Glutamate Receptor Gating

Kevin Erreger,¹ Philip E. Chen,² David J.A. Wyllie,² & Stephen F. Traynelis¹

¹Department of Pharmacology, Emory University School of Medicine, Rollins Research Center, Atlanta, Georgia, USA;
²Division of Neuroscience, University of Edinburgh, Edinburgh, UK

Address all correspondence to Kevin Erreger, Department of Pharmacology, 5062 Rollins Research Center, 1510 Clifton Rd., Atlanta, GA 30322-3090; kerrege@emory.edu

ABSTRACT: Ionotropic glutamate receptors (iGluRs) mediate the vast majority of fast excitatory synaptic transmissions within the mammalian central nervous system (CNS). As for other ion channel protein families, there has been astounding progress in recent years in elucidating the details of protein structure through the crystallization of at least part of the ion channel protein complex. The result is a new framework for the interpretation of both classic and emerging functional data. Here we summarize, compare, and contrast recent findings for the AMPA, kainate, and NMDA subtypes of glutamate receptor ion channels, with an emphasis on the functional and structural aspects of how agonist binding controls channel gating.

KEYWORDS: NMDA, AMPA, kainate, ion channel, gating, kinetics
I. INTRODUCTION

Ionotropic glutamate receptors (iGluRs) are recognized as playing a major role in physiological and pathophysiological processes in the brain. While our understanding of the detailed mechanisms of how these channels are activated has lagged behind other channel families, such as the voltage-gated potassium channels and the pentameric ligand-gated channels typified by the nicotinic acetylcholine receptor [Armstrong et al. 2003, Miyazawa et al. 2003], significant progress has been made recently in elucidating the structure and function of ionotropic glutamate receptors [Gouaux 2004, Mayer & Armstrong 2004, McFeeters & Oswald 2004]. Although sequence similarity suggests a common architecture for all glutamate receptor ion channels, they can be subdivided into the following three major groups based on pharmacological properties and sequence homology: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate, and N-methyl-d-aspartate (NMDA) receptors [Dingledine et al. 1999]. 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 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 [Dingledine et al. 1999]. It is thought that functional NMDA receptors exist as heteromers containing two NR1 and two NR2 subunits arranged so that like subunits are adjacent [Béhé et al. 1995, Laube et al. 1998, Schorge & Colquhoun 2003]. NMDA receptors can also contain NR3A or NR3B subunits that modulate channel function. Genetic knockouts of NR3A result in increased amplitude of NMDA currents in acutely dissociated cerebrocortical neurons and an increase in the number of dendritic spines in the cortex [Das et al. 1998]. Surprisingly, the NR3 subunit, when co-assembled with NR1, can form cation channels that are only activated by glycine [Chatterton et al. 2002]. AMPA, kainate, and NMDA receptors differ in many of their functional properties, including both activation and gating [Madden 2002, Qian & Johnson 2002]. In this review we summarize, compare, and contrast recent findings on glutamate receptor ion channels, focusing on the functional and structural aspects of how agonist binding controls channel gating. While some progress has been made in elucidating the function of the delta orphan receptors [Yuzaki 2003], we will restrict the scope of our discussion to activation and gating of AMPA, kainate, and NMDA receptors. We refer the reader to several excellent reviews for other topics such as receptor localization, receptor trafficking, subunit assembly, receptor modulation, desensitization, and detailed structural features of ligand binding [Wenthold et al. 2003, Bredt & Nicoll 2003, McGee & Bredt 2003, MacDonald et al. 2001, Song & Huganir 2002, Frandsen & Schousboe 2003, Gouaux 2004, Mayer & Armstrong 2004].

II. ARCHITECTURE OF THE GLUTAMATE RECEPTORS

Four distinct regions can be identified within each iGluR subunit (Fig. 1)—an extracellular amino terminal domain (ATD), an S1S2 ligand binding domain, a membrane associated domain, and an intracellular carboxy terminal domain. The ATD shows sequence homology with the bacteria periplasmic leucine/isoleucine/valine binding protein (LIVBP) and contains within it residues that, in the case of NMDA receptor subunits, interact with allosteric modulators [Gallagher et al. 1996, Choi & Lipton 1999, Low et al. 2000, Paolletti et al. 2000, Perin-Dureau et al. 2002] and influence desensitization [Villaruel et al. 1998, Krupp et al. 1998, Zheng et al. 2001]. This region also is proposed to be involved in the assembly of iGluRs [Leuschner & Hoch 1999, Meddows et al. 2001, Ayalon & Stern-Bach 2001],
FIGURE 1. The molecular organization of ionotropic glutamate receptors. The linear topology plot shows the location of functional domains and transmembrane regions of an individual subunit. Both NMDA and non-NMDA receptors have a tetrameric stoichiometry. A detailed cartoon is displayed for one subunit for each receptor showing the functional domains and sites of interaction with selected modulators.

Although NR2A or GluR4 with the ATD deleted can still form functional channels [Fayyazuddin et al. 2000, Pasternack et al. 2002].

The S1S2 ligand binding domain shares homology with the Escherichia coli glutamine binding protein and forms a clamshell-like structure from residues preceding the M1 transmembrane domain (S1) and residues located in the extracellular loop between transmembrane domains 3 and 4 (S2) [Stern-Bach et al. 1994, Paas 1998]. The crystal structure of this region for GluR2 and NR1 subunits, as well as bacterial GluR0, has been elucidated by the work of Gouaux and colleagues in recent years [reviewed by Gouaux 2004, Mayer & Armstrong 2004; see Table 1 for complete references] and has provided us with a wealth of information in regard to the ways that ligands interact with the binding pocket.

In contrast to the nicotinic acetylcholine receptor super-family of ligand-gated ion channels, iGluRs contain only three membrane spanning regions with an intervening re-entrant loop (P-loop) that contains residues that contribute to the lumen of the ion chan-
## TABLE 1. Glutamate Receptor Crystal Structures

<table>
<thead>
<tr>
<th>Crystal</th>
<th>Ligand</th>
<th>Closure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GluR2 S1S2I</td>
<td>kainate</td>
<td></td>
<td>Armstrong et al. 1998</td>
</tr>
<tr>
<td>GluR2 S1S2J</td>
<td>(apo)</td>
<td>2°</td>
<td>Armstrong et al. 2000</td>
</tr>
<tr>
<td>GluR2 S1S2J</td>
<td>glutamate</td>
<td>20°</td>
<td>Armstrong et al. 2000</td>
</tr>
<tr>
<td>GluR2 S1S2J</td>
<td>AMPA</td>
<td>20°</td>
<td>Armstrong et al. 2000</td>
</tr>
<tr>
<td>GluR2 S1S2J</td>
<td>kainate</td>
<td>12°</td>
<td>Armstrong et al. 2000</td>
</tr>
<tr>
<td>GluR2 S1S2J</td>
<td>DNQX</td>
<td>4°</td>
<td>Armstrong et al. 2000</td>
</tr>
<tr>
<td>GluR2 S1S2J</td>
<td>quisqualate</td>
<td>20°</td>
<td>J in et al. 2002</td>
</tr>
<tr>
<td>GluR2 S1S2J</td>
<td>ACPA</td>
<td>20°</td>
<td>Hogner et al. 2002</td>
</tr>
<tr>
<td>GluR2 S1S2J</td>
<td>2-Me-Tet-AMPA</td>
<td>20°</td>
<td>Hogner et al. 2002</td>
</tr>
<tr>
<td>GluR2 S1S2J</td>
<td>Br-HIBO</td>
<td>18°</td>
<td>Hogner et al. 2002</td>
</tr>
<tr>
<td>GluR2 S1S2J</td>
<td>willardiine</td>
<td>16°</td>
<td>J in &amp; Gouaux 2003, J in et al. 2003</td>
</tr>
<tr>
<td>GluR2 S1S2J</td>
<td>5-fluorowillardine</td>
<td>15°</td>
<td>J in &amp; Gouaux 2003, J in et al. 2003</td>
</tr>
<tr>
<td>GluR2 S1S2J</td>
<td>5-bromowillardine</td>
<td>15°</td>
<td>J in &amp; Gouaux 2003, J in et al. 2003</td>
</tr>
<tr>
<td>GluR2 S1S2J</td>
<td>5-iodowillardine</td>
<td>11°</td>
<td>J in &amp; Gouaux 2003, J in et al. 2003</td>
</tr>
<tr>
<td>GluR2 S1S2J</td>
<td>S-ATPA</td>
<td>21°</td>
<td>Lunn et al. 2003</td>
</tr>
<tr>
<td>GluR2 S1S2J (L483Y)</td>
<td>AMPA</td>
<td></td>
<td>Sun et al. 2002</td>
</tr>
<tr>
<td>GluR2 S1S2J (L483Y)</td>
<td>DNQX</td>
<td></td>
<td>Sun et al. 2002</td>
</tr>
<tr>
<td>GluR2 S1S2J (L483Y/L650T)</td>
<td>AMPA</td>
<td></td>
<td>Armstrong et al. 2003</td>
</tr>
<tr>
<td>GluR2 S1S2J (N 754D)</td>
<td>kainate</td>
<td></td>
<td>Sun et al. 2002</td>
</tr>
<tr>
<td>GluR2 S1S2J (N 754S)</td>
<td>glutamate/cyclothiazide</td>
<td></td>
<td>Sun et al. 2002</td>
</tr>
<tr>
<td>GluR2 S1S2J (Y702F)</td>
<td>ACPA</td>
<td>20°</td>
<td>Hogner et al. 2002</td>
</tr>
<tr>
<td>GluR2 S1S2J (Y702F)</td>
<td>Br-HIBO</td>
<td>18°</td>
<td>Hogner et al. 2002</td>
</tr>
<tr>
<td>GluR2 S1S2J (L650T)</td>
<td>AMPA</td>
<td></td>
<td>Armstrong et al. 2003</td>
</tr>
<tr>
<td>GluR2 S1S2J (L650T)</td>
<td>kainate</td>
<td></td>
<td>Armstrong et al. 2003</td>
</tr>
<tr>
<td>GluR2 S1S2J (L650T)</td>
<td>quisqualate</td>
<td></td>
<td>Armstrong et al. 2003</td>
</tr>
<tr>
<td>GluR0</td>
<td>glutamate</td>
<td></td>
<td>Mayer et al. 2001</td>
</tr>
<tr>
<td>GluR0</td>
<td>L-serine</td>
<td></td>
<td>Mayer et al. 2001</td>
</tr>
<tr>
<td>NR1 S1S2</td>
<td>glycine</td>
<td>21°</td>
<td>Furukawa &amp; Gouaux, 2003</td>
</tr>
<tr>
<td>NR1 S1S2</td>
<td>D-serine</td>
<td>21°</td>
<td>Furukawa &amp; Gouaux, 2003</td>
</tr>
<tr>
<td>NR1 S1S2</td>
<td>D-cycloserine</td>
<td>21°</td>
<td>Furukawa &amp; Gouaux, 2003</td>
</tr>
<tr>
<td>NR1 S1S2</td>
<td>5,7 dichlorokynurenate</td>
<td></td>
<td>Furukawa &amp; Gouaux, 2003</td>
</tr>
</tbody>
</table>
nel pore and control ion selectivity and permeability, including the so-called QRN site at which RNA editing can occur for the GluR2 subunit.

The presence of three transmembrane segments results in the carboxy terminus of iGluRs being located intracellularly. The carboxy terminus domain contains residues that interact with numerous membrane scaffolding and signal transduction proteins, and this region is an important control point for intracellular trafficking, regulation, and localization [Sheng 2001, Wenthold et al. 2003, Bredt & Nicoll 2003].

As yet, we do not know the crystal structure for any complete iGluR subunit; however, the recent studies of Gouaux and colleagues have provided us with much information about the structure of the ligand binding site of GluR2 and NR1 receptor subunits [Gouaux 2004, Mayer & Armstrong 2004]. In these studies the crystal structure of soluble proteins comprising the S1 and S2 domains joined (artificially) by a short linker (e.g., glycine-threonine) have been elucidated by X-ray diffraction. Although the ATD, the membrane-associated region, and the intracellular carboxy terminal are all absent in such structures, the information provided for these studies together with that from a variety of complementary functional studies has given us tremendous insight as to the way in which agonists, partial agonists, and antagonists interact with specific residues and waters within the binding pocket. A list of published crystal structures with ligands is given in Table 1.

III. MOLECULAR BASIS FOR LIGAND BINDING

III.A. Ligand Binding to GluR2 AMPA Receptor Subunit

Early investigations [Stern-Bach et al. 1994, Arvola & Keinanen 1996] of the S1S2 region suggested that this region could form an autonomous bi-lobed or clamshell-like structure when the intervening transmembrane regions between S1 and S2 are replaced by a short peptide linker. The elucidation of the ligand interactions with iGluRs that has come from studies of the crystal structure of the S1S2 agonist binding domain of the AMPA GluR2 subunit has shown this to be the case (Fig. 2).

Crystal structures with kainate [Armstrong et al. 1998] and subsequently glutamate, AMPA, and 5,6-dinitroquinoxalinedione (DNQX) [Armstrong & Gouaux, 2000] were the first ligand-bound structures to be reported, and have had a major impact on the way in which receptor structure and function are viewed.

The structure of the GluR2 S1S2 domain is similar to the related bacterial amino acid binding proteins [O’Hara et al. 1993, Felder et al. 1999]. Crystals form of dimerized subunits, suggesting that tetrameric AMPA receptors may be a dimer of dimers. It has subsequently been reported that electron microscopic images of the purified recombinant protein for the full GluR2 channel show two-fold rotation symmetry [Safferling et al. 2001]. There is also functional evidence for a dimer of dimers architecture for both AMPA and NMDA receptors [Robert et al. 2001, Bowie & Lange 2002, Sun et al. 2002, Schorge & Colquhoun 2003]. The crystal structure of agonists complexed with GluR2 S1S2I and S1S2J (I and J denote different length linkers) each showed the ligand recognition site being located in a pocket between the two lobes, which allowed the determination of the residues in the protein that form hydrogen bonds with the ligand that occupies the binding site.

These studies also suggested that indirect hydrogen bonding of ligands to residues in S1S2 via water molecules is important. Six residues in the GluR2 S1S2 construct are thought to hydrogen bond directly with glutamate, these being Pro478, Thr480, and Arg485 (S1 domain) and Ser654, Thr655, and Glu705 (S2 domain). In addition, Tyr450 in GluR2 forms an electron-dense ring structure above the ligand-binding site. Of these, four are conserved in the homologous positions in NR2 (glutamate binding) NMDA receptor subunits, with the exceptions being a histidine residue replacing Tyr450, a serine residue replacing Pro478, and an aspartic acid residue replacing Glu705. When either glutamate, AMPA, or kainate occupy the binding site, the orientation of their $\alpha$-carboxyl
FIGURE 2. The structure of the S1S2 ligand binding domain for GluR2 and implications for NMDA receptors. (A) Ribbon diagram of the structure of the S1 and S2 domains in the GluR2 receptor subunit showing kainate located in the binding site. The S1 and S2 domains predominantly contribute to lobe I and lobe II, respectively, of the ligand binding core. (B) Higher magnification of the binding pocket reveals the residues making direct contact with the ligand. The residues making direct contact with the ligand are highlighted. The structures were obtained using data from Armstrong et al. (1998) and generated with the program RasMol. (C) A schematic representation shows contact residues and hydrogen bonds for the glutamate complexed with GluR2. Color coding for atoms in the binding pocket are carbon = black, oxygen = red, and nitrogen = blue. Note that the tyrosine residue (Y450) and the glutamate residue (E705) sit “above” and “below” the binding site, respectively. (D) Partial amino acid alignments showing the location of known ligand binding residues (bold) identified by structural studies in the GluR2 and NR1 S1 S2 regions. The analogous residues in the NR2A and NR2B subunits have also been highlighted (see Table 2). The amino acid sequence is numbered according to the predicted mature polypeptide [Monyer et al 1992].

and α-amino groups and their hydrogen bonding to the residues Arg485, Glu705, Thr480, and Pro478 are superimposable with a tetrahedral-like structure formed between the α-amino group of the ligands and the residues Pro478, Thr480, and Glu705 (see Fig 2C). Mutagenesis studies have emphasized the importance
of the conserved Arg485 residue in mediating agonist binding, because all function is lost when this residue (or the analogous residue in NR2 NMDA receptor subunits) is changed to a charge-conserving lysine residue [Kawamoto et al. 1997, Lampinen et al. 2002, Laube et al. 1997, Chen et al. 2004].

In contrast, while the γ-carboxyl of glutamate and kainate interact with the same subsites, the isoxazole group of AMPA interacts with different subsites. The binding pocket has been hypothesized to contain two distinct regions (lobe I and lobe II, see Fig. 2A), with very little internal motion for residues contacting α-substituents compared to residues contacting γ-substituents [McFeeters & Oswald 2002]. When AMPA occupies the binding site, a water molecule is recruited to the position normally occupied by the γ-carboxyl group of glutamate, thus converting AMPA to a bioisosteric mimic of glutamate [Armstrong & Gouaux 2000]. Functional studies with GluR1-GluR3 chimeric and mutant receptors have suggested that the interactions of another water molecule within the binding cleft may have functional effects on ligand binding and receptor activation [Banke et al. 2001].

Elucidation of the structure of the GluR2 binding domain has allowed visualization of the way in which the competitive antagonist, DNQX, acts to block the action of glutamate (and other agonists). As is seen with the α-carboxyl binding of glutamate, the two carbonyl groups of DNQX form hydrogen bonds with Thr480 and Arg485 located in domain 1. Additional hydrogen bonds formed between DNQX and Pro478 and via a water molecule to Tyr405 hold this antagonist in the binding pocket, preventing access to glutamate (and other agonists). An aromatic residue (Tyr450) is oriented parallel to DNQX to produce stabilizing π-interactions [Armstrong & Gouaux 2000].

In all complexes of ligands with the GluR2 S1S2, there is discernable closure of the angle formed by the two lobes around the ligand compared to the unliganded apo state. Even antagonists such as DNQX cause a small amount (around 2.5°) of domain closure, but clearly this is insufficient to cause any conformational change that would lead to channel opening, because DNQX-gated currents have not been described for wild-type AMPA receptors.

One fascinating feature of the glutamate receptor family is the beautiful illustration of how agonist efficacy correlates with the change in closure of the clamshell-like domain as viewed in crystal structures [Gouaux & Armstrong, 2000, Jin et al. 2003, Armstrong et al. 2003]. Whereas antagonists cause minimal changes in degree of domain closure, agonists cause much larger changes. Partial agonists induced intermediate changes, and the degree of domain closure appears to correlate with the relative efficacy [Armstrong & Gouaux, 1998, Jin et al. 2003]. This relationship is most striking for a series of 5-substituted willardiines that differ in the size of a halide substituent, for which larger substituents sterically hinder domain closure [Jin et al. 2003]. Gouaux and colleagues have hypothesized that the closure of the domain creates sufficient intra-protein strain to cause either rearrangement of intramembrane helices that lead to gating or breakdown of the subunit dimer interface that leads to desensitization (see below).

Despite the important findings obtained with the crystal structure, caveats still remain regarding the interpretation of structural data from the engineered binding domain excised from a much larger protein. For example, the properties of the isolated domain in crystallized form may not correspond to those in solution [Abele et al. 1999]. Also, the short amino acid linker may impart artifactual restrictions in movement; perhaps the two lobes in native receptors have greater degrees of freedom. Furthermore, it remains unclear whether the partial degree of domain closure observed with ligand-complexed GluR2 S1S2 crystals reflects a similar physical conformation in native receptors. Alternatively, the degree of domain closure in the crystal structure instead may reflect the relative energetic stability of rapidly interconverting open and closed conformations of the S1S2 clamshell domain. The hinge of bacterial amino acid binding proteins has been demonstrated to exhibit motion in the time range of 10–1000 s⁻¹ [Careaga et al. 1995]. NMR and spectroscopic studies also support considerable intra-protein movement within the S1S2 ligand binding.

III.B. Ligand Binding to NR1 NMDA Receptor Subunit

To date, the only crystal structure of a ligand-binding domain of an NMDA receptor subunit is that of the co-agonist glycine binding, NR1 receptor subunit [Furukawa & Gouaux 2003], and thus our knowledge is well behind that of AMPA iGluRs. The NR1 S1S2 crystal, like that of GluR2 S1S2, shows a general clam-shell design, although there are differences in the organization of the various loops and helices between the two structures [see Furukawa & Gouaux 2003]. As is the case with ligand binding in the GluR2 S1S2 fragment, glycine makes both direct and indirect (via five water molecules) hydrogen bonding with residues located in both the S1 and S2 domains. The importance of the conserved arginine residue in the binding pocket is revealed in this structure, where the guanidinium group of Arg523 hydrogen bonds with the α-carboxyl group of glycine—the equivalent interaction in GluR2 S1S2 being mediated by Arg485. Furukawa & Gouaux [2003] superimposed the ligand-binding pockets of the NR1 and GluR2 S1S2 fragments to examine why the NR1 site cannot bind glutamate. The authors proposed that (1) position 655 in GluR2 (and indeed in all subunits that bind glutamate) is a threonine, in which the hydroxyl group hydrogen bonds to a γ-carboxylate oxygen of glutamate, whereas in NR1 this position (689) is occupied by a valine residue; and (2) the indole ring of a tryptophan residue at position 731 in NR1 sterically prevents the γ-carboxylate of glutamate from occupying the binding site, whereas in GluR2 the equivalent site is occupied by a leucine (Leu 704) that adopts a position such that it faces away from the binding pocket.

As is the case with GluR2 S1S2, agonist binding to the NR1 S1S2 causes domain closure. In contrast, however, to the findings that the degree of domain closure in GluR2 is related to agonist “efficacy,” the partial agonist, D-cycloserine, causes closure to a similar extent as that seen with the full agonists, glycine and D-serine. It remains to be seen whether other partial agonists at the glycine site also give rise to conformations that resemble those seen with full agonists, indicating perhaps a difference in the way that partial agonists exert their actions at this receptor site. As has been reported for DNQX at the GluR2 S1S2, the competitive glycine site antagonist, 5,7-dichlorokynurenic acid, holds the NR1 S1S2 in an open conformation and again, like DNQX, appears to interact with residues, mainly within the S1 domain. By depriving glycine of perhaps its initial contact sites with the open cleft of the binding pocket, 5,7-dichlorokynurenic acid can prevent glycine binding.

III.C. Ligand Binding to NR2 NMDA Receptor Subunit

Although as yet there is no high-resolution structural data on ligand binding to the NR2 NMDA receptor subunit, a number of predictions can be made. The resolution of crystal structures of the GluR2 and NR1 S1S2 domains have identified residues that make direct contact with the ligand (see, for example, Fig. 2C) and thus have provided insight into how the binding process may occur for NR2. Moreover, there is a great deal of sequence homology between the NMDA and non-NMDA receptor subunits in the agonist binding region. The six residues that lie in direct contact with the ligand are largely conserved among the glutamate binding sites for AMPA and NR2 subunits (see Fig. 2D), the exceptions being the serine in the S1 region of the NR2 subunits (S492 in NR2A, S486 in NR2B), which is replaced by a proline in GluR2 (Pro478) and an aspartate residue in the S2 region (D712 in NR2A and D706 in NR2B). 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 receptor family.

Most of the present ideas about glutamate binding to NR2 can be inferred from structure/function studies and site-directed mutagenesis in the NR2A/B
TABLE 2. Critical Residues for Ligand Binding to NMDA Receptors

<table>
<thead>
<tr>
<th>GluR2 S1S2</th>
<th>NR1 S1S2</th>
<th>NR2A</th>
<th>NR2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y450 ( ^a )</td>
<td>P516 ( ^b )</td>
<td>H466A (220) ( ^e,f )</td>
<td>H460F (9) ( ^d )</td>
</tr>
<tr>
<td>P478 ( ^a )</td>
<td>T518 ( ^b )</td>
<td>S492A (50) ( ^f )</td>
<td>S486A (43) ( ^d )</td>
</tr>
<tr>
<td>T480 ( ^a )</td>
<td>R523 ( ^b )</td>
<td>T494A (146) ( ^f )</td>
<td>ND</td>
</tr>
<tr>
<td>R485 ( ^a )</td>
<td>S688 ( ^b )</td>
<td>R499K (NF) ( ^f )</td>
<td>R493K (NF) ( ^d )</td>
</tr>
<tr>
<td>S654 ( ^a )</td>
<td>S670G (145) ( ^f )</td>
<td>S664G (118) ( ^d )</td>
<td>ND</td>
</tr>
<tr>
<td>T655 ( ^a )</td>
<td>T671A (1023) ( ^e )</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>E705 ( ^a )</td>
<td>D732 ( ^b )</td>
<td>D712E (NF) ( ^c,f \ast )</td>
<td>D706E (NF) ( ^c \ast )</td>
</tr>
</tbody>
</table>

Residues directly involved in agonist binding and the changes in glutamate potency (parentheses) found in recombinant NR1/NR2 receptors containing point mutations within the S1/S2 regions. Equivalent contact residues identified from the GluR2 and NR1 S1S2 structural data are included for comparison. NF indicates nonfunctional, ND indicates not determined. The amino acid sequence is numbered according to the predicted mature polypeptide.

\( ^a \) Armstrong et al. (1998, 2000).
\( ^b \) Furukawa and Gouaux [2003].
\( ^c \) Williams et al. (1996).
\( ^d \) Laube et al. (1997).
\( ^e \) Anson et al. (1998).
\( ^f \) Chen et al. [2004].
\( ^\ast \) Residues numbered as NR2A(D731) and NR2B(D732) in Williams et al. (1996).

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 may participate directly in hydrogen bonding.

A number of residues that probably make direct contact with the ligand have been identified using this approach, 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 (Thr655 in GluR2), and NR2A-Asp712/NR2B-Asp706 (Glu705 in GluR2). In addition, mutation of NR2A-His466/NR2B-His460 (Tyr450 in GluR2) also causes a reduction in glutamate potency [Williams et al. 1996, Laube et al. 1997, Anson et al. 1998, Chen et al. 2004; see Table 2]. The largest shift in potency reported occurs with the substitution of the Thr671 residue in the NR2A subunit with an alanine residue, which reduces glutamate potency by more than three orders of magnitude (1023-fold) and reduces D-AP5 (a competitive glutamate site antagonist) binding 255-fold [Anson et al. 1998]. Mutation of either of the charged residues within the binding pocket of NR2 subunits, NR2A-Arg499/NR2B-Arg493 and NR2A-Asp712/NR2B-Asp706, with lysine or glutamate, respectively, renders channels non-functional [Williams et al. 1996, Laube et al. 1997, Chen et al. 2004]. These functional studies confirm the importance for NMDA receptors of the contact residues identified in GluR2. Structural and functional data show that the guanidinium group from the arginine (Arg485 in GluR2) forms an essential tether with the $\alpha$-carboxyl group of glutamate (or glycine for NR1). The backbone amide group of Thr655 in GluR2 has been shown to interact with the $\gamma$-carboxyl group of glutamate. It is likely that similar interactions would occur in the NR2 binding site.
Interestingly, a nonconservative substitution within the binding site can result in an unaltered phenotype. A serine-to-alanine mutation at position 670 in NR2A (Ser654 in GluR2) [Anson et al. 1998] had little effect on glutamate potency. However, a substantial shift in potency was observed when this residue was substituted by a glycine [Laube et al. 1997, Chen et al. 2004]. How this could be interpreted should be clearer once the structural information from the NR2 binding site is obtained.

Finally, if one examines the amino acid sequence alignment of the non-glutamate binding NR3A and B subunits, the only direct contact residues absent are the two threonine residues in the S1 and S2 regions (Thr480 and Thr655 in GluR2). The threonine residue in the S2 region is conserved in all glutamate binding ionotropic receptors, and, intriguingly, in the NR3 subunits this is substituted with an alanine—this, in part, may explain the insensitivity of the NR3 subunits to activation by glutamate. However, because functional channels cannot be formed from the expression of NR3 subunits alone, it is possible that they themselves do not bind any ligand but simply act to modulate the function properties of receptor complexes [Perez-Otano et al. 2001].

IV. CHANNEL ACTIVATION AND GATING: AMPA/KAINATE RECEPTORS

IV.A. Functional Features of AMPA/KAINATE Receptor Activation

AMPA receptors bind the native transmitter glutamate with uniformly low affinity (Tables 3 and 4). Agonist binding induces rapid opening to multiple conductance levels with a high peak open probability (Tables 3 and 4). All AMPA receptors subsequently undergo rapid desensitization when activated by glutamate. The desensitized state is often hypothesized to be nonconducting and have a higher agonist affinity than the unbound undesensitized receptor (Table 4), but alternative hypotheses have been suggested [Bowie & Lange 2002, Robert & Howe 2003]. Agonist EC₅₀, affinity, activation, and desensitization properties can all vary with alternate splicing, with RNA editing, and among the various subunits (Tables 3 and 4), providing the CNS with a means for fine tuning excitatory synaptic function.

AMPA receptors exhibit subunit-specific functional properties that are additionally regulated by both alternative splicing and post-translational RNA editing at the glutamine–arginine–asparagine (QRN) site [for a review see Dingledine et al. 1999]. This site is located near the apex of the re-entrant pore loop of the second membrane associated region (Fig. 1) and is thought to form or be near the narrowest constriction of the ion permeation pathway. Amino acid substitutions at this position critically influence calcium permeability, rectification properties stemming from polyamine block, and single-channel conductance, suggesting that the QRN site may control permeation properties in wild-type channels. The overwhelming majority of GluR2 is edited post-transcriptionally from glutamine (Q) to arginine (R) in most tissues [Seeburg & Hartner 2003], but tissue from malignant brain tumor shows substantially lower editing [Maas et al. 2001]. GluR2(R)-containing receptors exhibit much lower calcium permeability than do receptors that lack this subunit [Hollman et al. 1991, Hume et al. 1991, Burnashev et al. 1992a]. At least three conductance levels are apparent in GluR2-lacking recombinant AMPA receptors (see Table 3). Incorporation of GluR2 receptor subunits to heteromeric assemblies of AMPA receptors dramatically reduces single-channel conductance. Moreover, homomeric GluR2(R) channels have a mean single channel conductance in the femtosiemen range (~0.3 pS) [Swanson et al. 1997b]. Similar to GluR2, Q to R editing of kainate receptors dramatically reduces both calcium permeability and channel conductance [Burnashev et al. 1995, Swanson et al. 1996]. Alternative splicing of the “flip/flop” region of AMPA receptors controls the rate of onset and recovery of desensitization, as well as providing a target for splice-variant specific pharmacology [Sommer et al. 1990, Mosbacher et al. 1994, Partin et al. 1994, 1995, 1996, Sekiguchi et al. 2002; see Table 3].
## TABLE 3. Kinetic Parameters Describing Glutamate Activation of Wild Type AMPA, Kainate, and NMDA Receptors

<table>
<thead>
<tr>
<th></th>
<th>Peak EC50&lt;sup&gt;a&lt;/sup&gt; µM</th>
<th>τ-Deactivate&lt;sup&gt;b&lt;/sup&gt; ms</th>
<th>τ-Desensitize&lt;sup&gt;b&lt;/sup&gt; ms</th>
<th>τ-Recovery&lt;sup&gt;c,h&lt;/sup&gt; ms</th>
<th>SS/Peak ratio</th>
<th>Peak Popen&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Open Time&lt;sup&gt;b&lt;/sup&gt; ms</th>
<th>Conductance pS&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>GluR1-flip</td>
<td>500–700&lt;sup&gt;11,12&lt;/sup&gt;</td>
<td>0.7–1&lt;sup&gt;12&lt;/sup&gt;</td>
<td>2.5–4&lt;sup&gt;12&lt;/sup&gt;</td>
<td>111–147&lt;sup&gt;12,12&lt;/sup&gt;</td>
<td>0.002–0.032&lt;sup&gt;2,4&lt;/sup&gt;</td>
<td>0.4–1&lt;sup&gt;0.4,5,d&lt;/sup&gt;</td>
<td>0.2–0.9&lt;sup&gt;4&lt;/sup&gt;</td>
<td>5, 14, 20&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>GluR1-fbp</td>
<td>0.86–1&lt;sup&gt;2,3,6&lt;/sup&gt;</td>
<td>3.2–3.8&lt;sup&gt;2,3,6&lt;/sup&gt;</td>
<td>147–155&lt;sup&gt;2,6&lt;/sup&gt;</td>
<td>0.023–0.080&lt;sup&gt;2,6,7&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GluR2-flipQ&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1390&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.62&lt;sup&gt;8&lt;/sup&gt;</td>
<td>5.9&lt;sup&gt;8&lt;/sup&gt;</td>
<td>11.7&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.066&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.61&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.32,1.47&lt;sup&gt;9&lt;/sup&gt;</td>
<td>7, 11, 18&lt;sup&gt;9&lt;/sup&gt;</td>
</tr>
<tr>
<td>GluR2-fbpQ&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1380&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.54&lt;sup&gt;8&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;8&lt;/sup&gt;</td>
<td>31.3&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.011&lt;sup&gt;8&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GluR3-flip</td>
<td>1970&lt;sup&gt;7,f&lt;/sup&gt;</td>
<td>0.56&lt;sup&gt;7&lt;/sup&gt;</td>
<td>3.0–5.1&lt;sup&gt;7,11&lt;/sup&gt;</td>
<td>15–70&lt;sup&gt;7,10&lt;/sup&gt;</td>
<td>0.024–0.054&lt;sup&gt;7,10&lt;/sup&gt;</td>
<td>0.82&lt;sup&gt;7,f&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GluR3-flop</td>
<td>1780&lt;sup&gt;7,f&lt;/sup&gt;</td>
<td>0.63–1.05&lt;sup&gt;6,7&lt;/sup&gt;</td>
<td>1.1–2.3&lt;sup&gt;6,7,11&lt;/sup&gt;</td>
<td>55–142&lt;sup&gt;6,7&lt;/sup&gt;</td>
<td>0.017</td>
<td>0.42&lt;sup&gt;7,f&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GluR4-flip</td>
<td>1810&lt;sup&gt;5,f&lt;/sup&gt;</td>
<td>0.63</td>
<td>3.6&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6–21&lt;sup&gt;10,12&lt;/sup&gt;</td>
<td>0.006–0.04&lt;sup&gt;10&lt;/sup&gt;</td>
<td>0.77&lt;sup&gt;5,f&lt;/sup&gt;</td>
<td>0.14, 3.3&lt;sup&gt;13&lt;/sup&gt;</td>
<td>8, 15, 24&lt;sup&gt;13&lt;/sup&gt;</td>
</tr>
<tr>
<td>GluR4-flop</td>
<td>1700&lt;sup&gt;5,f&lt;/sup&gt;</td>
<td>0.63</td>
<td>3.6&lt;sup&gt;3&lt;/sup&gt;</td>
<td>15–70&lt;sup&gt;7,10&lt;/sup&gt;</td>
<td>0.006–0.04&lt;sup&gt;10&lt;/sup&gt;</td>
<td>0.77&lt;sup&gt;5,f&lt;/sup&gt;</td>
<td>0.14, 3.3&lt;sup&gt;13&lt;/sup&gt;</td>
<td>8, 15, 24&lt;sup&gt;13&lt;/sup&gt;</td>
</tr>
<tr>
<td>GluR5a Q&lt;sup&gt;e&lt;/sup&gt;</td>
<td>630&lt;sup&gt;14&lt;/sup&gt;</td>
<td>4.1–8.9&lt;sup&gt;14,15&lt;/sup&gt;</td>
<td>50,5100&lt;sup&gt;16,9&lt;/sup&gt;</td>
<td>0.0116</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GluR6 Q&lt;sup&gt;e&lt;/sup&gt;</td>
<td>500&lt;sup&gt;18&lt;/sup&gt;</td>
<td>2.516</td>
<td>4.3–4.9&lt;sup&gt;18,19&lt;/sup&gt;</td>
<td>1900–3020&lt;sup&gt;18,20&lt;/sup&gt;</td>
<td>0.008–0.04&lt;sup&gt;18,19&lt;/sup&gt;</td>
<td>0.5–1.0&lt;sup&gt;18,d&lt;/sup&gt;</td>
<td>0.6,2.3&lt;sup&gt;17&lt;/sup&gt;</td>
<td>8, 15, 25&lt;sup&gt;17&lt;/sup&gt;</td>
</tr>
<tr>
<td>GluR7&lt;i&gt;a&lt;/i&gt;</td>
<td>5900&lt;sup&gt;21&lt;/sup&gt;</td>
<td>8.4–9&lt;sup&gt;21&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;21&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR1/NR2A</td>
<td>1.8–7.7&lt;sup&gt;22,23&lt;/sup&gt;</td>
<td>22–230&lt;sup&gt;24,31,34&lt;/sup&gt;</td>
<td>386–750, 2000&lt;sup&gt;24,34&lt;/sup&gt;</td>
<td>618&lt;sup&gt;24&lt;/sup&gt;</td>
<td>0.28–42&lt;sup&gt;25,26&lt;/sup&gt;</td>
<td>0.36–0.50&lt;sup&gt;27,36,37&lt;/sup&gt;</td>
<td>0.06,1.3,6&lt;sup&gt;28–30&lt;/sup&gt;</td>
<td>51, 38&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td>NR1/NR2B</td>
<td>0.9–4&lt;sup&gt;22,23,32&lt;/sup&gt;</td>
<td>71–95,538–67&lt;sup&gt;24,32&lt;/sup&gt;</td>
<td>100,495&lt;sup&gt;32&lt;/sup&gt;</td>
<td>1014–2100&lt;sup&gt;24,32&lt;/sup&gt;</td>
<td>0.027–0.53&lt;sup&gt;32,12&lt;/sup&gt;</td>
<td>0.07–0.17&lt;sup&gt;27,32,37&lt;/sup&gt;</td>
<td>0.6,2–3.2&lt;sup&gt;32&lt;/sup&gt;</td>
<td>51,39&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td>NR1/NR2C</td>
<td>1.0&lt;sup&gt;33,k&lt;/sup&gt;</td>
<td>260–382&lt;sup&gt;24,31,34&lt;/sup&gt;</td>
<td>NA</td>
<td>1.0&lt;sup&gt;34&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR1/NR2D</td>
<td>0.4&lt;sup&gt;35,k&lt;/sup&gt;</td>
<td>1700–4408&lt;sup&gt;24,28&lt;/sup&gt;</td>
<td>NA</td>
<td>1.0&lt;sup&gt;28&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;28&lt;/sup&gt;</td>
<td>0.10,9,2&lt;sup&gt;2,28&lt;/sup&gt;</td>
<td>35,17&lt;sup&gt;25&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Peak response to rapid agonist application. <sup>b</sup>Measurements from outside out patches; NMDA conductance measured in 1.0 Ca++. <sup>c</sup>See Lomeli et al. for RNA editing control of tau recovery. <sup>d</sup>Open depends on PKA. <sup>e</sup>Edited receptors or mutant receptors with a glutamine at the Q/R/N site. <sup>f</sup>Predicted from simulations using rate constants. <sup>g</sup>Onset and recovery from desensitization is variable from cell to cell. <sup>h</sup>The rate of recovery from desensitization is more complex; see Bowie 2002, Robert & Howe 2003. <sup>i</sup>Splice variants 7a and 7b have similar rates. <sup>j</sup>SS/Peak current ratio is typically higher in whole cell recordings. <sup>k</sup>Determined in Xenopus laevis oocytes. NA not applicable; NR1/NR2C and NR1/NR2D receptors show no desensitization in the continued presence of agonist.

### TABLE 4. Kinetic Modeling of AMPA and Kainate Receptor Function

<table>
<thead>
<tr>
<th>Reference</th>
<th>Receptor</th>
<th>Preparation</th>
<th>Agonist</th>
<th>Modulator</th>
<th>Agonist Koff/Kon mM</th>
<th>Desens. Koff/Kon mM</th>
<th>Opening rate s⁻¹</th>
<th>Peak P(open)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jonas et al. [1993]</td>
<td>Neuronal AMPA</td>
<td>hippocampal CA3 neurons, slices</td>
<td>L-glutamate</td>
<td></td>
<td>1.8, 0.057</td>
<td>0.018</td>
<td>4240</td>
<td>0.75</td>
</tr>
<tr>
<td>Raman &amp; Trussell [1995]</td>
<td>Neuronal AMPA</td>
<td>nucleus magnocellularis, slices</td>
<td>L-glutamate</td>
<td></td>
<td>0.02, 15</td>
<td>0.026</td>
<td>3000, 60000</td>
<td>0.57</td>
</tr>
<tr>
<td>Hausser &amp; Roth [1997]</td>
<td>Neuronal AMPA</td>
<td>cerebellar Purkinje cells, slices</td>
<td>L-glutamate</td>
<td></td>
<td>0.3, 0.39</td>
<td>0.29</td>
<td>17230</td>
<td>0.75</td>
</tr>
<tr>
<td>Lawrence et al. [2003]</td>
<td>Neuronal AMPA</td>
<td>n. magnocellularis, acute dissociation</td>
<td>L-glutamate</td>
<td></td>
<td>0.03, 3</td>
<td>0.0086</td>
<td>20000</td>
<td>0.65</td>
</tr>
<tr>
<td>Lawrence et al. [2003]</td>
<td>Neuronal AMPA</td>
<td>n. magnocellularis, acute dissociation</td>
<td>L-glutamate</td>
<td>aniracetam</td>
<td>0.015, 6</td>
<td>0.008</td>
<td>20000</td>
<td>0.87</td>
</tr>
<tr>
<td>Dzubay &amp; Jahr [1999]</td>
<td>Glial AMPA</td>
<td>cerebellar Bergmann glia, slices</td>
<td>L-glutamate</td>
<td></td>
<td>1.8</td>
<td>1.8</td>
<td>13000</td>
<td>0.62</td>
</tr>
<tr>
<td>Dzubay &amp; Jahr [1999]</td>
<td>Glial AMPA</td>
<td>cerebellar Bergmann glia, slices</td>
<td>L-glutamate</td>
<td>cyclothiazide</td>
<td>0.66</td>
<td>0.66</td>
<td>13000</td>
<td>0.90</td>
</tr>
<tr>
<td>Wadiche &amp; Jahr [2001]</td>
<td>Neuronal AMPA</td>
<td>cerebellar Purkinje cell, slices</td>
<td>L-glutamate</td>
<td></td>
<td>0.3, 0.4</td>
<td>0.039</td>
<td>17200</td>
<td>0.69</td>
</tr>
<tr>
<td>Diamond &amp; Jahr [1997]</td>
<td>Neuronal AMPA</td>
<td>CA1 hippocampal neurons</td>
<td>L-glutamate</td>
<td>kynurenate</td>
<td>0.46</td>
<td>0.235</td>
<td>5700</td>
<td>0.67</td>
</tr>
<tr>
<td>Clements et al. [1998]</td>
<td>Neuronal AMPA</td>
<td>hippocampal neurons</td>
<td>L-quisqualate</td>
<td>cyclothiazide</td>
<td>0.00065</td>
<td>—</td>
<td>1000</td>
<td>0.66</td>
</tr>
<tr>
<td>Clements et al. [1998]</td>
<td>Neuronal AMPA</td>
<td>hippocampal neurons</td>
<td>L-quisqualate</td>
<td>cyclothiazide</td>
<td>0.017</td>
<td>—</td>
<td>1000</td>
<td>0.50</td>
</tr>
<tr>
<td>Partin et al. [1996]</td>
<td>GluR1 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td></td>
<td>0.03, 5</td>
<td>0.0004</td>
<td>30000</td>
<td>0.71</td>
</tr>
<tr>
<td>Partin et al. [1996]</td>
<td>GluR1 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>aniracetam</td>
<td>0.03, 5</td>
<td>0.0004</td>
<td>30000</td>
<td>0.75</td>
</tr>
<tr>
<td>Partin et al. [1996]</td>
<td>GluR1 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>cyclothiazide</td>
<td>0.003, 0.25</td>
<td>0.0006</td>
<td>30000</td>
<td>0.95</td>
</tr>
<tr>
<td>Banke et al. [2000]</td>
<td>GluR1 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>PKA Ca</td>
<td>0.42</td>
<td>0.01</td>
<td>20000</td>
<td>0.74</td>
</tr>
<tr>
<td>Banke et al. [2000]</td>
<td>GluR1 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>calcineurin</td>
<td>0.42</td>
<td>0.01</td>
<td>20000</td>
<td>0.37</td>
</tr>
</tbody>
</table>
### TABLE 4. Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Receptor</th>
<th>Preparation</th>
<th>Agonist</th>
<th>Modulator</th>
<th>Agonist Koff/Kon mM</th>
<th>Desens. Koff/Kon mM</th>
<th>Opening rate s⁻¹</th>
<th>Peak P(open)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robert et al. [2001]</td>
<td>GluR1 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td></td>
<td>0.7</td>
<td>0.007</td>
<td>7,000</td>
<td>0.55</td>
</tr>
<tr>
<td>Robert et al. [2001]</td>
<td>GluR1 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>cyclothiazide</td>
<td>0.7</td>
<td>0.007</td>
<td>7,000</td>
<td>0.73</td>
</tr>
<tr>
<td>Robert et al. [2001]</td>
<td>GluR1 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>L497Y mutant</td>
<td>0.7</td>
<td>0.007</td>
<td>7,000</td>
<td>0.95</td>
</tr>
<tr>
<td>Robert &amp; Howe [2003]</td>
<td>GluR1 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td></td>
<td>0.45</td>
<td>0.45</td>
<td>16,000-32,000</td>
<td>0.69</td>
</tr>
<tr>
<td>Klein &amp; Howe [2004]</td>
<td>GluR1 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>lurcher mutant</td>
<td>0.0030</td>
<td>0.0030</td>
<td>1,400-30,800</td>
<td>0.81</td>
</tr>
<tr>
<td>Koike et al. [2000]</td>
<td>GluR2 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td></td>
<td>2.6</td>
<td>0.5</td>
<td>16,000</td>
<td>0.71</td>
</tr>
<tr>
<td>Sekiguchi et al. [2002]</td>
<td>GluR3 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td></td>
<td>0.03, 5</td>
<td>0.046</td>
<td>17,500</td>
<td>0.80</td>
</tr>
<tr>
<td>Sekiguchi et al. [2002]</td>
<td>GluR3 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>PEPA</td>
<td>0.03, 5</td>
<td>1.8</td>
<td>17,500</td>
<td>0.88</td>
</tr>
<tr>
<td>Sekiguchi et al. [2002]</td>
<td>GluR3 flap</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td></td>
<td>0.03, 5</td>
<td>0.0016</td>
<td>30,000</td>
<td>0.39</td>
</tr>
<tr>
<td>Sekiguchi et al. [2002]</td>
<td>GluR3 flap</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>PEPA</td>
<td>0.008, 0.5</td>
<td>0.109</td>
<td>30,000</td>
<td>0.30</td>
</tr>
<tr>
<td>Robert &amp; Howe [2003]</td>
<td>GluR4 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td></td>
<td>1</td>
<td>1</td>
<td>40,000-80,000</td>
<td>0.83</td>
</tr>
<tr>
<td>Heckmann &amp; Dudel [1997]</td>
<td>Neuronal GluR</td>
<td>Drosophila larval muscle</td>
<td>L-glutamate</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td>19,000</td>
<td>0.64</td>
</tr>
<tr>
<td>Heckmann et al. [1996]</td>
<td>GluR6 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td></td>
<td>0.004</td>
<td>0.008</td>
<td>7,000</td>
<td>0.71</td>
</tr>
<tr>
<td>Bowie et al. [1998]</td>
<td>GluR6 flip</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td></td>
<td>0.03, 5</td>
<td>0.007</td>
<td>5,000</td>
<td>0.25</td>
</tr>
<tr>
<td>Li et al. [2003]</td>
<td>GluR6 flip</td>
<td>HEK cell</td>
<td>caged glutamate</td>
<td></td>
<td>0.45</td>
<td>—</td>
<td>11,000</td>
<td>0.96</td>
</tr>
</tbody>
</table>

---

a When two numbers present, they indicate Kd for first and then second binding step.
b Opening rate from fully liganded closed, nondesensitized state.
c Determined from running model driven by a supra maximal (30-100 mM) concentration of glutamate.
d Model postulates multiple gating steps with no single opening rate.

Model summaries and configuration files for Channelab are downloadable from http://www.pharm.emory.edu/straynelis/Downloads/
IV.B. Conceptual Models of AMPA/KA Receptor Activation

Initial quantitative descriptions of AMPA receptor activation kinetics involved analysis of macroscopic currents and suggested that there are at least two agonist binding steps required for agonist activation, although these data could not exclude the possibility of more than two such steps [Häusser & Roth 1997, Raman & Trussell 1995, Clements et al. 1998, Jonas et al. 1993] (Table 4).

The discovery of a non-desensitizing GluR3/GluR6 chimera allowed more direct examination of single-channel AMPA activation [Stern-Bach et al. 1994]. The absence of desensitization simplified analysis and allowed for the unequivocal isolation of patches containing a single functional AMPA channel [Rosenmund et al. 1998]. The authors used an elegant experimental design employing a high-affinity antagonist (DNQX) that dissociated slowly from the channel one subunit at a time.

DNQX unbinding frees the agonist binding site to bind the agonist quisqualate (Fig. 3). This protocol allowed direct measurement of the activity of

![Recombinant GluR3/R6](image1.png)

![Native AMPAR](image2.png)

**FIGURE 3.** Channel conductance for AMPA receptors depends on the number of subunits binding agonist. (A) A non-desensitizing chimeric channel shows incremental conductance increases as the antagonist NBQX dissociates and each subunit binds the agonist quisqualate. (B) Native receptors show a concentration dependence of unitary conductance. As the number of subunits occupied by agonist increases, so does the channel conductance. Reprinted with permission from Rosenmund et al. Science 280:1596-9. © 1998 American Association for the Advancement of Science and Smith & Howe, Nat. eN eN 2002 992-7. © 2000 Nature Publishing Group (www.nature.com/)
the channel with incremental changes in the subunit occupancy of the agonist binding sites. The channel shows three clear conductance levels (5, 15, 23 pS) with the transition times from each conductance state fit by 2, 1, and 1 exponentially decaying kinetic functions, respectively. The most parsimonious interpretation of these data is that the largest conductance level (23 pS for the R3/R6 chimera) represents a fully liganded conformation (4 agonists), 15 pS the three-agonist conformation, and 5 pS the two-agonist conformation. The singly liganded channel is either nonconducting or possesses a conductance that is too low to resolve.

In addition to suggesting that there are four functional agonist binding sites, this study suggested a direct relationship between the number of agonists bound and the subconductance states of the channel. This is similar to, but simpler than, cyclic nucleotide gated channels that show concentration-dependent subconductance levels but with fluctuations between multiple conductance levels, even when the channel is occupied by a fixed number of ligands [Ruiz & Karpen 1997, Root & MacKinnon 1994]. Similar subunit-dependent gating has also been suggested for K+ channels [Zheng & Sigworth 1997]. Rosenmund et al. [1998] further generalized the importance of their results by demonstrating the same phenomenon in wild-type GluR3 in the presence of cyclothiazide (an inhibitor of desensitization) and by demonstrating the agonist-concentration dependence of subconductance state occupancies.

Drawing on the results of this and other studies, Smith & Howe [2000] examined the concentration dependence of the subconductance states of native AMPA and kainate receptors. They focused on native channels from developing cerebellar granule neurons, which are known to exhibit multiple resolvable single-channel conductance states [Wyllie et al. 1993; Smith et al. 2000]. Consistent with the results of Rosenmund et al. [1998], Smith & Howe [2000] recorded from patches with a single active AMPA channel and observed an agonist-concentration dependence of the relative occupancy of the subconductance states using mean low-variance amplitude distributions (Fig. 3). Furthermore, half the patches in this study exhibited four different open channel conductances. This is different from the three levels observed by Rosenmund et al. [1998] but may indicate the presence of a singly liganded receptor that opens to a resolvable conductance.

In addition, Smith and Howe [2000] observed that kainate receptors exhibit multiple subconductance states but lack a concentration dependence to the occupancy of these conductances. The weighted mean unitary conductance of recombinant homomeric GluR6 is similarly concentration independent [Traynelis & Wahl 1997]. Even at high agonist concentrations, kainate receptors appear to occupy multiple conductance levels, including low conductance states, highlighting a potentially important functional distinction between AMPA and kainate receptors.

Howe and coworkers conceptualized a straightforward kinetic model that has each of four binding steps capable of opening to a different conductance level or desensitizing [Robert & Howe 2003] (Table 5). This model can predict many features of AMPA receptor function, including the Hill slope of dose response curves, the concentration dependence of desensitization, and the concentration dependence of single-channel properties. Moreover, the model does not require negative cooperativity between binding sites [for example, see Partin et al. 1996]. This model constitutes a first approximation of physical properties of the receptor in a kinetic scheme and seems likely to be an improvement over the previous two binding-site models.

AMPA and kainate receptors are more closely related to each other than to NMDA receptors, based on sequence homology. Nonetheless, there are important distinctions separating AMPA and kainate receptors, including the difference in sensitivity to agonist concentration described by Smith and Howe [2000].

Moreover, the pharmacology of kainate receptor subunits (GluR5-7, KA1-2) is more richly developed and heterogeneous than that of AMPA receptor subunits (GluR1-4). For example, selective activators and antagonists of GluR5 exist [Lubisch et al.
Furthermore, AMPA and (S)-5-iodowillardine can activate heteromultimers of GluR6/KA2, whereas homomeric GluR6 receptors are not activated by these agonists [Herb et al. 1992, Swanson et al. 1998]. There are at least two possible interpretations: first, incorporation of KA2 could change the conformation of GluR6 to make it able to bind and be activated by AMPA or IW; second, KA2 binding of AMPA or IW allows it to contribute to the opening of the ion permeation pore independent of activation of GluR6.

Swanson et al. [2002] used the subtype selective agonist dysiherbaine (DH) to differentiate between these two hypotheses. By comparing responses of homomeric GluR5 or GluR6 to glutamate and DH to the responses of heteromeric GluR5/KA2 or GluR6/KA2, they demonstrated that individual subunits within the heteromeric complex make independent contributions to the activation of the channel. This conclusion is similar to the model of activation for AMPA receptors proposed by Rosenmund et al. [1998] but is perhaps inconsistent with the observation of Smith & Howe [2000] that native KA receptor single-channel conductances are insensitive to agonist concentration. It is possible that desensitization may obscure underlying subunit occupancy dependence of conductance states, and that this may be the basis for these two apparently different observations. In addition, the molecular composition of the kainate receptors in cerebellar granule cells has not been determined and thus may differ from GluR6/KA2 studied by Swanson et al. [2002].

IV.C. Structural Features of AMPA/KA Receptor Activation and Gating

Armstrong et al. [1998] provided the first crystal structures for glutamate complexed with ligands, which immediately lead to several important advances in our understanding of glutamate receptor function. First, the pharmacological properties of the isolated agonist binding domain for GluR2 match that for functional receptors, reinforcing the idea that glutamate receptor function reflects the action in part of semiautonomous domains. Second, the clamshell that forms the agonist binding state is mostly open in the apo (not ligand bound) state and is stabilized in this position when competitive antagonists occupy the ligand recognition site.

Evaluation of the structure of the ligand binding core with full and partial agonists showed unambiguously differential degrees of domain closure [Armstrong & Gouaux 2000]. This observation led to the intriguing hypothesis that agonist binding and activation/desensitization are correlated with the degree of domain closure induced by a given agonist. For example, binding of agonists such as glutamate and AMPA cause a conformation change in the S1S2 structure such that the two lobes are drawn closer to one another—it is predicted that the structure is approximately 20° more closed compared to that of the apo state. In contrast, kainate causes only approximately 12° of domain closure, leading to the prediction that this may provide a physical (and novel) basis of partial agonism at AMPA receptors [Armstrong & Gouaux 2000, Table 1]. It has been proposed that closure of the agonist binding domain exerts strain on the subunit assembly that can be relieved either by rearrangement of the transmembrane elements in a manner that leads to opening of the pore, or by breakdown of the subunit interface that leads to desensitization [Armstrong & Gouaux 2000, Sun et al. 2002, Horning & Mayer 2004].

Verification of the functional hypotheses of gating from static structures clearly requires a combination of structural and functional data. For example, further insights into the structural determinants of AMPA receptor activation and desensitization have been gleaned from a combination of functional and crystallographic studies. The S1S2J fragment, in either the apo or in the ligand-bounded state, crystallizes into a dimeric arrangement, and the area of the dimer interface is estimated to be around 1500 Å². This interface is thought to be functionally important in determining the extent of desensitization of some iGluRs because it is in this region that cyclothiazide, a blocker of AMPA iGluR desensitization, binds.
dimer interface is also the location of a leucine residue (Leu507 in GluR3, numbering from the beginning of the open reading frame), which when mutated to a tyrosine residue abolishes desensitization in GluR3 containing AMPA receptors [Stern-Bach et al. 1998]. This supports the idea that the breakdown of the dimer interface is central to the phenomenon of desensitization of AMPA iGluRs. Receptor mutations or ligands that prevent the destabilization of the dimer interface result in nondesensitizing current responses [Sun et al. 2002, see also Horning & Mayer 2004]. Mutating residues predicted to be at the dimer interface for kainate receptors can also have dramatic effects on desensitization [Fleck et al. 2003].

Jin et al. [2003] used a similar combination of structural and functional studies to extend the work of Armstrong et al. [1998, 2000] with an elegant series of experiments in which a series of five substituted willardines that have previously been described as partial agonists [Patneau et al. 1992, Wong et al. 1994] were crystallized in the agonist binding domain (Fig. 4). Alterations in the five-substituent creates compounds of different sizes that still retained the same atomic contacts within the binding cleft.

The increasing size of the substituent creates steric hindrance for domain closure. Cleft closure induced by these compounds correlated with the degree of efficacy of the agonist, such that I-willardiine induced the least domain closure and was the weakest partial agonist. A series of single-channel studies supported and extended the working hypothesis of Rosenmund et al. [1998] and Smith & Howe [2000], showing that agonist occupancy appears to correlate with single-channel conductance. It was proposed that each agonist-bound subunit has an independent probability of making a contribution to gating and that for full agonists the aggregate probability that all subunits will become activated is higher because the probability (quantified as a coupling efficiency) for an individual subunit is higher. Conversely, partial agonists that lower the coupling efficiency also lower the probability that at any given moment all four subunits will be activated. This idea converts the agonist dependence of subunit activation to changes in single-channel conductance, which can be measured. The data support a first approximation of this idea and further suggest that modulators such as CaMKII that increase single-channel conductance [Derks et al. 1999] may do so by altering the efficiency of coupling of agonist binding to gating within individual subunits [Kristensen et al. 2003].

V. CHANNEL ACTIVATION AND GATING: NMDA RECEPTORS

V.A. Functional Features of NMDA Receptor Activation

NMDA receptors exhibit a higher apparent affinity for glutamate than AMPA and kainate receptors following a brief application of agonist (Table 2). This increased affinity is reflected in the slower deactivation following removal of agonist, and it is this slow deactivation (Table 2) that underlies the prolonged time course of the NMDA component of excitatory postsynaptic currents (EPSCs) [Lester et al. 1990]. This slow deactivation results in the NMDA component of the excitatory postsynaptic potentials (EPSP), making a dominant contribution to temporal integration of synaptic inputs [Maccarferri & Dingledine 2002]. This potential role in temporal integration, together with both their relatively high calcium permeability and voltage-dependent block by Mg²⁺, has given rise to the description of NMDA receptors as “coincidence” detectors, primarily for the roles they play in certain forms of synaptic plasticity [Nicol 2003]. Given the pivotal role that NMDA receptors play in many physiological and pathophysiological conditions [Carroll & Zunin 2002, Marino & Conn 2002, Petrenko et al. 2003, Rogowki & Wenk 2003, Smith 2003], it is not surprising that these receptors are regulated by a variety of extracellular factors, including protons, Mg²⁺, Zn²⁺, reduction/oxidation environment [Low et al. 2003, Williams 1996, Choi & Lipton 2000], as well as intracellular modulators such as Na⁺, kinases, phosphatases, and scaffolding proteins [Yamakura & Shimoji 1999, Yu & Salter 1998, Wenthold et al. 2003].
FIGURE 4. The degree of domain closure determines the efficacy of partial agonists for the GluR2 subunit. (A) A partial agonist induces less domain closure than the full agonist glutamate. Analysis of the crystal structures for glutamate and iodowillardiine reveals that glutamate induces greater domain closure. (B) Both relative macroscopic current and domain closure correlate directly with the efficiency for coupling agonist binding to channel opening. The coupling efficiency \( \varepsilon \) describe the probability that an activating conformational change within an individual subunit will occur when an agonist is bound. Activation of each subunit makes an incremental increase in ion channel conductance. Reprinted with permission from Jin et al., *Nature Neuroscience* 6:803-10. © 2003 Nature Publishing Group (www.nature.com/).

Although there is a vast literature on modulation of these receptor channels, in order to begin to understand the mechanisms by which allosteric modulators act, it is first necessary to understand at a basic level how the channels operate, which will be the topic discussed here. The complex functional behavior of NMDA channels has been appreciated since the first description of multiple conductance levels—prolonged periods of intense activity as well as multiple shut-time components within single activations [Cull-Candy & Usowicz 1987, Jahr & Stevens 1987, Ascher et al. 1988, Gibb & Colquhoun 1991]. This begins with the fact that NMDA receptors are heteromultimeric receptors composed of NR1 and NR2 subunits, with the additional complication that there are four different NR2 gene products (NR2A-D), while NR3 subunits and multiple alternative splicing sites for NR1 also influence channel function [Ishii et al. 1992, Durand et al. 1993, Das et al. 1998, Al-Hallaq et al. 2002, Rumbaugh et al. 2000]. The NR1 subunit binds the co-agonist, glycine [Furukawa & Gouaux 2003], and all functional NMDA receptors require this subunit to be present. Given the likely tetrameric nature of the receptor, it is to be expected that each heteromeric assembly contains two of these subunits [Béhé et al. 1995, Schorge & Colquhoun 2003].
While recombinant studies have indicated that NMDA receptor can possess two different types of glutamate-binding NR2 subunits [Wafford et al. 1993, Cheffings & Colquhoun 2000, Brimecombe et al. 1997], it is less certain that native NMDA receptors can possess two different types of NR2 subunit. Evidence for this has come mainly from the study of single channels [Brickley et al. 2003, Pina-Crespo & Gibb 2002]. Each of the four NR2 subunits are temporally and spatially regulated and are a major determinant of pharmacological and biophysical properties of the heteromeric channel when expressed with the NR1 subunit [Monyer et al. 1992, 1994, Vicini et al. 1998, Wyllie et al. 1998]. For example, NMDA receptors containing NR2A subunits deactivate more rapidly than those containing either NR2B or NR2C subunits, which in turn deactivate at least 10 times more rapidly than NR2D containing receptors (see Table 3, Fig. 5). Moreover, NMDA receptors containing the NR2A subunit have a higher channel-open probability than those containing the NR2B subunit [Chen et al. 1999b; Erreger et al. 2005] (Table 3). The fact that different NR2-containing NMDA receptors possess unique “signatures” means that the pharmacological and biophysical properties determined from studies of recombinant receptors can be used to suggest the molecular composition of native receptors [Momiyama et al. 1996, Wyllie et al. 1996, Misra et al. 2000, Vicini & Rumbaugh 2000, Brickley et al. 2003].

**FIGURE 5.** The identity of the NR2 subunit regulates NMDA channel kinetic properties. The time course of deactivation for recombinant receptors expressed in HEK293 cells following a brief (1 ms) pulse of 1 mM glutamate is shown for each NR2 subunit. The upper trace for each panel shows the open tip current used to measure the duration of drug application. Vicini et al., 1998, *J. Neurophysiol.* 79:555-66 used by permission from the American Physiological Society.
Even with a defined subunit composition, as can be achieved in recombinant heterologous expression systems, the properties of activations of NMDA receptors are far more complex than for those seen for the nicotinic acetylcholine receptor [for example see Salamone et al. 1999], as indicated by the presence of multiple open and closed states [Wyllie et al. 1998; Popescu & Auerbach 2003; Banke & Traynelis, 2003]. Furthermore, although the NR1 and NR2 agonist binding sites are on distinct subunits, there is strong evidence that they communicate with each other, adding yet another layer of complexity. For example, the identity of the NR2 subunit influences the EC₅₀ and apparent affinity for glycine binding to NR1 [Kutsuwada et al. 1992, Ikeda et al. 1992]. Also, a negative interaction between the glutamate and glycine binding sites has been hypothesized to underlie some forms of desensitization that are dependent on the glycine or glutamate concentration, with the occupancy of the glutamate binding site influencing the affinity of the glycine binding site for its agonist and vice versa [Benveniste et al. 1990, Vyklicky et al. 1990, Lester et al. 1993, Priestley & Kemp 1994, Nehum-Levy et al. 2001, 2002; see Table 5].

In addition to the slow kinetics of deactivation, NMDA receptors also exhibit slow activation (7–15 ms, 10–90% rise time) even following rapid saturation (<1 ms) of agonist binding sites [Chen et al. 1999b, Lester et al. 1990]. This demonstrates that there are relatively slow conformational changes of the fully liganded receptor that precede and/or mediate the final gating or pore dilation process. Indeed, the relatively slow activation kinetics contribute to the slow deactivation kinetics [see Wyllie et al. 1998], which is reflected in virtually all kinetic models where the opening rate is 10–100-fold lower than that for AMPA receptors (Tables 4 and 5).

V.B. Conceptual Models of NMDA Receptor Activation

The first widely accepted quantitative description of the activation kinetics of NMDA receptors came from Lester and Jahr’s [1992] study of native NMDA receptors in outside-out patches using rapid agonist application techniques. In addition to establishing that glutamate is most likely the endogenous amino acid responsible for activating synaptic NMDA receptors, this landmark article demonstrated that the time course of synaptic NMDA EPSCs is controlled by both the agonist unbinding rate and the entry into and recovery from a desensitized state. The macroscopic data were accurately described by a relatively simple model that is conceptually similar to that which had previously been employed to describe the behavior of the nicotinic acetylcholine receptors (nAChRs) [Colquhoun & Sakmann 1985, Sine et al. 1990]. The proposed activation process was similar to the muscle nACh receptor in the sense that both the NMDA receptor and the nACh receptor have two agonist binding sites. The model consisted of two glutamate binding steps, after which the channels could enter either an “open” ion-conducting conformation or a relatively long-lived or “desensitized” nonconducting conformation, or the agonist could unbind. The Lester and Jahr (LJ) model was an important advance for the field, providing a mechanistic understanding of the receptor channel activation process and establishing a framework for understanding and predicting how a variety of agonists function at NMDA receptors. For example, in the context of this model, one could differentiate and quantify how agonists other than glutamate influence parameters that represent channel opening/closing rate, desensitization (and recovery), and agonist binding/unbinding (Table 5).

Despite its ability to predict macroscopic waveforms, there are two major limitations of the LJ model that need to be overcome in order to establish a more complete understanding of channel activation. First, it is known that both glutamate and glycine binding are required to activate the channel, but the LJ model contains no explicit glycine binding step and is therefore generally applied to data recorded in the continuous presence of saturating glycine. A complete model for NMDA receptor activation needs to take glycine binding into account and include explicit binding reactions for glycine as well as glutamate because
### TABLE 5. Kinetic Modeling of NMDA Receptor Function

<table>
<thead>
<tr>
<th>Reference</th>
<th>Receptor</th>
<th>Preparation</th>
<th>Agonist</th>
<th>NR1 co-agonist</th>
<th>Modulator</th>
<th>Agonist Koff/Kon mM(^a)</th>
<th>Opening rate s(^{-1}) (^{1b})</th>
<th>Peak P(open) (^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benveniste et al. [1990]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td></td>
<td>0.030</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Benveniste &amp; Mayer [1991a]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td>AP5</td>
<td>0.00011, 0.011</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Benveniste &amp; Mayer [1991b]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td>AP5</td>
<td>0.00011, 0.011</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Benveniste &amp; Mayer [1991b]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td>AP7</td>
<td>0.00011, 0.011</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Benveniste &amp; Mayer [1991b]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td>D-CPP</td>
<td>0.00011, 0.011</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Benveniste &amp; Mayer [1991b]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td>CGP37849</td>
<td>0.00011, 0.011</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Benveniste &amp; Mayer [1991b]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td>CGS19755</td>
<td>0.00011, 0.011</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Clements et al. [1991]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>L-glutamate</td>
<td>glycine</td>
<td></td>
<td>0.0010</td>
<td>10</td>
<td>0.03</td>
</tr>
<tr>
<td>Clements et al. [1992]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>L-glutamate</td>
<td>glycine</td>
<td>D-amino-adipate</td>
<td>0.0019</td>
<td>10</td>
<td>0.03</td>
</tr>
<tr>
<td>Lester &amp; Jahr [1992]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>L-glutamate</td>
<td>glycine</td>
<td></td>
<td>0.00094</td>
<td>46.5</td>
<td>0.29</td>
</tr>
<tr>
<td>Lester &amp; Jahr [1992]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>L-cysteate</td>
<td>glycine</td>
<td></td>
<td>0.013</td>
<td>39.1</td>
<td>0.25</td>
</tr>
<tr>
<td>Jahr [1992]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>L-glutamate</td>
<td>glycine</td>
<td>MK801</td>
<td>0.0009</td>
<td>46.5</td>
<td>0.29</td>
</tr>
<tr>
<td>Lester et al. [1993]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>L-glutamate</td>
<td>glycine</td>
<td></td>
<td>0.0010</td>
<td>104</td>
<td>0.25</td>
</tr>
<tr>
<td>Priestley &amp; Kemp [1993]</td>
<td>Neuronal NMDA</td>
<td>cortical neurons, in vitro</td>
<td>L-glutamate</td>
<td>glycine</td>
<td></td>
<td>0.0003</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Priestley &amp; Kemp [1993]</td>
<td>Neuronal NMDA</td>
<td>cerebellar neurons, in vitro</td>
<td>L-glutamate</td>
<td>glycine</td>
<td></td>
<td>0.005</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
### TABLE 5. Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Receptor</th>
<th>Preparation</th>
<th>Agonist</th>
<th>NR1 co-agonist</th>
<th>Modulator</th>
<th>Agonist Koff/Kon mM&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Opening rate s&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Peak P(open)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kleckner &amp; Pallotta [1995]</td>
<td>Neuronal NMDA</td>
<td>cortical neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td></td>
<td>—</td>
<td>60-230&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.33</td>
</tr>
<tr>
<td>Donnelly &amp; Pallotta [1995]</td>
<td>Neuronal NMDA</td>
<td>cortical neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td></td>
<td>—</td>
<td>5,500&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>Jackson [1997]</td>
<td>Neuronal NMDA</td>
<td>cortical neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td></td>
<td>0.001-0.01</td>
<td>60-230&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.68</td>
</tr>
<tr>
<td>Nahum-Levy et al. [2001]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>L-glutamate</td>
<td>L-alanine</td>
<td></td>
<td>0.00024, 0.0010</td>
<td>83.8</td>
<td>0.47</td>
</tr>
<tr>
<td>Nahum-Levy et al. [2001]</td>
<td>Neuronal NMDA</td>
<td>hippocampal neurons, in vitro</td>
<td>L-cysteine-sulfate</td>
<td>L-alanine</td>
<td></td>
<td>0.0008, 0.0025</td>
<td>83.8</td>
<td>0.48</td>
</tr>
<tr>
<td>Blanpied et al. [1997]</td>
<td>Neuronal NMDA</td>
<td>cortical neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td>memantine</td>
<td>0.020</td>
<td>5.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Blanpied et al. [1997]</td>
<td>Neuronal NMDA</td>
<td>cortical neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td>memantine</td>
<td>0.020</td>
<td>0.3</td>
<td>—</td>
</tr>
<tr>
<td>Blanpied et al. [1997]</td>
<td>Neuronal NMDA</td>
<td>cortical neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td>amantadine</td>
<td>0.020</td>
<td>1.64</td>
<td>—</td>
</tr>
<tr>
<td>Sobolevsky &amp; Koshelev [1998]</td>
<td>Neuronal NMDA</td>
<td>CA1 neurons, acute dissociation</td>
<td>L-aspartate</td>
<td>glycine</td>
<td></td>
<td>0.00094</td>
<td>46.5</td>
<td>0.34</td>
</tr>
<tr>
<td>Sobolevsky &amp; Koshelev [1998]</td>
<td>Neuronal NMDA</td>
<td>CA1 neurons, acute dissociation</td>
<td>L-aspartate</td>
<td>glycine</td>
<td>memantine</td>
<td>0.00094</td>
<td>46.5</td>
<td>—</td>
</tr>
<tr>
<td>Sobolevsky &amp; Koshelev [1998]</td>
<td>Neuronal NMDA</td>
<td>CA1 neurons, acute dissociation</td>
<td>L-aspartate</td>
<td>glycine</td>
<td>MR22/78</td>
<td>0.00094</td>
<td>46.5</td>
<td>—</td>
</tr>
<tr>
<td>Dilmore &amp; Johnson [1998]</td>
<td>Neuronal NMDA</td>
<td>forebrain neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td></td>
<td>0.011</td>
<td>3.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Dilmore &amp; Johnson [1998]</td>
<td>Neuronal NMDA</td>
<td>forebrain neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td>NEFA</td>
<td>0.011</td>
<td>0.23</td>
<td>—</td>
</tr>
<tr>
<td>Kew et al. [1998]</td>
<td>Neuronal NMDA</td>
<td>cortical neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td></td>
<td>0.01</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Kew et al. [1998]</td>
<td>Neuronal NMDA</td>
<td>cortical neurons, in vitro</td>
<td>NMDA</td>
<td>glycine</td>
<td>Ro 8-4304</td>
<td>0.0027</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
**TABLE 5. Continued**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Receptor</th>
<th>Preparation</th>
<th>Agonist</th>
<th>NR1 co-agonist</th>
<th>Modulator</th>
<th>Agonist Koff/Kon mM&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Opening rate s&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Peak P(open)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rycroft &amp; Gibb [2002]</td>
<td>Neuronal NMDA</td>
<td>dentate granule cells, slices</td>
<td>NMDA</td>
<td>glycine</td>
<td>—</td>
<td>0.0050</td>
<td>280</td>
<td>0.48</td>
</tr>
<tr>
<td>Rycroft &amp; Gibb [2002]</td>
<td>Neuronal NMDA</td>
<td>dentate granule cells, slices</td>
<td>NMDA</td>
<td>glycine</td>
<td>calmodulin</td>
<td>0.0050</td>
<td>150</td>
<td>0.17</td>
</tr>
<tr>
<td>Chen et al. [2001]</td>
<td>NR1/NR2A</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>glycine</td>
<td>—</td>
<td>0.0050</td>
<td>150</td>
<td>0.49</td>
</tr>
<tr>
<td>Popescu &amp; Auerbach [2003,2004]</td>
<td>NR1/NR2A</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>glycine</td>
<td>H-mode</td>
<td>0.0030</td>
<td>285&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>0.97</td>
</tr>
<tr>
<td>Popescu &amp; Auerbach [2003,2004]</td>
<td>NR1/NR2A</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>glycine</td>
<td>M-mode</td>
<td>0.0030</td>
<td>285&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>0.83</td>
</tr>
<tr>
<td>Popescu &amp; Auerbach [2003,2004]</td>
<td>NR1/NR2A</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>glycine</td>
<td>L-mode</td>
<td>0.0030</td>
<td>169&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>0.40</td>
</tr>
<tr>
<td>Erreger et al. [2005]</td>
<td>NR1/NR2A</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>glycine</td>
<td>—</td>
<td>0.0320</td>
<td>230, 3140&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.50</td>
</tr>
<tr>
<td>Chen et al. [2001]</td>
<td>NR1/NR2B</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>glycine</td>
<td>—</td>
<td>0.00060</td>
<td>80</td>
<td>0.49</td>
</tr>
<tr>
<td>Banke &amp; Traynelis [2003]</td>
<td>NR1/NR2B</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>glycine</td>
<td>—</td>
<td>0.0031</td>
<td>89, 1557&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.28</td>
</tr>
<tr>
<td>Banke &amp; Traynelis [2003]</td>
<td>NR1/NR2B</td>
<td>HEK cell</td>
<td>NMDA</td>
<td>glycine</td>
<td>—</td>
<td>45, 1557&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Banke &amp; Traynelis [2003]</td>
<td>NR1/NR2B</td>
<td>HEK cell</td>
<td>quinolinate</td>
<td>glycine</td>
<td>—</td>
<td>42, 1557&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Banke et al. [2005]</td>
<td>NR1/NR2B</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>glycine</td>
<td>protons</td>
<td>—</td>
<td>118, 1482&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.22</td>
</tr>
<tr>
<td>Erreger et al. [2005]</td>
<td>NR1/NR2B</td>
<td>HEK cell</td>
<td>L-glutamate</td>
<td>glycine</td>
<td>—</td>
<td>0.0135</td>
<td>48, 2836&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.12</td>
</tr>
</tbody>
</table>

<sup>a</sup> When two numbers present, they indicate Kd for first and then second binding step.

<sup>b</sup> Opening rate from fully liganded closed, nondesensitized state.

<sup>c</sup> Determined from running model driven by a saturating (30-100 mM) concentration of glutamate.

<sup>d</sup> Model postulates multiple gating steps with no single opening rate.

<sup>e</sup> Apparent opening rate, inverse of first latency to opening from fully liganded receptor.

Model summaries and configuration files for Channelab are downloadable from www.pharm.emory.edu/straynelis/Downloads/downloads.html.
the glycine concentration at synapses is regulated by active transport and has been demonstrated to be sub-saturating, at least at some synapses under certain conditions [Berger et al. 1998, Bergeron et al. 1998]. Moreover, high affinity agonists at the glycine site such as D-serine may be in dynamic equilibrium with glycine and could alter the nature of NMDA receptor activations, depending on the degree to which other glycine site agonists bind to the NR1 subunit [Snyder & Kim 2000].

A number of models have incorporated glycine and glutamate binding [Clements & Westbrook 1991, Benveniste & Mayer 1991a, Lester et al. 1993, Nahum-Levy et al. 2001; see Table 5]. For example, Nahum-Levy et al. [2001] have partially addressed this problem by expanding the LJ model to include two glutamate and two glycine binding steps that show allosteric interactions that can replicate glycine-dependent desensitization. A second major limitation to the LJ model is that it is inconsistent with what is known about NMDA channel gating from single-channel experiments. For example, because the LJ model was designed initially to predict macroscopic waveforms, it assumes a single open state of the channel with a mean lifetime of approximately 10 ms for simplicity. Yet single-channel recordings show that there are at least two open states of the receptor with mean lifetimes less than 5 ms and that there are correlations between adjacent openings and closings [Gibb & Colquhoun 1991, 1992]. Furthermore, the distribution of shut times also reveals far more complexity than can be explained by the LJ model [Gibb & Colquhoun 1991, 1992, Wyllie et al. 1998, Popescu & Auerbach 2003, Banke & Traynelis 2003].

A recent study [Banke & Traynelis 2003, also see Gibb 2004] expanded on the principle of the LJ model in an attempt to account for the single-channel observations and to provide a hypothesis about how the binding of the two co-agonists on separate subunits could lead to channel opening. This study used the concentration jump technique to study the response of recombinant NR1/NR2B receptors to a brief (synaptic-like) exposure to glutamate; the key to this approach was to study patches in which only one active channel was observed [Banke & Traynelis 2003]. The experimental design allowed the authors to examine the behavior of a single receptor complex following a single agonist binding event, effectively yielding information about gating of the fully liganded receptor. In such an approach all shut times observed between separate channel openings within an individual sweep must reflect a nonconducting agonist-bound conformation of the channel. The authors were able to quantitatively describe their data with a modified version of the LJ model.

The hypothesis behind the new scheme is that following agonist binding, each subunit must undergo a conformational change that is permissive for gating. Once all subunits have undergone this change, the channel pore rapidly dilates. This concept of all subunits changing to an activated conformation before the channel gate may open is in contrast to the current working hypothesis for the gating of AMPA receptors, wherein each individual subunit activates independently and makes an incremental and permissive contribution to ion permeation [Rosenmund et al. 1998, Jin et al. 2003].

Banke & Traynelis [2003] provided support for their hypothesis by using partial (low efficacy) agonists at either the glycine binding site on NR1 or the glutamate binding site on NR2. They found that the use of a glycine-site partial agonist (HA-966 or D-cycloserine) specifically shifted the “fast” (1 ms) component of the shut time distribution, which in their model is dominated by the rate of NR1 activation. Similarly, the use of a glutamate-site partial agonist (NMDA or quinolinic acid) specifically shifts the “slow” (10 ms) shut time component, which in the model is dominated by the rate of NR2 activation. This model, obtained from analyzing single-channel data, was expanded to include glutamate binding steps, so that it could successfully describe the time course of macroscopic currents activated by both brief and long applications of both high and low concentrations of glutamate. Nonetheless, the main evidence in support of the model is the association of time constant shifts with specific physical events, a link that is not entirely straightforward. While
the data presented provides support for a two-step activation process, the authors have not shown that these two steps necessarily reflect independent subunit movements. The model assumes that a change associated with a particular agonist reflects a specific local conformational change confined to that subunit. In the light of the crosstalk that is known to occur between subunits, this may or may not be a valid assumption. For example, there may be a two-step process, but the fast step may be a more global conformational change that includes movement of residues that form the glycine binding site. While much work remains to either validate or refute the hypothesized two-step independent-subunit transition model, it represents an important conceptual advance for the field and provides a new starting point for design of future studies.

Another recent study using a different approach [Popescu & Auerbach 2003, also see Magleby 2004] postulated a series of kinetic schemes to describe NMDA channel activation. Their experimental approach was to study a single NR1/NR2A channel in the cell-attached patch configuration at steady-state in the continuous presence of saturating concentrations of glutamate and glycine.

This design has two main advantages. First, each patch containing a single active channel could be studied for a relatively long time period, and a large number of events could be recorded and analyzed. Second, the cell-attached method avoids the changes in functional properties of NMDA receptors that are known to accompany the process of patch excision.

Analysis of transitions within bursts of activity revealed that there are at least three closed states and two open states of the channel. This is similar to previous reports for both native and recombinant channels [Gibb & Colquhoun 1991,1992, Wyllie et al. 1998, Banke & Traynelis 2003]. The authors went on to directly fit the sequence of openings and closings observed in the data record to various kinetic models. Their analyses suggested that the two open states are connected and reflect conformational changes of the receptor complex that can directly interconvert. While the specific details of their model are different from that proposed by Banke and Traynelis, the kinetic scheme determined by Popescu and Auerbach [2003] encapsulates the same basic concept that, following agonist binding, there is a sequence of two or more conformational changes that precedes channel opening and that multiple sojourns into the conducting states contribute to slow deactivation of NMDA receptors in response to a synaptic pulse of glutamate.

In addition to establishing a kinetic model of activation, Popescu and Auerbach [2003] observe that NMDA receptors do not behave uniformly over time but instead shift between three discrete modes of activity referred to as H-, M-, and L-mode for high, medium, and low channel open probability (Fig. 6). These three modes are characterized by differences in open probability caused in large part by changes in the mean channel open time. In terms of a kinetic mechanism, all three modes can be described by the same model but differ in the relative occupancy of the open versus closed states. That is, that the relative energetic stability of the open versus closed states is different in different modes. The transitions between modes are abrupt and reversible, occurring on the time scale of minutes. Modal gating is observed under a variety of experimental conditions, including low concentrations of glutamate or glycine, other agonists, and different pHs.

The specific molecular events regulating the transitions between gating modes are not currently known. However, an intriguing study by Rycroft and Gibb [2004] suggests that binding of alpha-actinin and calmodulin to the cytoplasmic domain of NMDA receptors can regulate the channel-open probability. Binding of calmodulin, which would be increased under conditions of high intracellular calcium, causes native NMDA channels from hippocampal neurons to exhibit low open probability behavior, whereas alpha-actinin competes with calmodulin for the same cytoplasmic binding sites and increases channel-open probability. This is one candidate for the molecular basis of modal gating, although many other potential intracellular modulators are present at the postsynaptic density and can be dynamically regulated [Husi et al. 2000, Sheng 2001, Ehlers 2003].
Recently, Popescu et al. [2004] extended their model of NMDA channel activation to include agonist binding steps whose rates are determined by analysis of the agonist concentration dependence of single-channel kinetics. The agonist binding rates reported by Popescu et al. [2004] were consistent with previous estimates for both native and recombinant receptors (Table 5). Driving model simulations with a brief synaptic-like pulse of glutamate suggests that synaptic NMDA receptors are fully saturated with agonist but are only likely to open about half of the time in response to a single synaptic release of glutamate. Receptors enter a nonconducting state with approximately equal probability of channel opening or releasing agonist, demonstrating how intrinsic properties of channel gating can shape the time course of synaptic NMDA currents.

Erreger et al. [2005] measured the response of a single recombinant NMDA channel to a brief synaptic-like pulse of glutamate for both NR2A- and NR2B-containing receptors in outside-out patches. The results show that NR2A-containing receptors have a higher probability of opening at least once in response to a brief synaptic-like pulse of glutamate than NR2B-containing receptors (NR2A 0.80, NR2B 0.56), a higher peak-open probability (NR2A 0.50, NR2B 0.12), and a higher open probability within an activation (NR2A 0.67, NR2B 0.37). Analysis of the sequence of single-channel open and closed intervals showed that both NR2A- and NR2B-containing receptors undergo multiple conformational changes prior to opening of the channel, with at least one of these steps being faster for NR2A than for NR2B. These distinct properties produce profoundly dif-

FIGURE 6. Modal gating of NMDA receptors. (A) The continuous record of NR1/NR2A in a cell-attached patch with only one active channel. H, M, and L denote periods of high, medium, and low open probability. (B) The box in panel A is displayed on an expanded time scale. The arrow marks the transition from high to medium open probability. (C) Mean channel open time is plotted for each 1 s of active time. Three distinct populations are apparent with the channel remaining in a single mode on the time scale of tens of seconds before switching. Reprinted with permission from Popescu & Auerbach, Nat Neurosci 6:476–83. © 2003 Nature Publishing Group (www.nature.com/).

ferent temporal signaling profiles for NR2A- and NR2B-containing receptors. Simulations of synaptic responses demonstrated that at low frequencies typically used to induce LTD (≤1 Hz), NR1/NR2B makes a larger contribution to total charge transfer and therefore calcium influx than does NR1/NR2A. However, under the high-frequency tetanic stimulation (100 Hz) typically used to induce LTP, the charge transfer mediated by NR1/NR2A considerably exceeds that of NR1/NR2B. This may provide one mechanism for the differential contribution to long-term plasticity hypothesized for NR2A- and NR2B-containing receptors [Liu et al. 2004, Massey et al. 2004].

The models described above have created a conceptual framework for activation of the receptor that involves some pregating conformational changes within the protein that are required before the channel ion permeation pathway becomes conducting. The next challenge for the future is to link specific regions and movements within the protein to these kinetically distinct pregating steps.

V.C. Structural Features of NMDA Receptor Activation

As detailed above, an emerging property of ligand-gated ion channels is the seemingly modular nature of their mechanics. For example, the extracellular ligand-binding domain of GluR2 binds agonists in the absence of the rest of the protein, and the conclusions drawn from these experiments are entirely consistent with functional experiments on the intact channel [Jin et al. 2003]. In addition, NMDA receptors have recently been demonstrated to form functional channels when truncated after transmembrane domain M3 and having the M4 domain and intracellular C-terminal provided as a separate protein [Schorge & Colquhoun 2003].

While there has been rapidly emerging information about the structural determinants of agonist binding and the subsequent rearrangements of the agonist binding domains, the specifics of how these conformational changes are communicated to effect changes in the pore-forming transmembrane domains remains more of a mystery. Nonetheless, significant progress has been made in this area. It has been recognized for some time that the tip of the reentrant M2 P-loop (and specifically the QRN site) forms the narrowest constriction of the ion channel pore and controls ion permeation properties, including block by Mg²⁺ ions [Burnashev et al. 1992b, Mori et al. 1992, Kawajiri & Dingledine 1993, Kuner et al. 1996, Williams et al. 1998, Wollmuth et al. 1998a/b, Opella et al. 1999]. A similar P-loop is present and controls ion permeation in potassium channels but is oriented on the extracellular side of the membrane.

While there is strong evidence for an evolutionary and functional link between glutamate receptors and potassium channels [Wood et al. 1995, Chen et al. 1999a, Panchenko et al. 2001], iGluRs lack the glycine hinge thought to be critical for gating in potassium channels [Sobolevsky et al. 2003]. The nature of the gate itself and how it is controlled in NMDA receptors is still the subject of intense investigation and speculation. In this section, we summarize recent studies on the structural basis for both the coupling of ligand binding to channel gating and for the conformation changes that mediate the channel gating process.

The M3 transmembrane region has received particular attention as a candidate for transducing agonist binding to channel gating [Kohda et al. 2000, Taverna et al. 2002, Jones et al. 2003, Low et al. 2003]. At the extracellular end of M3 there is a sequence of nine amino acids that are entirely conserved throughout glutamate ion channels, suggesting a critical function. This region is homologous to the end of the S6 domain in K⁺ channels, for which mutations within S6 perturb gating properties, and the S6 helix bundle has been suggested to be the gate that controls ion flux in some K⁺ channels [Holmgren et al. 1998, Armstrong 2003].

The corresponding motif in glutamate receptors contains the so-called “lurcher” residue, originally identified as a naturally occurring Ala to Thr mutation in the mouse delta2 orphan receptor proposed to cause
constitutive channel activity and subsequent cerebellar dysfunction [Yuzaki 2003]. When introduced into other glutamate receptors (NR1, GluR1, or GluR6), the lurcher substitution did not result in constitutive activity, but these receptors have increased apparent agonist affinity and slower deactivation [Taverna 2000, Kohda 2000, Klein & Howe 2004]. Notably, GluR1c shows altered agonist pharmacology with CNQX (a competitive antagonist for wild-type AMPA receptors) acting as a potent agonist [Taverna 2000]. While the lurcher substitution A653T (numbering from the beginning of the open reading frame) does not cause constitutive activity for NR1, a T648A mutation in the same region apparently does [Kashiwagi et al. 2002, Williams et al. 2003]. This should be qualified by the caveat that it is difficult to differentiate constitutive activity from receptors with such high agonist affinity that they become activated by low contaminant background levels of glutamate.

Experiments using substituted cysteine accessibility measurements (SCAM) have demonstrated that the solvent accessibility of amino acids within M3 of NMDA receptors is regulated by the activation state of the channel [Sobolevsky et al. 2002a, Jones et al. 2002]. Jones et al. [2002] found that the Ala residue immediately preceding the lurcher residue (A652 for NR1) has an unusual pattern of accessibility and functional effects when mutated to Cys and treated with the cysteine-modifying agent MTSEA. This residue is inaccessible in the closed state, but applying MTSEA in the presence of glutamate and glycine appears to lock the channel in an open conformation, even when glutamate and glycine are removed from the extracellular solution. This study found that the presence of both glutamate and glycine is required for this modification and the rate of modification correlates with agonist concentration and efficacy, suggesting that a conformational change of both agonist binding domains must precede the conformational change in M3 transducing binding to gating. This is consistent with the functional model of gating proposed by Banke and Traynelis [2003] based on their analysis of single-channel kinetics of activation by partial agonists.

Both the glycine-binding site on NR1 and the glutamate-binding site on NR2 are absolutely required for channel function, and conformational changes in both subunits appear to precede conformational changes in the transmembrane domains. While NR1 and NR2 share the same conserved residues near the extracellular end of M3, there is evidence that these structural elements are arranged asymmetrically between NR1 and NR2. Sobolevsky et al. (2002b) tested the voltage dependence of cysteine modification and the capacity of pairs of substituted cysteines on NR1 and NR2C to coordinate a Cu²⁺ ion. These data suggest that the NR1 and NR2 subunits are staggered such that the same amino acids on the NR2 subunit are approximately 4 residues (~1 helical turn) closer to the extracellular side than the corresponding residues on NR1. In conjunction with other structure information emerging from the current literature, these data bring the field a step closer to a three-dimensional picture of how channel subunits are assembled and oriented.

VI. CONCLUSIONS

Recent advances in glutamate receptor ion channel structure, especially for the agonist binding domains, have yielded new insights into the process of receptor channel activation and gating. It is now possible to connect detailed structural information with established and developing functional knowledge of ion channel gating from electrophysiological studies. Moreover, structural ideas are becoming central to understanding functional data. Studies combining both structural and functional approaches [for example, Sun et al. 2002, Jin et al. 2003] yield a richer image of ionotropic glutamate receptors than can be obtained though the study of either in isolation. The picture emerging is that of an interplay between relatively autonomous functional domains: the agonist binding S1S2 domain, the transmembrane ion channel pore, as well as the modulatory extracellular N-terminal domain and intracellular C-terminal domain.
The specifics of agonist binding and structural rearrangements of the agonist-binding domains are now well established, and the linker regions between the agonist-binding and transmembrane domains are recognized as critically involved in gating. The next challenge is to determine precisely how these conformational changes are transduced to control ion flux through the channel pore for all three classes of glutamate receptor ion channels.

ACKNOWLEDGMENTS

This work was supported by NIH (NS36654, NS39419, SFT), NARSAD (SFT), a Howard Hughes Medical Institute predoctoral fellowship (KE), and the Biotechnology and Biological Sciences Research Council (PEC, DJAW). We thank Alasdair Gibb, Jim Howe, and Geoff Swanson for critical comments on the manuscript.

REFERENCES


Banke TG, Greenwood JR, Christensen JK, Liljefors T, Traynelis SF, Schousboe A, Pickering DS (2001) Identification of amino acid residues in GluR1 re-


Burnashev N, Monyer H, Seeburg PH, Sakmann B (1992a) Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. Neuron 8:189-98.


Lomeli H, Mosbacher J, Melcher T, Hoger T, Geiger JR, Kuner T, Monyer H, Higuchi M, Bach A, Seeburg...
Critical Reviews™ in Neurobiology

K. Erreger et al.


Mosbacher J, Schoepfer R, Monyer H, Burnashev N, Seeburg PH, Ruppersberg JP (1994) A molecular de-


Schiffer HH, Swanson GT, Heinemann SF (1997) Rat GluR7 and a carboxy-terminal splice variant, GluR7b, are functional kainate receptor subunits with a low sensitivity to glutamate. Neuron 19:1141-6.


Glutamate receptor gating.


Williams K, Uttillo M, Sabado TN, Kashiwagi K, Igarashi K (2003) Pharmacology of delta2 glutamate recep-
Wollmuth LP, Kuner T, Sakmann B (1998b) Intracellular Mg2+ interacts with structural determinants of the narrow constriction contributed by the NR1-subunit in the NMDA receptor channel. J Physiol 506:33-52.