pocket shows that the guanidinium group from the arginine (Arg485 in GluR2) forms an essential tether with the α-carboxyl group of glutamate (or glycine for NR1) and modelling predicts that substitution of the arginine residue with a shorter but similarly charged lysine is a detriment to glutamate binding (Chen *et al*., 2005). Nonetheless, the elucidation of the NR2A crystal structure (Furukawa *et al*., 2005) has shown that the predicted interaction of the S2 aspartate residue (NR2A-Asp731, located in a homologous position to Glu705 in the GluR2 subunit) with the α-amino group of glutamate does not occur. Rather, this α-amino group interacts with Tyr761 *via* a water-mediated interaction. Thus, the reason that mutation of the S2 aspartate residue results in a nonfunctional receptor (Williams *et al*., 1996; Laube *et al*., 1997; Chen *et al*., 2005; Hansen *et al*., 2005) is probably due to a disruption of the binding pocket and/or interaction of other residues in this locality, rather than the prevention of direct interaction of the acidic side chain with the ligand. These data illustrate the importance of correlating information gathered from both structural and mutagenesis studies.

Nonetheless, small decreases in agonist potency due to mutagenesis of a certain residue may also mislead us in determining their role in agonist binding. One such example is

the Ser670 residue in the S2 domain (Figure 5a). Mutation of Ser670 to glycine, in the NR2A NMDA receptor subunit causes a far greater shift in potency (>100-fold) than that seen when the residue is mutated to an alanine residue (possibly due to altered chain flexibility) (Anson *et al*., 1998; Chen *et al*., 2005, see Figure 5a). In this respect, the NR2A (S670G) mutation causes a similar reduction in glutamate potency to that seen with the homologous mutation in the NR2B receptor subunit (S664G, 118-fold) (Laube *et al*., 1997). We see a similar situation in the NR1 subunit in terms of glycine binding. At the equivalent position in the NR1 subunit, mutation of the serine residue (Ser688) to an alanine only produced a four-fold reduction in glycine potency (Kuryatov *et al*., 1994). However, the role of this serine residue in glycine binding was only appreciated following the analysis of the NR1S1S2 crystal structure (Furukawa & Gouaux, 2003).

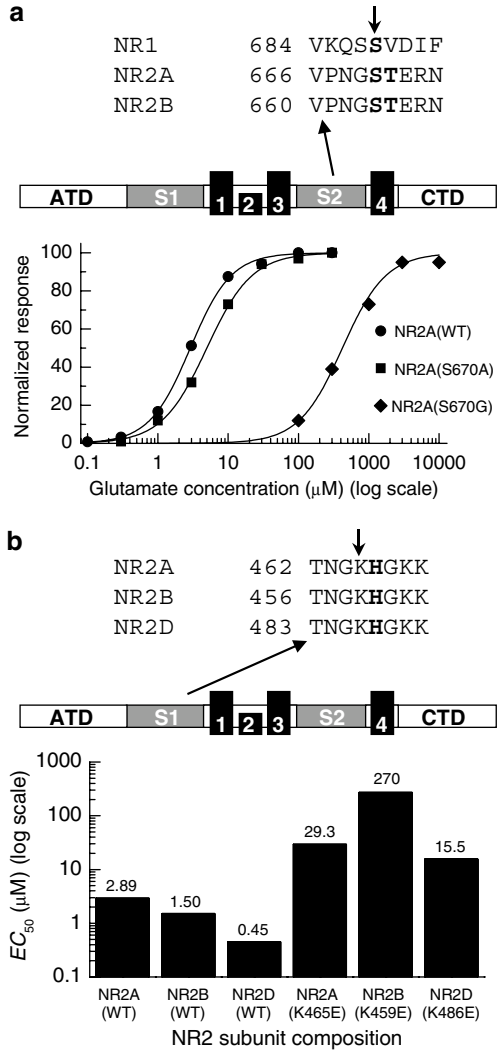

Figure 5 (a) Different mutations at one of the serine residues known to be involved in glutamate binding can exert drastically different effects on glutamate potency (location shown by arrowhead in the alignment above). Glutamate concentration–response curves for NR2A wild type, NR2A(S670A)- and NR2A(S670G)-containing receptors. The NR2A(S670A) mutation only increased glutamate EC_{50} to 4.97 μM (approximately two-fold more than wild type); however, the NR2A(S670G) mutation reduced glutamate potency by 145-fold to 421 μM. This compares well with observations of the equivalent mutations in NR2B-containing receptors (Laube *et al*., 1997; 2004). Data from Anson *et al*. (1998) and Chen *et al*. (2005). Error bars omitted for clarity. (b) The equivalent lysine to glutamic acid mutation within the S1 domain can produce differential glutamate sensitivities among NR2A-, NR2B- and NR2D-containing receptors. The bar graph compares mean glutamate EC_{50} values for wild-type NR2A, NR2B- or NR2D-containing receptors and their Lys to Glu mutant counterparts, NR2A(K465E), NR2B(K459E) and NR2D(K486E). The NR2B(K459E) mutation reduces glutamate potency by about 180-fold compared to NR2A(K465E) and NR2D(K486E) (10- and 34-fold). Location of the lysine residue (arrowhead) among the NR2 subunits is highlighted above. NR2A and NR2B data from Laube *et al*. (1997) and Anson *et al*. (1998) respectively, NR2D data from Chen, 2000.

Another perplexing observation is the influence of the same point mutation in either NR2A or NR2B subunits on glutamate potency (Figure 5b). Mutation of the NR2A-Lys465/NR2B-Lys459 residue to a negatively charged glutamic acid exerts varying effects on glutamate potency between NR2A- and NR2B-containing receptors. In NR2A, only a 10-fold reduction in glutamate potency was observed; however, a far greater reduction was found in the NR2B mutant (180-fold). Although modelling studies suggest that this lysine residue is probably not in direct contact with glutamate within the pocket, it may support, indirectly, certain secondary structural folding and further investigation may reveal interesting differences in how glutamate may interact with the NR2A or NR2B pockets.

Full and partial agonist modelling in the NR2 NMDA receptor subunits: molecular basis of selectivity

The crystal structures of the GluR2- or NR1-binding pockets have served as templates for a number of homology models of the NR2-glutamate-binding site (Chohan *et al*., 2000; Tikhonova *et al*., 2002; Blaise *et al*., 2004; Laube *et al*., 2004; Chen *et al*., 2005; Kinarsky *et al*., 2005; Hansen *et al*., 2005). The NR2-glutamate pocket shares similar features to the GluR2- and NR1-binding pockets, although there are slight differences in loop and helical structure, the general pharmacophoric pattern formed by these residues is conserved among the ionotropic glutamate receptor family. Generally, these studies have been accurate in predicting the molecular interactions within the NR2 pocket; however, some differences have emerged with the NR2A-glutamate crystal structure due to the precise positioning of water-mediated interactions. For example, the interaction of NR2A-Tyr730 with the γ-carboxyl group of glutamate had not been predicted.

Gouaux and co-workers modelled NMDA into the crystal structure of the NR2A-glutamate-binding pocket and suggested that by displacing the water molecule W2 (Figure 4), NMDA can be accommodated within the pocket because the Asp731 residue does not clash with the *N*-methyl group (Furukawa *et al*., 2005). The equivalent Glu705 residue in GluR2 is one methylene group longer and would clash with the *N*-methyl group of NMDA (Figure 6a). Homology modelling of NR2A- and NR2B-binding pockets have suggested that a methionine residue that is conserved throughout the AMPA

receptor family (Met708) may obstruct NMDA binding (Laube *et al*., 2004; Chen *et al*., 2005) (Figure 6b and c), whereas in NR2A and NR2B receptor subunits this methionine is replaced by a valine residue (Val715 in NR2A, Val 709 in NR2B) which does not intrude into the hydrophilic pocket as much as Met708. Indeed, mutating this valine residue, in either NR2A or NR2B NMDA receptor subunits, to a methionine residue causes a decrease in the potency of NMDA (Laube *et al*., 2004; Chen *et al*., 2005). Nonetheless, the molecular determinants underlying NMDA selectivity still remain to be resolved and future studies examining the roles of these and other residues within the pocket will provide a fuller picture of agonist selectivity.

Although previous work has allowed us to postulate possible mechanisms of agonist action in the GluR2 and NR1 subunits, we are still unclear of the structural consequences of glutamate binding at the NR2 ligand-binding pocket and how that may translate into conformational changes which trigger NMDA receptor activation. Recent efforts using partial agonists and molecular modelling have shed light on the possible mechanisms involved in this process. Homoquinolinate acts as a partial agonist on NR1/NR2A-containing NMDA receptors and allows us to probe the mechanism of activation of this subtype of glutamate receptor (Figure 7a and b). Kinetic modelling of single-channel data obtained from recombinant NR1/NR2A NMDA receptors suggested that a rate-limiting pre-gating conformational step precedes slower for the partial agonist homoquinolinate than for glutamate (Erreger *et al*., 2005). Molecular dynamics simulations of homoquinolinate docked within the NR2A-glutamate pocket predict that this may be due to differences in the ability of homoquinolinate to induce local conformational changes in the NR2 subunit compared to that caused by glutamate (Figure 7c and d). For example, intrapocket motion is increased when homoquinolinate is docked than with glutamate (Figure 7e and f; see molecular dynamics movies available from http://www.pharm.emory.edu/straynelis/StructuralModels). This implies that agonist-specific conformational movements regulate the efficacy of an activation step that precedes the global changes required for channel gating.

Another issue that is unresolved is whether the degree of domain closure is correlated to agonist potency in NR2-glutamate complexes as observed in GluR2 and GluR5/6 (Jin *et al*., 2003; Mayer, 2005a; Nanao *et al*., 2005). Recent structure–function data show that engineering steric clashes within the NR2B-glutamate-binding pocket is correlated to the degree of agonist efficacy (Hansen *et al*., 2005). Thus it is intriguing to think that within the same NMDA receptor complex, the action of glutamate and glycine on the NR2 and

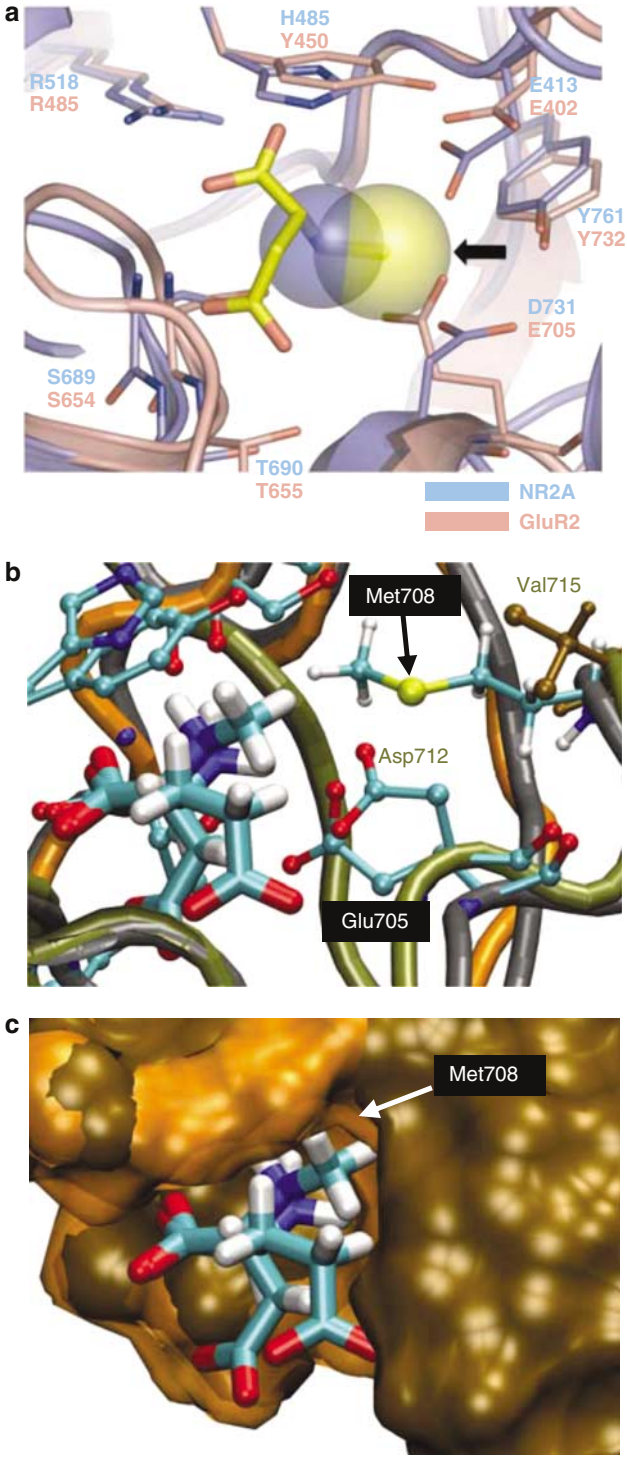

Figure 6 (a) Possible mechanism underlying NMDA selectivity as judged by comparing both GluR2 and NR2 pockets. The position of the side chain from Glu705 in GluR2 would sterically occlude the *N*-methyl group of NMDA (yellow sphere), whereas in NR2A the side chain group of the Asp731 residue would not hinder NMDA binding. (b) Superimposing the ball and stick schemes of the homology model of NR2A-NMDA from Chen *et al*. (2005) with the GluR2S1S2-glutamate crystal structure reveals that a methionine residue (Met 708) in GluR2 may sterically hinder the *N*-methyl group of NMDA. In NR2A, this residue is replaced by the smaller valine residue facilitating NMDA binding. The GluR2 backbone is shown in grey, while the NR2A S1 and S2 regions are coloured orange and green, respectively. Equivalent GluR2 and NR2A residues are labelled in white or green text, respectively. (c) Surface profiles of the GluR2 (light brown) and NR2A schemes (dark brown) shown in (b). Location of Met708 in GluR2 is shown by the white arrow. Panel (a) reprinted with permission from Furukawa *et al*. (2005) (*Nature* **438**, 185–192. © 2005 Nature Publishing Group (www.nature.com/)). Panels (b) and (c) reprinted, with permission, from Chen *et al*. (2005) (*Molecular Pharmacology* **67**, 1470–1484. © 2005 American Society for Pharmacology and Experimental Therapeutics).

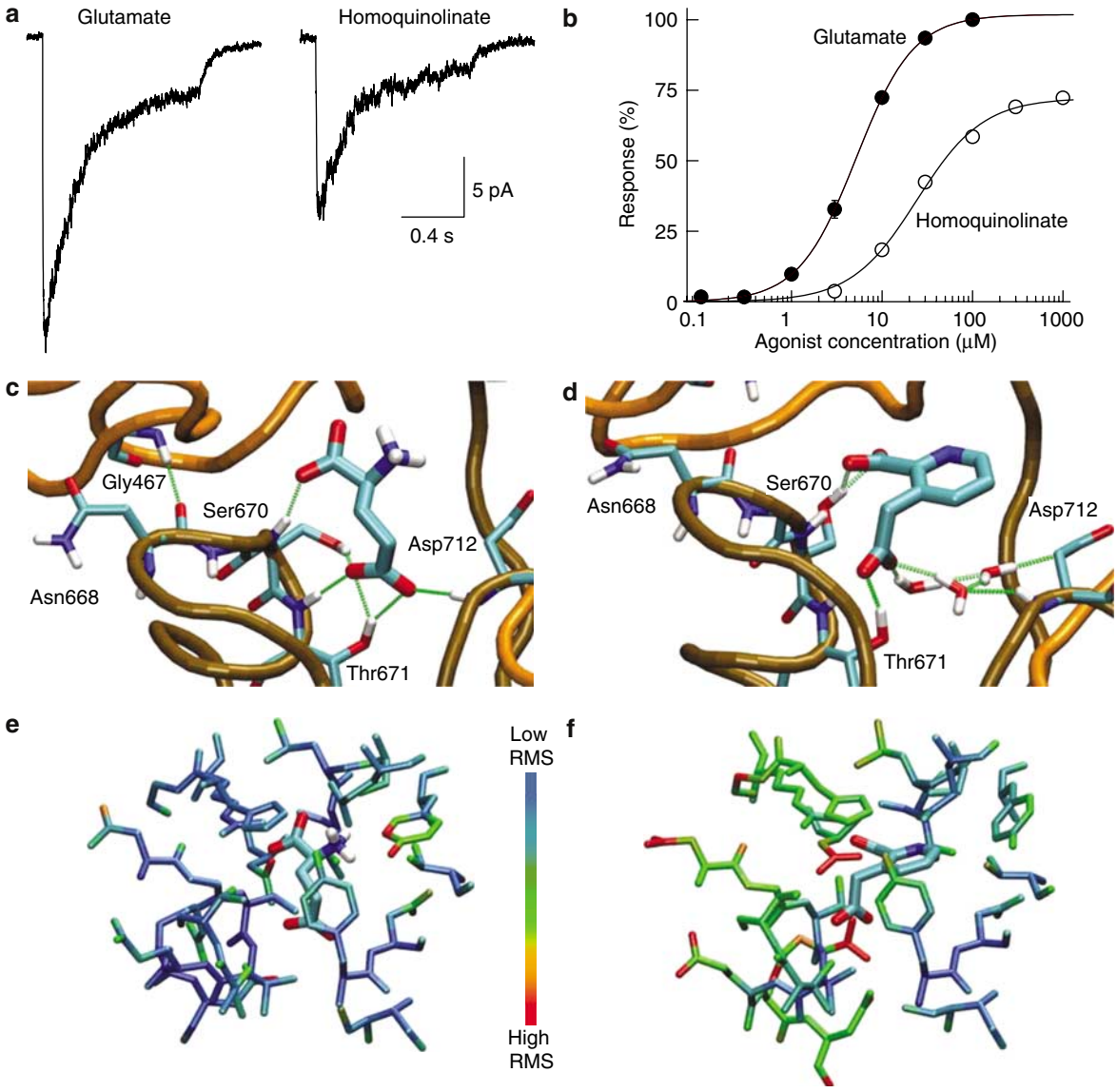

Figure 7 (a) Examples of currents, evoked by saturating concentrations of either glutamate or homoquinolinate and recorded in the same outside-out membrane patch excised from a HEK293 cell expressing NR1/NR2A NMDA receptors. (b) Concentration–response curves for glutamate and homoquinolate-evoked responses recorded in *Xenopus laevis* oocytes expressing recombinant NR1/NR2A NMDA. Responses are normalized to 100 μM glutamate response. The EC_{50} for glutamate-evoked responses is 4.6 μM, while for homoquinolinate-mediated responses it is 24.4 μM. (c) Illustration of glutamate residing in the binding pocket of the NR2A NMDA receptor subunit. The γ-carboxyl of glutamate makes hydrogen bonds with Ser670, Thr671 and Asp712. (d) Illustration of homoquinolinate residing in the binding pocket of the NR2A NMDA receptor subunit. Note that in order to make hydrogen bonds with Asp712, bridging water molecules are required; this is thought to result in an increased mobility of the pocket. (e, f) The root mean square (rms) of atomic positions of residues in the binding site of NR2A when it is occupied by either glutamate (e) or homoquinolinate (f). The positions are pseudo-coloured from blue to red to indicate low to high rms values. Figure adapted and reprinted, with permission, from Erreger *et al*. (2005) (*Journal of Neuroscience* **25**, 7858–7866 © 2005 Society for Neuroscience).

NR1 subunits may induce different structural mechanisms underlying agonist efficacy.

Finally, while agonist binding is essential for receptor gating, there is evidence to suggest that it may also be needed for cell surface expression. This was first observed by Grunwald & Kaplan (2003) with binding site and ion pore mutations in the non-NMDAR GLR-1 subunit from *Caenorhabditis elegans*. This agonist binding-dependent trafficking also occurs in mammalian cells for the cell surface expression of kainate receptors containing the rat GluR6 and KA2 subunits. Mutations in the GluR6 and KA2 subunits at equivalent positions to contact residues Arg485 and Thr655 in the GluR2 S1S2 cause the subunits to be retained in the endoplasmic reticulum although normal protein folding is still unchanged. How ligand binding occurs within the cell and its significance remains unclear (Mah *et al*., 2005; Valluru *et al*., 2005). Perhaps a 'quality assurance' mechanism is necessary to verify correct receptor protein folding and function in advance of cell surface expression. Hence for kainate receptors, at least, a ligand-binding site interaction

is necessary within the cell before the receptor reaches the cell surface.

Antagonist modelling at the NR2-glutamate pocket

The simulated docking of antagonists within NR2 homology models have predicted that the common NMDA competitive antagonist, D-AP5 (D-2-amino-5-phosphonopentanoic acid) shares similar binding pocket interactions to those of the α-amino-and α-carboxyl groups of glutamate (Tikhonova *et al*., 2002; Laube *et al*., 2004; Blaise *et al*., 2005). The phosphonium group of D-AP5 is predicted to interact with two lysine residues (Lys459 and Lys462 in NR2B) and agrees with mutagenesis studies on NR2B-containing recombinant NMDA receptors where point mutations at these residues reduce both agonist and antagonist potencies by more than 50-fold compared to wild type (Laube *et al*., 2004). However, this may not be apparent in the other NR2 subunits. The equivalent mutation at one of the lysine residues in NR2A and the NR2D subunits (NR2A-Lys465 and NR2D-Lys486) produced less than a 50-fold reduction in glutamate potency compared to a 180-fold reduction in the equivalent NR2B mutation (Laube *et al*., 1997; Anson *et al*., 1998; Chen, 2000; see Figure 5b). Although D-AP5 affinity was not examined in the NR2A mutation, it implies that antagonists and possibly agonists may interact differently at the NR2A and NR2B pockets.

Another inconsistent result is the role played by the threonine residue that interacts with the γ-carboxyl group of glutamate (NR2A-Thr671; NR2B-Thr665; Thr655 in GluR2). Expression of recombinant receptors containing a mutation at this position (NR2A(T671A)) reduced D-AP5 affinity (using Schild analysis) by over 250-fold compared to wild-type NR2A-containing receptors (Anson *et al*., 1998). However, NR2B models docked with D-AP5 predict that this residue does not interact with the antagonist (Tikhonova *et al*., 2002; Laube *et al*., 2004). In contrast to this, docking D-AP5 into a NR2A model has shown that the phosphonic group actually interacts with a number of residues in the S2 domain (Thr671 being one of them) rather than the two lysine residues predicted by NR2B docked models (Grazioso *et al*., 2005). Molecular dynamics simulations of competitive antagonists docked in the modelled pockets support this view and suggest that the distinct geometries of the NR2A- and NR2B-binding pockets create alterations in electrostatic potential that may contribute to differential antagonist selectivity (Blaise *et al*., 2005). Hence, either D-AP5 interacts differently within the NR2A- or NR2B-binding pockets or other unknown interactions (such as water bonds) exist which are beyond the predictive power of modelling studies. These discrepancies will be resolved with the availability of antagonist-bound NR2S1S2 crystal structures for each of the NR2 subunits.

One of the main driving forces behind antagonist modelling is in the design of subtype-specific drugs for each NR2 subunit. Since all residues identified by modelling studies as ligand binding are conserved among all four NR2 subunits, subtype selectivity is likely to be specified by other residues not involved in direct contact with the ligand. Thus, NR2 selective antagonists may have to be designed to interact with NR2 subunit-specific residues at the edge of the binding pocket but close enough to prevent agonist interaction (Kinarsky *et al*., 2005).

Conclusions

Although the information gathered from structural studies has allowed us to speculate the possible mechanisms behind glutamate receptor action following ligand interaction, we should pay attention to the fact that many of these observations have been made from studies of an isolated part of a receptor protein that is free from the influence of the rest of the subunit and other subunits within the receptor complex (Abele *et al*., 1999). In some studies, the use of mutations to facilitate structure–function studies has been employed. For example, the study of GluR2 by Jin *et al*. (2003) removed the confounding effects of desensitization when studying the relationship between agonist efficacy and domain closure by using the L483Y mutation or by carrying out their experiments in the presence cyclothiazide. Thus, it remains unclear how the variation in degrees of domain closure seen in the GluR2S1S2 protein between antagonists, full and partial agonists reflects actual domain motion within the native receptor complex. This will only be resolved when we are able to visualize the interactions and mechanics of the entire receptor in action. In this respect, it is interesting to note that different conformational states of the entire homomeric GluR2 receptor have been observed upon the application of glutamate and cyclothiazide (Nakagawa *et al*., 2005).

While homology modelling studies provide a plausible scheme of ligand-binding pocket interactions, they are limited and should be used to make predictions and test hypotheses concerning the interaction of a ligand with its binding site. For example, the contribution of water-bridged interactions within the NR2A ligand-binding pocket play important roles in ligand interactions and have not been considered in work using homology modelling of this and other NR2 subunits.

Despite these caveats, the structural information obtained from the GluR2-, GluR5/6-, NR1- and NR2-binding pockets have provided a great deal of information concerning the molecular basis of agonist and antagonist interactions and have also suggested mechanisms distinguishing the action of full agonists, partial agonists and antagonists – the central theme of receptor pharmacology. Hopefully this and information obtained from future studies will greatly aid in the design and development of new subtype-specific ligands and provide further insights into how conformational changes in the ligand-binding site lead to channel opening.

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