# 4

# Glutamate Receptors

## *Ionotropic*

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## 1. INTRODUCTION

L-Glutamate is the primary excitatory neurotransmitter in the vertebrate central nervous system (CNS) *(1–5)*. This conclusion, based on innumerable pharmacological, physiological, and biochemical studies, is now succinctly confirmed by the characterization of the vesicular glutamate transporters and their localization throughout the brain *(6)*. In contrast to the neuromodulatory neurotransmitters that are commonly released by brainstem nuclei projecting diffusely to large regions of the brain, and in contrast to the inhibitory, nonprojecting, local circuit neurons that use γ-aminobutyric acid (GABA) or glycine, glutamate-using pathways provide fast signaling between discrete brain regions. (For further discussion of glutamate-using pathways, *see* Chapter 3). L-Glutamate released from presynaptic nerve terminals binds to glutamate receptors on the receiving neuron. The ionotropic glutamate receptors span the plasma membrane and the binding of L-Glutamate causes a conformational change that opens a pore in the membrane formed by the receptor complex. The opened ion channel allows the influx of $Na^+$, and sometimes $Ca^{++}$ ions, causing the cell to depolarize. If sufficiently depolarized, the neuron is activated. It is the fast-acting ionotropic glutamate receptors that underlie fast electrical responses in the CNS. Unexpectedly, there is also a wealth of slower-acting G protein-coupled glutamate receptors, the metabotropic glutamate receptors. The metabotropic receptors are the subject of Chapter 5 in this volume. The discovery and characterization of L-glutamate as the major CNS neurotransmitter was a major breakthrough and has opened the door to understanding many essential aspects of brain function at all levels of investigation.

L-Glutamate was first shown to be excitatory by two independent groups. In 1954 Hayashi reported in the *Keio Journal of Medicine* that L-glutamate and L-aspartate caused convulsions after intracerebral injections into dog brain *(7)*. Independently, Watkins and colleagues, in the process of screening several known brain chemicals for excitatory and inhibitory activity, found that L-glutamate directly excited spinal cord neurons *(8)*. Of the many active agents identified in this study, Watkins and colleagues

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focused the next three decades on glutamate and aspartate (excitatory amino acids). This work characterized the excitatory action of various excitatory amino acid agonists and developed and identified antagonists that could block their action. Ultimately, these studies led to the conclusion that the excitatory actions of glutamate and aspartate were mediated by at least three distinct types of receptors, which were named for agonists by which the receptors were selectively activated: *N*-methyl-D-aspartate (NMDA), kainate, and quisqualate. The quisqualate receptor subsequently was renamed as the "AMPA" receptor (named for the selective agonist α-amino-3-hydroxy-5-methylisoxazole-4-propionate) to help avoid confusion generated by the observation that quisqualate is also a potent metabotropic glutamate receptor agonist *(3,9)*. These physiologically identified receptors differed in their physiological properties, pharmacological profiles, and anatomical distributions *(10,11)*. This receptor classification was subsequently confirmed using various radioligands (L-[$^3$H]glutamate, which bound to all three receptor types *(12)*, and the subtype-specific radioligands [$^3$H]AMPA *(13)*, [$^3$H]kainate *(14)* and D-[$^3$H]AP5 *(15)*, which bound specifically to quisqualate, kainate, and NMDA receptors, respectively). Using receptor autoradiography to map out each of these radioligand binding sites *(16–21)*, revealed discrete distributions, but collectively, glutamate receptors are found in nearly every region of the CNS. This is consistent with observations that essentially all neurons are excited by L-glutamate.

With the realization that glutamate mediates most fast synaptic transmission throughout the brain, came the pessimistic perception that the glutamate system was too widespread and of fundamental importance to be involved in subtle neurological and psychiatric disease states. Along these lines, it was felt that drugs that modulate L-glutamate receptors would be too nonspecific in their actions (e.g., general anesthetics) to be useful as therapeutic agents. Unexpectedly, however, the receptors that mediate the synaptic actions of L-glutamate were found to each be a family of receptors with discrete brain distributions and with significant differences in physiological activity and biochemical signaling. Furthermore, these glutamate receptors can have fast signaling (<10 ms), intermediate timescale signaling (100–1000 ms), and slow excitatory actions (>1 s). Consequently, there is a rich diversity of glutamate receptors and their corresponding actions. Within this diversity there is significant potential for specific receptor systems to be involved in the etiology and/or therapeutic treatment of neurological and psychiatric disorders.

Several years after the initial physiological and biochemical characterization of AMPA and kainate receptors, the distinction between these two receptors became controversial, leading to the term "non-NMDA" receptors to signify AMPA and/or kainate receptors. However, with the cloning of separate genes coding for AMPA and kainate receptors, it is clear that these two receptor families are distinct. In this chapter, we discuss the AMPA and kainate receptors together to better compare and contrast these two closely related receptors. NMDA receptors, which are functionally quite different, though closely related, will be discussed separately.

The cloning of proteins related to ionotropic glutamate receptors not only confirmed the three-receptor classification scheme initially proposed by Watkins and colleagues *(11)*, but revealed an additional subunit family termed delta (δ) *(22,23)*. These subunits have their closest homology to the kainate and AMPA subunits, but in contrast to the other glutamate ionotropic receptors, they do not form glutamate or glycine-responsive channels. Presently these receptors are considered orphan receptors. These receptors do

**Table 1**
**Glutamate Receptor Subunits and Subunit Families[a]**

| Receptor | Subunit Family | Subunits |
|---|---|---|
| AMPA | GluR1-4 | GluR1 (GluRA, α1) |
| | | GluR2 (GluRB, α2) |
| | | GluR3 (GluRC, α3) |
| | | GluR4 (GluRD, α4) |
| Kainate | KA | KA1 (γ1) |
| | | KA2 (γ2) |
| | GluR5-7 | GluR5 (β1) |
| | | GluR6 (β2) |
| | | GluR7 (β3) |
| GluRδ | GluRδ | GluRδ1 |
| | | GluRδ2 |
| NMDA | NR1 | NR1a-h (ζ1) |
| | NR2 | NR2A (ε1) |
| | | NR2B (ε2) |
| | | NR2C (ε3) |
| | | NR2D (ε4) |
| | NR3 | NR3A (χ1) |
| | | NR3B (χ2) |

[a]Alternative nomenclature is noted in parentheses.

appear to be involved in some aspect of synaptic transmission and synapse formation. In the δ-2 knockout, there is an impairment in cerebellar long-term depression (LTD) and Purkinje cell synapse formation *(24,25)*. In the lurcher mouse, the defect is a mutation in δ-2 that renders the channel constitutively active and associated with cerebellar neuronal cell loss and ataxia *(26)*. Recently, Yuzaki and colleagues have presented evidence for heteromeric complex formation between δ-2 and AMPA and kainate receptor subunits *(27)*. For an overview of glutamate receptor subunit families, *see* Table 1.

## 2. AMPA AND KAINATE RECEPTORS

### *2.1. AMPA/Kainate Receptor Function*

Agonist binding to either AMPA or kainate receptors opens a channel permeable to $Na^+$ and $K^+$ ions. With the influx of $Na^+$ ions, the cell membrane is depolarized. In special circumstances, some receptor channels also exhibit high $Ca^{2+}$ permeability, depending on subunit composition and posttranscriptional editing *(28)*. For both AMPA and kainate receptor ion channels, they are rapidly activated and, in the presence of L-glutamate, are rapidly inactivated owing to desensitization. It is this rapid activation/deactivation that allows fast synaptic transmission to accurately follow high-frequency CNS activity. AMPA receptors can activate in the submillisecond time-scale and then desensitize in the 1- to 10-ms range (e.g., ref. *29*). Recovery from desensitization takes longer, in the 10s of ms scale. Kainate receptors also have fast activation/deactivation kinetics; however, they can differ from AMPA responses in being slower *(30)*. On the basis of pharmacological experiments and receptor distribution studies, AMPA receptors are thought to be the primary signal for fast excitatory synaptic transmission in the vertebrate CNS. Only in recent

years have the pharmacological tools become available to study kainate receptor function. As found for AMPA receptors, kainate receptors also mediate fast synaptic transmission, but also play other roles in synaptic signaling.

In addition to ionotropic effects, each of the ionotropic glutamate receptors have been suggested to have G protein-coupled receptor-like activity. For example, some physiological responses mediated by kainate receptors have been reported to require G protein activation *(31)*. Though such signaling mechanisms are difficult to explain, recent evidence for ionotropic receptor subunit association with G protein-coupled receptors indicates that there may be many potential signals arising from glutamate ionotropic receptors *(32,33)*.

### 2.2. AMPA/Kainate Receptor Subunits

Molecular cloning has led to the isolation of four AMPA receptor subunits, GluR1-GluR4, and five subunits that combine to form kainate receptors. There are two types of kainate receptor subunits: GluR5-GluR7 are low-affinity kainate receptor subunits that have been shown to form functional ion channels when homomerically expressed in HEK 293 cells or Xenopus oocytes *(34,35)*. KA1 and KA2 are high-affinity kainate binding proteins that combine with GluRs 5–7 in native receptors but do not form functional homomeric channels (for reviews, *see* refs. *28,36*).

AMPA and kainate iGluR subunits are approx 900 amino acids long with a molecular weight of around 100 kDa. Hydrophobicity analysis originally suggested that each subunit contained four membrane-spanning domains but *N*-glycosylation, site-specific antibodies, and mutagenesis studies have since led to the currently accepted topology where the second proposed transmembrane domain is actually a re-entrant loop *(28,37)*. Functional non-NMDA iGlu receptors have been postulated to consist of homo- or heteromeric assemblies of either four or five subunits, although the tetrameric assembly is now the more widely accepted stoichiometry *(38,39)*.

Studies using chimeric assemblies of AMPA and kainate receptor subunits and site-directed mutagenesis demonstrated that the agonist-binding site of the receptor is formed between two segments, termed S1 and S2 *(40,41)*. S1 is a 130 amino acid section preceding the M1 transmembrane domain and S2 is made up of most of the extracellular amino acids between transmembrane domains M3 and M4 *(40)*. *See* Fig. 1 for the general structure of ionotropic glutamate receptors.

### 2.3. Receptor Subunit Structure

#### 2.3.1. Ligand-Binding Domain Structure

A major advance in the study of glutamate receptors in recent years has been the development of crystal structure analysis of the ligand-binding core of the receptors. In order to attain high-resolution X-ray structures, a soluble form of the ligand-binding core of GluR2 was made by substituting the first two transmembrane regions, M1 and M3, with a peptide linker and removing the extreme amino terminal domain and M4 *(42)*. After the original description of this ligand binding core in complex with kainate *(42)*, several studies have described the high-resolution X-ray structure of the GluR2 construct in complex with agonists, antagonists and in its unbound, apo state *(43–47)*. A representation of the GluR2 ligand-binding domain crystal structure is shown in Fig. 2.

The crystal structure of the truncated GluR2 subunit in complex with kainate indicated that two domains are involved in the binding of an agonist. One domain is formed by the

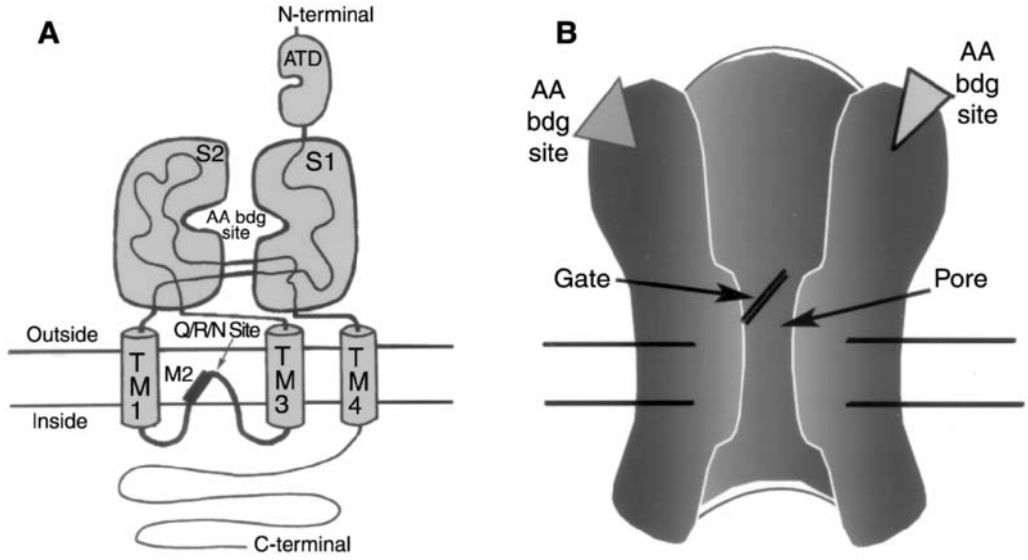


**Fig. 1.** Glutamate receptor subunit and complex structure. **(A)** A schematic representation of the ionotropic glutamate receptor subunit topology. The S1 and S2 domains together form the amino acid binding site (AA bdg site) for glutamate or glycine. The critical site for determining channel permeability (Q/R/N site) is shown at the center of the M2 domain. Four subunits assemble **(B)** to form a pore structure through the membrane with the M2 region of each subunit contributing to the pore constriction.

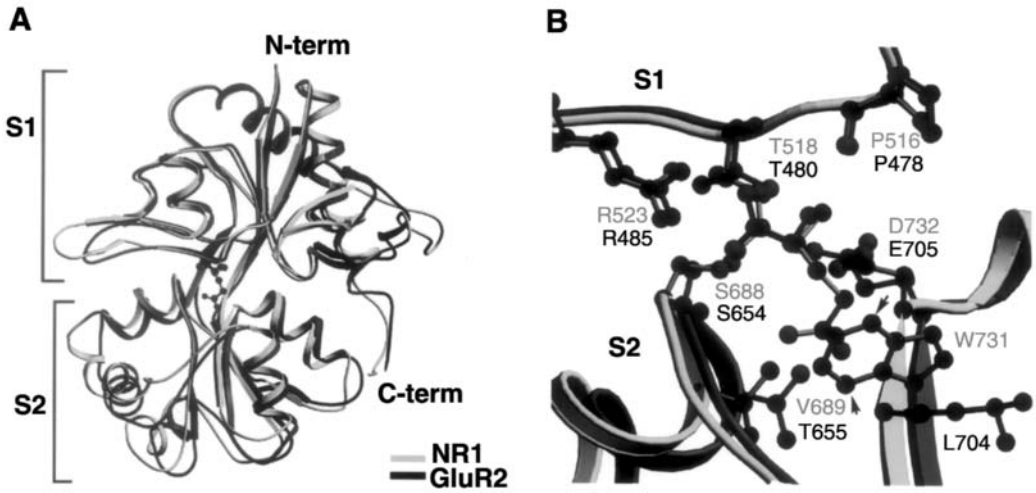


**Fig. 2.** Crystal structure of the S1/S2 ligand-binding domain of GluR2 and NR1. **(A)** The S1 and S2 domains of GluR2 and NR1 are superimposed. The N-terminals (N-term) and C-terminals (C-term) are shown. **(B)** Glutamate and glycine are shown docked into their respective binding sites. The peptide amino acid residues critical to ligand binding are shown. Although the α amino and carboxy groups of glutamate and glycine bind in a similar fashion, tryptophan (W) 731 of NR1 blocks the binding of the longer glutamate structure, but allows binding of glycine. Figure adapted from Furukawa and Gouaux *(47a)* and used by permission of the publishers.

**Table 2**
**Key Residues in the Agonist-Binding Cavity of AMPA and Kainate Receptors[a]**

| GluR1-4 | GluR5/6/7 | KA1/2 | Interaction with kainate |
|---|---|---|---|
| Arg485 | Arg | Arg | α-carboxyl group of kainate |
| Thr480 | Thr/Ala/Thr | Thr | protonated amino group of kainate |
| Glu705 | Glu | Glu | protonated amino group of kainate |
| Ser 654 | Ser/Ala/Ser | Ser | ω-carboxyl group of kainate |
| Thr655 | Thr | Ser/Thr | ω-carboxyl group of kainate |

[a]The GluR2 crystal structure was used to demonstrate the residues that interact with kainate and the residues present at equivalent positions in the other subunits are indicated *(48)*.

S1 segment and a 33 amino acid segment in the C-terminal end of S2 (which includes the flip/flop site). The second domain is made up of the 134 amino acids in the N-terminal end of the S2 segment. When kainate was docked in the receptor construct it bound between the two domains with its glutamate-like backbone forming a bridge between domains 1 and 2. The study also identified amino acids that are likely to be essential for agonist binding. Proposed interactions between kainate and amino acids in the GluR2 agonist binding pocket are summarized in Table 2. Mutagenesis studies have shown that these five residues, at equivalent positions in other glutamate receptors, are important for agonist interactions. An example of another residue that appears to be important for agonist interaction in GluR2 is Tyr450. This amino acid residue appears to prevent full closure of the binding domain by forming a wedge between the pyrrolidine ring and the isopropenyl group of kainate and domain 1. The authors proposed that this steric clash is what causes kainate to be a partial agonist, whereas AMPA and glutamate, whose structures would not clash with this residue, can allow further closure of the binding domains and therefore act as full agonists *(42)*. The degree of domain closure has also been correlated to the extent of desensitization induced by agonists *(46)*.

In Table 2 some of the key residues present in the agonist-binding core at equivalent sites in AMPA and kainate receptor subunits are compared. The five key residues believed to interact with the glutamate backbone of kainate in the GluR2 subunit are conserved in GluR1-4, GluR5, GluR7, and KA2. The differences seen in the GluR6 agonist-binding cleft may explain why various classes of compound act selectively at AMPA and GluR5 receptors but not GluR6 receptors. Another residue, Met708, of GluR2 is replaced by a serine residue in GluR5 and this amino acid switch has been proposed as an explanation for the GluR5 selectivity of ATPA and 5-iodowillardiine *(46,47)*.

Crystallographic studies have also demonstrated that the extent of cleft closure seems to correlate well with the activity of ligands at the GluR2 subunit. For example, the full agonists glutamate and AMPA induce a cleft closure of approx 20° when compared to the ligand free (apo) state whereas the partial agonist kainate leads to a cleft closure of only approx 12° *(43)*. A further study demonstrated that AMPA receptor agonists with an isoxazole ring bind in slightly different ways, depending on the substituents added to the isoxazole ring, and there was a strong correlation between the degree of domain closure and efficacy in electrophysiological studies *(44)*. Evidence from studies using antagonists in complex with the ligand-binding core of GluR2 agree with the concept that cleft closure is related to activation. Two structurally unrelated antagonists, DNQX and ATPO, have been shown to stabilize an open form of the ligand-binding core *(43,44)*.

### *2.3.2. Structural Basis of Desensitization*

A recent study by Sun and colleagues *(49)* used structural and functional studies to develop a mechanistic scheme for the process of desensitization in AMPA and kainate receptors. The authors suggest that the four subunits of each receptor form dimers. They demonstrated that cyclothiazide can promote dimerization of the subunits and, using crystallography, showed that cyclothiazide interacts with a pocket formed at the interface of two subunits. It was proposed that after agonist binding the agonist is trapped in a cleft between two domains of the subunit, which leads to conformational strain causing the opening of the ion channel. When desensitization occurs the dimer interface changes and the domain closure no longer leads to ion channel opening. Normally, the energy barrier for activation is lower than that for desensitization. Once the receptor is desensitized, however, it is more stable than an active receptor and therefore prolonged agonist application leads to the desensitization of most of the receptor population *(49)*.

### *2.3.3. Ion Channel Structure*

Glutamate receptor ion channels are thought to be formed as a tetramer of M2 pore-lining segments *(50)*. The M2 loop penetrates only partially into the membrane with a key amino acid residue position, termed the "Q/R" site, at the tip of the loop *(51)*. The constriction of the pore appears to be two amino acid postions C-terminal to the Q/R site. On the N-terminal side of the Q/R site, M2 forms an α helix with a dipole that has the negative end inside the membrane near the Q/R site and the positive end near the cytoplasmic surface. Near the Q/R site is a kink and C-terminal to this is an extended form of polypeptide chain (descending random coil) that returns to the cytoplasmic surface. This structure is generally similar to that found for various potassium channels. Recently, bacterial glutamate receptors have been identified that gate potassium channels and have specific structural similarities to both mammalian glutamate receptors and potassium channels *(49)*.

## *2.4. Multiple Isoforms of AMPA and Kainate Receptor Subunits*

### *2.4.1. Alternative Splicing*

Different forms of AMPA and kainate receptors exist owing to alternative splicing and RNA editing (Table 3). The AMPA receptor subunit GluR4 can exist in an alternative splice variant form, GluR4c, which has a short C-terminus. Of the kainate receptor subunits, both GluR5 and GluR7 exist as various splice variants. GluR5-1 has an additional 15 amino acid section in the N-terminal region *(52)*. GluR5-2 has three further variants, termed GluR5-2a, -2b, and -2c, each of which varies in its C-terminal domain. Variation in the C-terminal domain also gives rise to the two splice variants of GluR7, GluR7a and GluR7b *(35)*. No alternative splicing has been reported for GluR6, KA1, or KA2 subunits.

AMPA receptor subunits (GluR1–4) contain an alternatively spliced cassette of 38 amino acids in the extracellular loop preceding the M4 transmembrane domain. Two variants of this cassette exist, termed "flip" and "flop" isoforms, which differ in their desensitization profiles. The flip isoform displays less desensitization after application of glutamate or AMPA than does the flop isoform *(53)*.

### *2.4.2. RNA Editing*

When the genomic sequence for GluR2 receptor subunits was determined, a mismatch was discovered between the genomic sequence and the cDNA sequence. Whereas the

**Table 3**
**Summary of the Multiple Isoforms of AMPA and Low-Affinity Kainate Receptor Subunits**

| Receptor subunits (splice variants in brackets) | Contains flip/flop cassette | Contains Q/R RNA editing site in M2 | Contains R/G editing site preceding flip/flop cassette |
|---|---|---|---|
| GluR1 | Yes | No | No |
| GluR2 | Yes | Yes | Yes |
| GluR3 | Yes | No | Yes |
| GluR4 (4c) | Yes | No | Yes |
| GluR5 (1, 2a, 2b, 2c) | No | Yes | No |
| GluR6 | No | Yes | No |
| GluR7 (7a, 7b) | No | No | No |

initially characterized cDNA sequence coded for an arginine in the middle of GluR2's M2 re-entrant loop, the genome codes for a glutamine. The genes for all AMPA and kainate receptor subunits code for a neutral glutamine (Q) at this position, however, RNA for GluR2, GluR5, and GluR6 can undergo site-specific posttranscriptional RNA editing that leads to the replacement of this amino acid with a positively charged arginine (R) *(54–56)*. This modification is highly significant; animals without Q/R editing have seizures and die young. Thus, the genome codes for a lethal mutation. Accordingly, editing from Q to R at this site in the GluR2 subunit is very efficient and is thought to be almost complete in rat brain *(54)*. The replacement of glutamine by arginine at the Q/R editing site in GluR2 subunits results in ion channels with low calcium permeability and linear current–voltage relationships *(57,58)*. Edited GluR2 subunits determine the channel properties when coassembled with other AMPA receptor subunits (which code for Q and are not edited). Because most native AMPA receptors have a GluR2 subunit, most AMPA receptors in the brain are not calcium permeable owing to the presence of edited GluR2 subunits.

Q/R editing is a result of the actions of ADAR2 (adenosine deaminase acting on RNA – 2) *(59)*. This enzyme recognizes a specially folded RNA structure and deaminates the critical adenosine to make an inosine. This changes the three-letter code from CAG (which codes for glutamine) to CIG, which codes for arginine. The ADAR2 knockout in mice is lethal, but not in mice where the GluR2 Q/R site is mutated at the genome to code for R *(60)*.

Q/R site editing of GluR5 and GluR6 subunits also results in lower $Ca^{2+}$ permeability, although homomeric GluR6 edited receptors are not purely cation selective as they also permit anions through their channels *(55,58,61,62)*. GluR5 and GluR6 editing is less efficient with 35 and 75% of these subunits being edited, respectively *(61)*.

Another site of RNA editing is found in the segment immediately preceding the flip/flop site. Three of the AMPA receptor subunits, GluR2, GluR3, and GluR4, undergo editing of an arginine (R) to a glycine (G) at this site; this modification increases the rate of onset and the rate of recovery from agonist-induced desensitization in receptors containing these subunits *(63)*.

In the first transmembrane-spanning segment, M1, GluR6 receptors contain two further editing sites. An isoleucine/valine site is encoded by the gene and a tyrosine/cysteine site is encoded by the edited transcipt *(64)*. The $Ca^{2+}$ permeability of these kainate receptors can vary depending on editing of both M1 and M2 regions *(61)*. Calcium-permeable AMPA

and kainate receptors have an inwardly rectifying current-voltage relationship, which is a result of a polyamine (spermine) block of the channels at positive potentials *(65)*.

### *2.5. Homomeric and Heteromeric Assemblies of AMPA and Kainate Receptors*

Each of the AMPA receptor subunits, GluR1–GluR4, and the low-affinity kainate receptor subunits, GluR5–GluR7, can form functional channels when expressed homomerically. However, the ability of the subunits to form heteromeric complexes greatly increases the functional diversity of AMPA and kainate receptors. Several studies have demonstrated that changing the subunit composition slightly can dramatically change the pharmacology of receptors. For example, (*S*)-5-iodowillardiine (300 μ*M*) shows no activity at homomeric GluR6 or GluR7 receptors but elicits small currents in GluR6/KA2 and GluR7/KA2 heteromers *(66)*. Also, the reportedly GluR5-selective agonist ATPA gave an $EC_{50}$ value of 2.1 μ*M* at recombinant GluR5 subunits and was inactive at GluR6 homomeric channels, yet when tested at heterologous subunit assemblies it, gave an $EC_{50}$ of 6.3 μ*M* at GluR5/KA2 receptors and 84 μ*M* at GluR6/KA2 receptors *(67,68)*. Heteromeric assemblies of subunits also display different rates of desensitization than homomeric receptors. For example, in one study 10 m*M* glutamate currents in GluR6 homomers and GluR6/KA2 receptors gave desensitization times ($\tau_{des}$) of 3.8 ± 0.2 ms and 2.3 ± 0.2 ms, respectively, and 30 m*M* glutamate currents gave $\tau_{des}$ times of 7.6 ± 0.53 ms in GluR7 homomers but 6.6 ±1.0 ms in GluR7/KA2 heteromeric receptors, *(66)*. (*S*)-5-Iodowillardiine currents displayed a $\tau_{des}$ of 8.9 ± 1.6 ms in GluR5 receptors, which was significantly reduced to 2.6 ± 0.2 ms in GluR5/KA2 heteromers *(66)*.

A recent study making use of the selective kainate receptor agonist dysiherbaine demonstrated that each type of subunit within a heteromeric kainate receptor can contribute a distinct conductance upon activation by agonist binding *(69)*. The authors reported how a long-lasting interaction between dysiherbaine and GluR5 subunits elicits a tonic current from GluR5/KA2 heteromers; then subsequent cooperative gating of the KA2 subunits can be elicited by another agonist, such as glutamate *(69)*.

Examples of both homomeric and heteromeric AMPA and kainate receptors have been detected *in situ*, although the subunit composition of most native receptors remains unknown.

### *2.6. Pharmacology of AMPA and Kainate Receptors*

Only a brief introduction of the pharmacology of AMPA and kainate receptors is given here; for more in-depth reviews, *see* refs. *28,36,70*. For structures of the most useful pharmacological tools, *see* Figs. 3 and 4.

#### *2.6.1. AMPA Receptor Agonists*

Originally defined using the agonist quisqualate, AMPA was shown to be a more selective agonist for this receptor type in the 1980s *(71)*. This agonist is still widely used in the study of AMPA receptors, although the willardiine derivative (*S*)-5-fluorowillardiine is also a potent and selective AMPA receptor agonist *(72,73)*. As kainate-evoked AMPA receptor responses are nondesensitizing, this agonist is also widely used to activate AMPA receptors.

#### *2.6.2. Kainate Receptor Agonists*

The standard agonists at kainate receptors, kainate and domoic acid, have limited use owing to their activation of AMPA receptors *(28)*. More recently, however, ligands have been

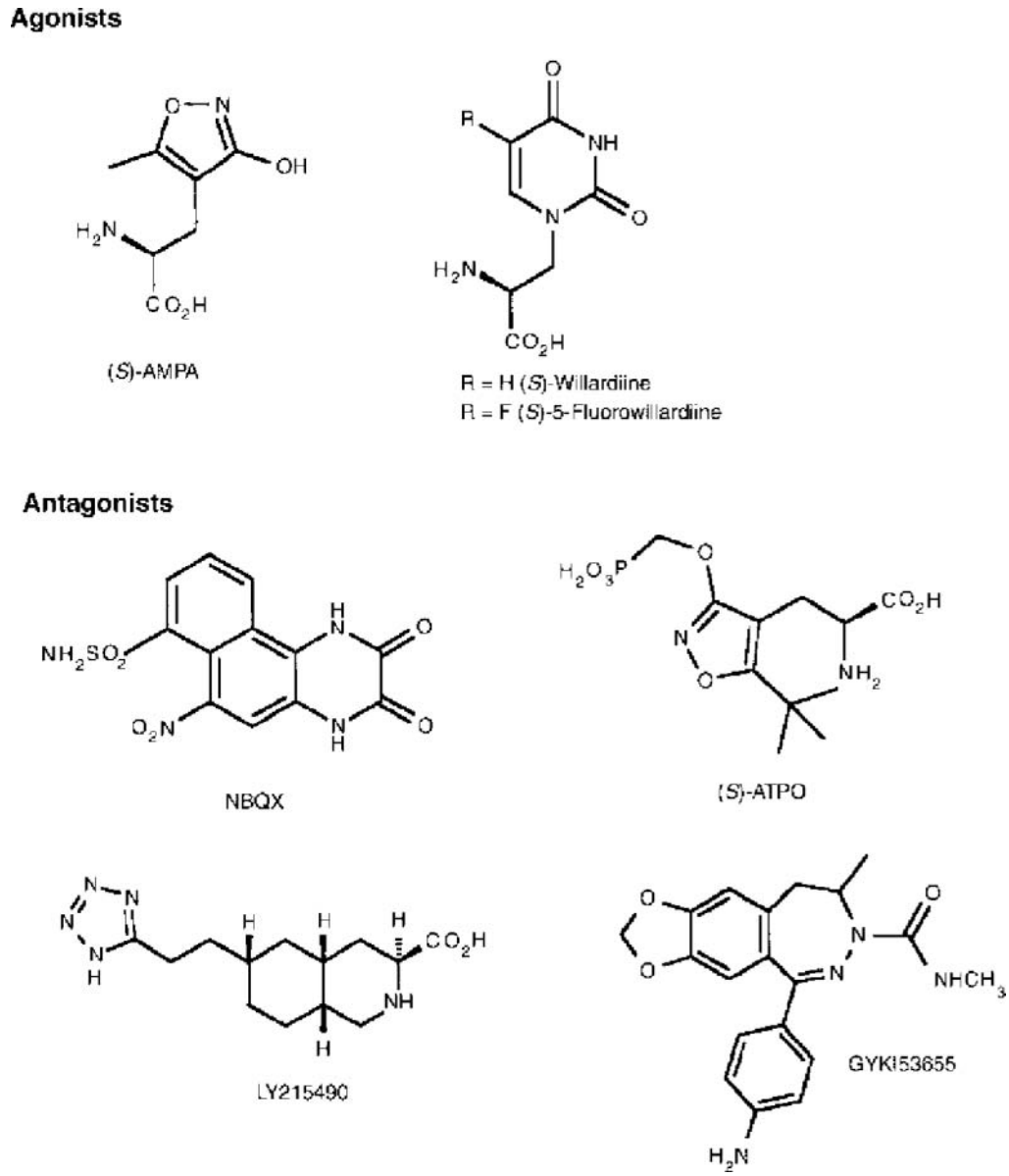


**Fig. 3.** Structures of key AMPA receptor compounds.

developed that show selectivity for kainate over AMPA receptors. (*S*)-5-Iodowillardiine and ATPA both show selectivity for the GluR5 kainate receptor subunit over other kainate or AMPA receptors *(67,72)*. (2*S*,4*R*)-4-methylglutamate shows potent agonist activity at both GluR5 and GluR6 receptors *(74)*, although the fast desensitization properties of this agonist often lead to its use as a functional antagonist of these receptors *(75)*.

*2.6.3. AMPA Receptor Antagonists*

The most commonly used competitive antagonists of AMPA receptors are the quinoxalinediones. Of these NBQX is the most selective, although it displayed only 30-fold selectivity for AMPA over kainate receptors in binding studies *(76)*. CNQX is less potent

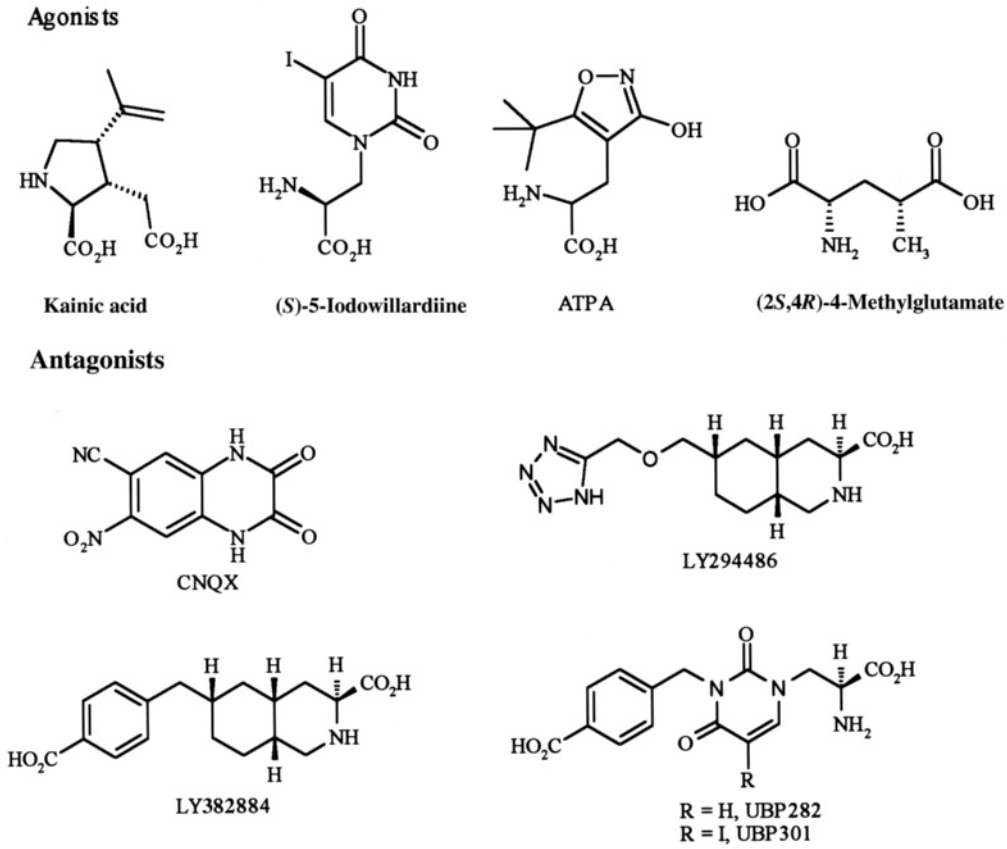


**Fig. 4.** Structures of key kainate receptor compounds.

and selective so its use is limited to studies where the nonselective antagonism of both AMPA and kainate receptors is required *(76,77)*.

A more selective pharmacological tool for blocking AMPA receptor responses is the noncompetitive antagonist GYKI53655 *(75,78)*. This 2,3-benzodiazepine and a related compound GYKI52466 have been used widely in physiological studies to selectively depress AMPA over kainate receptor responses *(28,36)*. They appear to bind to an allosteric site on the AMPA receptor acting as negative allosteric modulators *(79,80)*.

### *2.6.4. Kainate Receptor Antagonists*

Until recently there was a paucity of selective kainate receptor antagonists, which limited information about the physiological roles of this receptor family. A series of decahydroisoquinolines have been developed as antagonists of AMPA and kainate receptors and several of these compounds, including LY382884, LY294486, and LY293558, show selectivity for the GluR5 kainate receptor subunit over other AMPA or kainate receptor subunits *(36,81)*. They have therefore been used to demonstrate the importance of the GluR5 receptor subunit in CNS functions. More recently, novel antagonists based on the structure of willardiine, such as UBP282 and UBP301, have been developed, the latter showing selectivity for the GluR5-containing kainate receptors present on neonatal dorsal root fibers over the AMPA receptors expressed on spinal motoneurons *(81)*. There is still a lack of antagonists with selective activity at GluR6 or GluR7 receptor subunits.

#### 2.6.5. Allosteric Modulators

Several positive allosteric modulators of AMPA receptors have been reported, the most commonly used being the benzothiadiazide cyclothiazide (for a review, *see* ref. *28*). For kainate receptors, the lectin concanavalin A is often used to reduce receptor desensitization. These two allosteric modulators can be used to differentiate between physiological responses of AMPA or kainate receptors *(83)*.

### 2.7. AMPA and Kainate Receptor Radioligands

Radiolabeled ligands are available for both AMPA and kainate receptors. These are useful for determining the pharmacological properties, as well as distributions and densities of receptors in a variety of tissues or experimental conditions. As expected [$^3$H]AMPA labels AMPA receptors *(13)*. [$^3$H]5-fluorowillardiine is another AMPA receptor agonist that can be used to radiolabel AMPA receptors *(84)*. In addition, the AMPA antagonist [$^3$H]NBQX labels a larger population of AMPA receptors *(85)*. To label kainate receptors, there are two agonists available, [$^3$H]kainate *(14)* and [$^3$H]4-methylglutamate *(86)*. The high- and low- affinity sites labeled by [$^3$H]kainate can be distinguished by adding calcium, which inhibits binding to the high-affinity site *(87)*. Both AMPA and kainate receptors are labelled by L-[$^3$H]glutamate *(12,88)*, but this signal is relatively small owing to the labeling of NMDA receptors and thus the more selective radioligands are preferred.

### 2.8. Physiological Roles of AMPA and Kainate Receptors

#### 2.8.1. Presynaptic Modulation by AMPA and Kainate Receptors

In addition to their role in mediating postsynaptic excitatory transmission in the CNS, AMPA and kainate receptors have also been reported to exist presynaptically, acting to regulate synaptic transmission (for reviews, *see* refs. *89* and *90*). Presynaptic kainate receptors reportedly modulate both GABAergic and glutamatergic terminals and in recent years there has been conjecture as to whether presynaptic kainate receptors act by ionic or metabotropic mechanisms *(89,90)*.

Studies have suggested that kainate causes a biphasic modulation of NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) on CA1 neurons in the hippocampus and this action is sensitive to the GluR5 receptor antagonist LY294486 *(91,92)*. The activation of presynaptic kainate receptors has also been shown to suppress glutamate release from primary afferent spinal cord neurons *(93)*.

In the hippocampus and other brain areas, it has been shown that kainate application, possibly acting at GluR5-containing kainate receptors, causes a decrease in evoked inhibitory postsynaptic current amplitude in the presence of GYKI53655 *(66,94–96)*. Most studies agree that the depression of GABAergic transmission by kainate is owing to a direct presynaptic effect *(97–99),* although some reports have suggested that depression of evoked GABA release is secondary to an enhancement of GABA release, with the GABA then acting on presynaptic $GABA_B$ regulatory receptors to downregulate release *(93,95)*.

In some cases kainate receptor agonists can also act to facilitate GABAergic transmission. Researchers have attributed this to a direct effect at presynaptic terminals of interneurons with kainate possibly acting at non-GluR5 receptors *(100,101)*. However, in a recent study, Braga and colleagues reported that low concentrations of GluR5 kainate receptor agonists could enhance GABAergic transmission whereas high concentrations depressed transmission in the basolateral amygdala *(99)*. The authors concluded that

both these effects were owing to a direct effect of the kainate receptor agonists at GABAergic terminals and the bidirectional effects of the agonists may be a result of activation of two receptor populations, each with different affinities for the agonists and mechanisms of action *(99)*. The development of more selective ligands for kainate receptors may help to clarify this.

Although fewer examples have been described, evidence for presynaptic AMPA receptors is also beginning to emerge. Presynaptic AMPA receptors have been reported to inhibit the release of GABA from GABAergic interneurons in the cerebellum *(102)*, yet to increase the release of GABA from cerebellar stellate cells *(103)*. A recent study has also reported the functional expression of AMPA receptors on the central terminals of dorsal root ganglion neurons which, when activated, inhibit the synaptic release of glutamate *(104)*.

The increase in reports of presynaptic AMPA and kainate receptors led to the question of how these receptors regulate transmitter release. Although many of the presynaptic effects can be explained by ionotropic mechanisms, there have also been reports of metabotropic functions. Rodriguez-Moreno and Lerma *(97)* reported a metabotropic function for the presynaptic kainate receptors on CA1 interneurons and a kainate receptor-mediated metabotropic function has also been reported in CA1 pyramidal cells where kainate exposure leads to an inhibition of an afterhyperpolarization potassium current ($I_{sAHP}$), which follows short bursts of action potentials *(105)*. Inhibition of this $I_{sAHP}$ by excess glutamate may lead to hyperexcitability via a positive feedback loop of glutamate release *(97,105)*.

### *2.8.2. AMPA and Kainate Receptor Function: Information From Transgenic Mice*

As there are few subunit selective pharmacological tools, researchers have used gene targeting to provide some information about the roles of different AMPA and kainate receptor subunits. Mice lacking the GluR2 subunit contain AMPA receptors with increased $Ca^{2+}$ permeability, enhanced neuronal excitability, and increased synaptic plasticity in the CA1 region of the hippocampus *(106,107)*. Kainate-evoked AMPA receptor-mediated currents and AMPA receptor-mediated neurotoxicity were also increased in GluR2$^{-/-}$ neurons *(108)*. Transgenic mice overexpressing the AMPA receptor subunit GluR2-flip have been used to demonstrate the crucial role of these receptor subunits in the pathogenesis of focal hypoxic-ischaemic neuronal cell death *(109)*.

In GluR1 knockout mice associative long-term potentiation was absent in CA3 to CA1 synapses *(110)*. Knockout mice have also implicated the importance of GluR1-containing AMPA receptors in the synaptic plasticity in the basolateral nucleus of the amygdala, which underlies conditioned reinforcement *(111)*.

In one GluR5 mutant where all the Q/R sites are edited (i.e., encode arginine), fewer and smaller responses to the kainate receptor agonist domoate were recorded in isolated dorsal root ganglion neurons *(112)*. In GluR5 global knockout mice, there is a loss of kainate receptor-mediated potentiation of evoked excitatory synaptic transmission in perforant path inputs to CA3 neurons and loss of kainate-induced enhancement of mEPSC frequency in mossy fiber synapses, although overall neuroanatomy and general health is normal *(100,113,114)*.

In GluR6 global knockout mice the following are absent: small kainate-induced currents in CA1 hippocampal neurons, kainate receptor-mediated responses in CA3 neurons, high-affinity kainate binding in CA3 neurons or in the dentate gyrus, and synaptic activation

of kainate receptors in the mossy fiber pathway. There is also loss of kainate receptor-mediated depression of evoked excitatory synaptic transmission in both mossy fiber and associational-commisural inputs to CA3 neurons and loss of kainate receptor-mediated potentiation of evoked excitatory synaptic transmission in perforant path inputs to CA3 neurons. In addition, there is a reduction in mossy fiber LTP and GluR6$^{-/-}$ mice are less susceptible to seizures induced by kainate injection *(113–116)*. Mutant mice in which the Q/R site cannot be edited have been used to demonstrate that kainate-induced seizure susceptibility is inversely correlated with the degree of editing of the GluR6 subunit *(117)*.

In KA2 global knockout mice, there is a loss of the facilitatory effect of low doses of kainate at mossy fiber synapses and the depressant effect of kainate occurs at lower concentrations than in wildtype mice. The heterosynaptic facilitation of mossy fiber EPSCs is also absent in KA2 knockouts *(118)*.

## 3. NMDA RECEPTORS

### *3.1. NMDA Receptor Function: Physiological Properties*

#### *3.1.1. Voltage Dependency and $Mg^{++}$ Blockade*

NMDA receptors have a distinctive role in synaptic transmission because of several unique physiological and biochemical properties. Several years passed after identifying the first NMDA receptor antagonist before the first demonstration of an NMDA receptor-mediated synaptic response *(119)*. This delay occurred because NMDA receptors do not mediate the primary fast synaptic response in a glutamate-using synapse. Instead, they are more robustly activated under special conditions, such as high-frequency synaptic activation or with concurrent depolarization. Under normal physiological conditions, a single activation of a synaptic pathway results in an AMPA receptor-mediated synaptic response but with little detectable NMDA receptor-mediated component *(120)*. The apparent absence of a NMDA receptor synaptic component is owing to the voltage-dependent properties of the NMDA receptor. In contrast to most other ligand-gated ion channel receptors, NMDA receptor currents are both ligand gated and voltage gated *(121,122)*. Greater NMDA receptor responses occur when the cell is moderately depolarized from the resting membrane potential. Thus, partial depolarization results in larger NMDA-evoked currents, even though the voltage-gradient driving force responsible for these currents is reduced. The voltage dependency of NMDA receptors is because of the preferential blockade of NMDA receptor channels by $Mg^{++}$ ions at negative membrane potentials *(123,124)*. Hence, at the normal negative resting membrane potential, physiological concentrations of $Mg^{++}$ ions potently block NMDA receptor channels. However, if the cell has been previously depolarized, such as during high-frequency stimulation, $Mg^{++}$ ions can no longer block the NMDA receptor channel and a larger current results. Thus, NMDA receptor responses are dependent upon the immediately preceding history of the cell, with larger NMDA receptor responses occuring if the cell is currently depolarized from a previous synaptic signal.

#### *3.1.2. Calcium Permeability*

NMDA receptor ion channels are highly permeable to calcium *(125)*. Whereas most ligand-gated cation channels are permeable to just $Na^+$ and $K^+$ ions, the NMDA receptor is also permeable to $Ca^{++}$ ions. Calcium itself is a potent second messenger, able to regulate the functions of a large variety of intracellular signaling systems. It is this influx of calcium

that is thought to be responsible for many of the subsequent biological actions induced by NMDA receptor activation. With the combination of the two distinctive physiological properties, voltage dependency and calcium permeability, NMDA receptors are able to use calcium as a trigger for experience-dependent plasticity, most notably the phenomenon known as long-term potentiation (LTP).

### *3.1.3. Slow, Long-Lasting Channel Currents and Desensitization*

The AMPA and kainate receptors can activate in less than a millisecond and desensitize in 1–10 ms. In contrast, NMDA receptor activation occurs much more slowly, peaking well after the AMPA receptor response has desensitized (20–30 ms; ref. *120*). Furthermore, the NMDA receptor response has a half-life of 100–5000 ms. Thus, at fairly low frequencies, some NMDA receptors are continuously activated.

Unlike AMPA and kainate receptors, NMDA receptors do not show as complete or rapid desensitization. They do, however, desensitize. At least three different mechanisms contribute to NMDA receptor desensitization: (a) $Ca^{++}$-dependent inactivation, (b) glycine/$Ca^{++}$-independent desensitization, and (c) glycine-sensitive desensitization (for review, *see* ref. *126*). The $Ca^{++}$-dependent inactivation is displayed by NR1a/NR2A and NR1a/NR2D receptors, but not by NR1a/NR2B and NR1a/NR2C receptors *(127)*. The glycine/$Ca^{++}$-independent desensitization is most robust with NR1a/NR2A, less apparent with NR1a/NR2B, and not seen in NR1a/NR2C *(127)*. Glycine-sensitive desensitization is reversed by saturating concentrations of glycine. The apparent desensitization is thought to be a result of glutamate binding causing a reduction in glycine affinity *(128)*.

### *3.1.4. Glycine or D-Serine Coagonist*

Although NMDA receptors are termed glutamate receptors, they require the presence of another agonist, glycine or D-serine, to achieve channel activation *(129,130)*. Thus, L-glutamate or NMDA alone is insufficient to evoke an NMDA receptor-mediated response. Likewise, glycine alone is insufficient to evoke an NMDA receptor response, but glycine together with L-glutamate results in full receptor activation. In many preparations, the glycine-binding site appears to be saturated, or nearly saturated, in the absence of exogenous glycine or D-serine. Hence the receptor is responsive to L-glutamate. Because of the assumed tonic saturation of the glycine site, relatively few studies have examined the role of cellular mechanisms that modulate glycine levels in the synapse. However, there is some evidence that both glycine and D-serine may have their extracellular levels regulated at subsaturating concentrations, and hence may play a role in modulating the excitability levels of NMDA receptors *(131)*. In recent years, the role of D-serine has become an important topic and the enzymes that regulate its synthesis have been characterized *(132)*. A provocative finding is that some schizophrenics contain a genetic mutation in a protein that modulates the enzyme oxidizing D-serine—a finding consistent with the NMDA-receptor hypofunction hypothesis of schizophrenia *(133)* and the recent report of reduced D-serine in schizophrenics *(134)*. (*See* Chapter 7 for further discussion).

### *3.1.5. NMDA Receptor Cellular Functions*

It is now well established that NMDA receptor activation is necessary for most forms of LTP that have been observed in brain tissue (for reviews, *see* refs. *135–137*). In LTP, high-frequency stimulation of an afferent input leads to long-lasting enhancement of the synaptic response when the afferent is tested later with a single stimulation. This phenomenon is thought to represent a cellular mechanism for learning and accordingly,

NMDA receptor blockade (or NMDA receptor knockout) can block some forms of learning *(138)*. Interestingly, NMDA receptor activation is also required for some instances of the opposing phenomenon of LTD. In LTD, the use of other stimulus paradigms leads to a depression of synaptic responses. Currently, it is thought that the entry of low levels of calcium causes LTD whereas higher levels of calcium influx lead to LTP *(139)*. Alternatively, we have presented evidence that the NMDA receptor subtypes triggering LTP and LTD are pharmacologically distinct *(140)*.

NMDA receptor activation is also key to several forms of experience-dependent plasticity wherein experience (e.g., visual stimulation) causes both the pruning and expansion of afferent terminals onto their target fields. The most well-characterized example is the formation of ocular dominance columns owing to binocular visual stimulation. For a review of this field, see ref. *139*. NMDA receptor activation is also required for somatosensory mapping of the whisker representations in cortex and trigeminal nucleus (the "barrels") *(141,142)*.

In addition to the above-mentioned plasticity mechanisms, NMDA receptor activation is required for plasticity related to pain enhancement in some model systems *(143,144)* and in the development of opiate tolerance *(145)*. In still other neuronal systems such as in the spinal cord, NMDA receptor activation is necessary for proper rhythm generation *(146)*. With the presence of NMDA receptors throughout the CNS, and a diversity of signaling systems with which NMDA receptors may be interacting, NMDA receptors are likely to have many other presently unknown functions in the CNS.

### 3.2. Molecular Properties of NMDA Receptors

#### 3.2.1. NMDA Receptor Subunits

NMDA receptors are a multimeric complex composed of subunits derived from three related families: NR1, NR2, and NR3 subunits (for reviews, *see* refs. *147–149)*. Both NR1 and NR2 subunits are required for receptor function. The NR1 subunit contains the glycine-binding site whereas the NR2 contains the L-glutamate-binding site *(150)*. In contrast, the NR3 subunit appears to modulate receptor function in a limited number of situations *(151)*. Multiple lines of evidence suggest that there are two NR1 subunits and two NR2 in a single NMDA receptor complex. Thus NR1/NR2 complexes may exist as a tetramer *(152)*. However, the effect of including NR3 subunits upon the stoichiometry is unknown.

The NR1 subunit (also termed NMDAR1 for rat and ζ1 for mouse), is over 900 amino acids in length and displays 22–26% identity with AMPA and kainate receptor subunits *(153,154)*. The NR1 gene consists of 22 exons; exons 5, 21, and 22 can be alternatively spliced, resulting in eight distinct NR1 isoforms *(155,156)*. Exon 22 includes a stop codon, and hence has a different C-terminal than proteins that do not have exon 22.

Subsequent to the cloning of the NR1 subunit, the NR2 subunits were identified *(157–162)*. The four members of the NR2 subunit family (NR2A–NR2D for rat and ε1–ε4 for mouse) are the products of four separate genes. The physiological and pharmacological properties of native and recombinant NMDA receptors vary with the specific NR2 subunit present in the heteromeric complex *(160,163–165)*.

#### 3.2.2. NR3 Subunits: When a Glutamate Receptor Is Not a Glutamate Receptor

The NR3A was initially termed χ − 1 *(166,167)*. Among various glutamate receptor subunits, NR3A has highest identity with NR1 and NR2 subunits (27%). When coexpressed with NR1/NR2B in oocytes, it reduces the magnitude of NMDA-evoked current responses. Further suggesting that NR3 is an NMDA receptor subunit is that NR1

subunits are required for the surface expression of NR3 subunits, that NR3 subunits are associated with both NR1 and NR2 subunits, and that NR3 subunits alter the channel properties of NR1/NR2 receptor complexes *(168)*. Intriguingly, in the NR3A knockout mouse *(151)*, NMDA receptor-mediated responses are larger and spine density is increased. More recently, an additional protein has been identified, NR3B, which in the human is 57% identical to NR3A *(169)* and in the mouse is 51% identical to NR3A *(170)*. This subunit is highly expressed in midbrain and lower regions *(171)*, particularly in motoneurons of the brainstem and spinal cord *(170)*.

A striking finding is that NR1/NR3 subunit complexes are functional when coexpressed in *Xenopus* oocytes *(172)*. Consistent with the presence of an NR1 subunit and the absence of an NR2 subunit, this receptor is activated by glycine and not by glutamate. Thus, this receptor is formed from two glutamate receptor subunits but is not a receptor for glutamate. This receptor complex is inhibited by D-serine, calcium impermeable, and insensitive to the classic NMDA receptor channel blockers MK-801 and $Mg^{++}$. There is evidence that these may exist in brain.

### *3.2.3. NMDA Receptor Subunit Structure*

#### 3.2.3.1. The Amino Terminal Domain

The N-terminal NMDA receptor subunits (the ATD or amino terminal domain; *see* Fig. 1) has homology to bacterial amino acid-binding protein LIVBP (leucine- isoleucine- valine-binding protein; ref. *173* and is the general site where modulators such as zinc and ifenprodil influence the desensitization properties of NMDA receptors *(174)*. As in the other glutamate receptor subunits, this region has been proposed to play a role in subunit assembly; however, functional receptors are formed even if this domain is removed *(175)*.

#### 3.2.3.2. The S1/S2 Domain

The S1/S2 domain has homology to other bacterial amino acid-binding proteins (glutamine-binding protein QBP and lysine, arginine, ornithine binding protein—LAOBP; refs. *173,176*), and it is these domains on NR1 and NR2 that is thought to be the primary ligand binding sites for glycine and glutamate respectively *(40)*. The crystal structure of the S1/S2 region of the NR1 subunit has recently been described. This study provides detailed description of the mechanism by which glycine and 5,7-dichlorokynurenic acid bind to the NR1 subunit *(47a)*. As expected, this structure is very similar to the GluR2 crystal structure with the S1/S2 structure forming a "clamshell" bilobed structure that closes in the agonist-bound state.

#### 3.2.3.3. The M2 Domain

NMDA receptor ion channels are thought to have the same general structure as postulated for AMPA and kainate receptors. Thus, the M2 re-entrant loop is the primary portion of the sequence lining the ion channel pore. Portions of M1 and M3 also appear to line the pore. An important difference between NMDA and non-NMDA receptors is that the amino acid at the critical Q/R site of AMPA and kainate receptors is an asparagine, which contributes to the calcium permeability of NMDA receptors *(177)*.

#### 3.2.3.4. The C-terminal Domain: Site of Intracellular Protein–Protein Interactions

The C-terminal tail of NMDA receptor subunits, the region following the last transmembrane domain, is located intracellularly and is the primary site for intracellular

protein–protein interactions. For both the NR1 and NR2 subunits, a variety of proteins are known to be interacting with this domain. NR2 subunits with truncated C-termini form functional ion channels, but their ability to be properly localized within the cell is impaired. Furthermore, since NR1 and NR2 C-termini bind to various signaling and cytoskeletal proteins, various downsteam signals require the specific localization at the NMDA receptor ion channel. Mice expressing truncated NR2 subunits act much like mice missing the subunit altogether even though functional ligand-gated ion channels are formed *(178)*.

A key protein family that organizes many of these protein-protein interactions are the membrane-associated guanylate kinase proteins, of which PSD-95 (SAP90) is the prototype. The family of PDZ domain-containing proteins related to PSD-95 are characterized by having three N-terminal PDZ domains followed by an SH3 (src homology 3) domain and a guanylate kinase (GK) homology domain *(179)*. Each of these domains mediate specific protein–protein interactions. Specific PDZ domains interact with specific sequences in the C-terminal of proteins and specific SH3 domains bind to specific, proline-rich sequences in their target proteins. The GK domain is enzymatically inactive and binds to specific sequences in GKAPs (GK-associated proteins). The PSD-95 family includes PSD-95, SAP97, SAP102, and Chapsyn-110.

The C-terminal of the NR2B (and the NR2A) subunit ends with the sequence SIESDV, which preferentially interacts with the second PDZ domain (PDZ2) of PSD-95 *(180)*, SAP102 *(181,182)*, and potentially other PDZ-containing proteins. The NR2C and NR2D subunits end in SLESEV, which presumably slightly changes the PDZ-selectivity of the C-terminal. NR1-3a has been reported to bind to PSD-95 *(183)*. In addition to the roles of clustering and anchoring, these scaffolding proteins allow for spatially ordered signal transduction systems. Hence NMDA receptor activation can preferentially activate multiple calcium-activated processes by virtue of the localization of the calcium-responsive systems. For example, calcium-sensitive neuronal nitric oxide synthase *(184)*, and a $Ca^{++}$-calmodulin kinase II-phosphorylated neuronal ras-GAP *(185,186)* can also associate with PSD-95. Thus, NMDA receptor $Ca^{++}$ influx can selectively modulate NO production and the ras effector pathways such as MAP kinase. In recent years, the list of identified proteins that interact with NMDA, AMPA, and kainate receptors has become quite long. For recent reviews, *see* refs. *187,188*.

### *3.2.4. NMDA Receptor Heteromeric Complexes*

Most studies presently favor a tetrameric complex for the NMDA receptor *(152)*. This is consistent with evidence of two glutamate- and two glycine-binding sites *(189)* and evidence for two NR1 subunits in a complex *(190)*. A tetrameric structure is also supported by the construction of a functional receptor consisting of four subunits joined in tandem *(191)*. This work also suggests that the complex may form as a dimer of dimers in the arrangement NR1/NR1/NR2/NR2. However, there is other evidence that supports a pentameric structure *(192)*. For NMDA receptors that contain an NR3 subunit, there is presently no information regarding subunit stochiometry.

Because NR2 subunits confer distinct physiological and pharmacological properties to NMDA receptors, an important question is whether there are heteromeric complexes that contain more than one type of NR2 subunit. Most studies support the existence of heteromeric complexes. When coexpressed in *Xenopus* oocytes NR1/NR2A/NR2C subunits have properties consistent with a heteromeric structure *(193)*. Likewise, coexpressed

NR1/NR2A/NR2B *(194,195)*, coexpressed NR1/NR2B/NR2D *(196)*, and coexpressed NR1/NR2A/NR2D *(197)* form receptors, which have properties suggestive of heterotrimeric receptor complexes. Such heterotrimeric complexes may exist in brain. Some NR2A subunits are coimmunoprecipitated with NR2B antibodies *(198)*. Furthermore, from single channel analysis, NR1/NR2B/NR2D receptors appear to be found in cerebellar Golgi cells *(199)*.

### *3.3. Pharmacology of NMDA Receptors*

#### *3.3.1. Glutamate Recognition Site Agonists*

L-Aspartate and L-glutamate are potent NMDA receptor agonists *(11)*. These typify the structural requirements for agonist activity: two negative charge groups (preferably both carboxys) separated by three or four carbon–carbon bond lengths (aspartate and glutamate, respectively); the α-carbon should be in the S- (or L)-configuration, and the ω-charge group should be a carboxy but can also be a sulfonic acid, or a tetrazole group.

Several rigid glutamate analogs have been constructed that are potent NMDA receptor agonists that provide insight into the optimal configuration of charges to obtain agonist activity. These compounds include homoquinolinate, (2S,3R,4S) 2-(carboxycyclopropyl)glycine (L-CCG-IV) *(200)*, (1R,3R) 1-aminocyclopentane-1,3-dicarboxylic acid (ACPD), and 1-aminocyclobutane-1,3-dicarboxylic acid (ACBD). The high potency of these structures suggests that L-glutamate is active in a folded conformation *(201)*. (For structures of key NMDA receptor compounds, *see* Fig. 5).

#### *3.3.2. Glutamate Recognition Site Competitive Antagonists*

Structure-activity studies indicate several features that are important for antagonist action at the glutamate recognition site of the NMDA receptor complex (for a detailed review, see ref. *202*). Antagonists have the same general structural requirements as do agonists, with the exception that antagonist activity occurs (a) by increasing the chain length between the two negative charges, (b) by replacing the S-chiral center (as in *s*-glutamate) with an R stereoisomer (as in *R*-AP5), and (c) by replacing the distal negative carboxy with a phosphate group (as in AP5 compared to amino-adipate). Experimentally useful antagonists include: The R isomers of AP5, AP7, CPP, CPPene, CGS19755, and CGP37849 *(69)*. A variety of other multiring structures and additional groups have also been shown to increase the antagonist potency of the basic AP5/AP7 structure. These compounds include EAB515 *(203)*, LY 274614 *(204)*, and PBPD *(196)*.

Because the NR2 subunit has a glutamate-binding site, the four different NR2 gene products might be expected to each contain pharmacologically distinct glutamate-binding sites. Indeed, studies have confirmed that four distinct pharmacological profiles can be seen for native and recombinant NMDA receptors containing the different NR2 subunits *(163,164,196,205)*. However, at the present, glutamate site antagonists only weakly discriminate between the different NR2 subunits. In general, AP5-like antagonists (e.g., AP5, CPP, CGS19755) display a NR2 subunit selectivity pattern of NR2A > NR2B > NR2C > NR2D (high to low affinity). Interestingly, the larger, multiring antagonists (e.g., EAB515, LY 274614, and PBPD) display varied patterns of NR2 selectivity *(196,206)*. We have recently identified the large multiring antagonist PPDA as a high-affinity antagonist that has significantly higher affinity at NR2C and NR2D subunits than at NR2A/NR2B *(140)*.

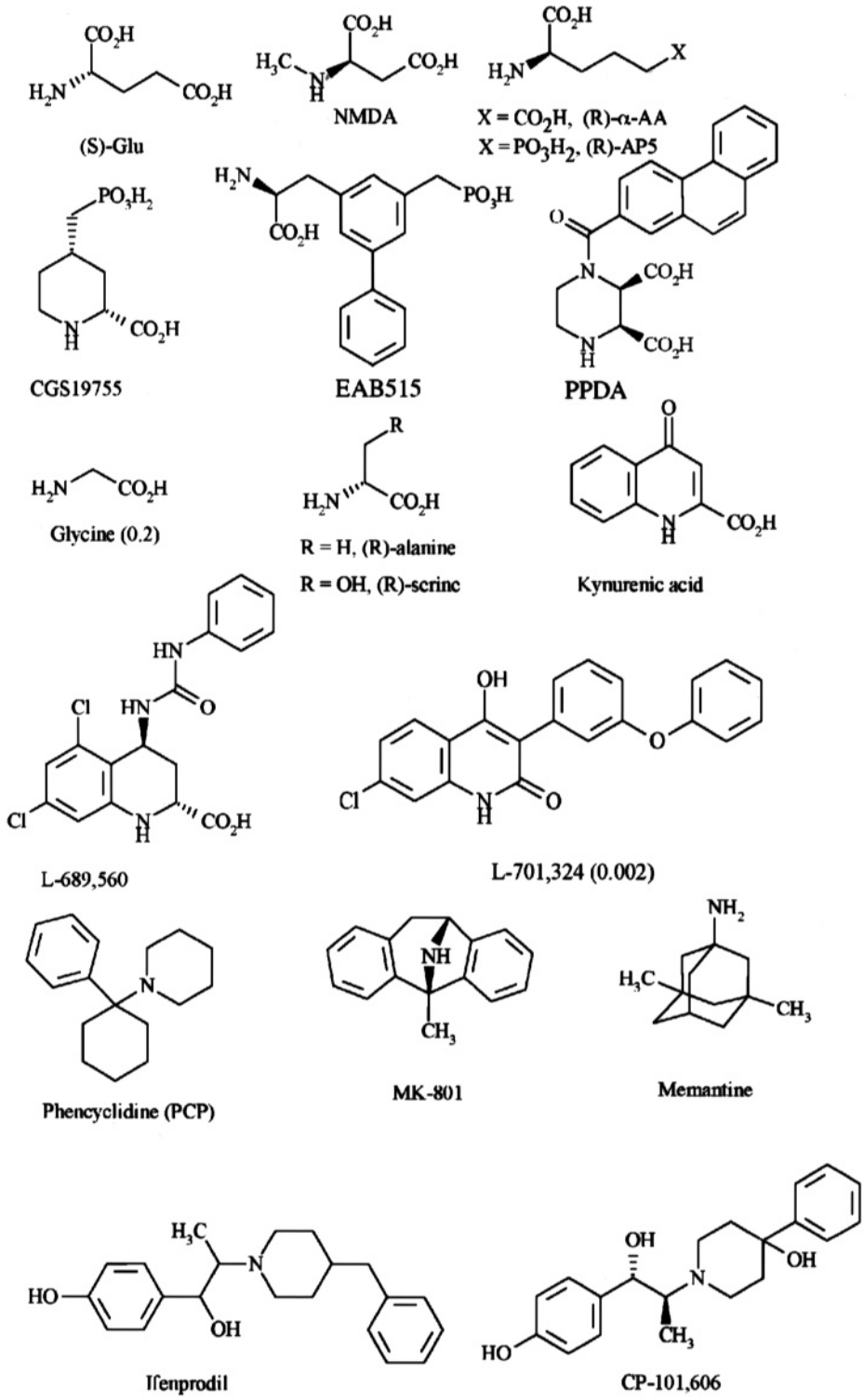


**Fig. 5.** Structures of key NMDA receptor compounds.

### *3.3.3. NMDA Receptor: Glycine Recognition Site Agonists*

Glycine binds specifically to the NR1 subunit *(207)*, however, the NR2 subunits confer subtype-specific pharmacological properties to the glycine-binding site in a heteromeric receptor complex. Potencies for the agonists glycine, D-serine, D-alanine, and 1-aminocarboxycyclobutane are significantly lower at NR1/NR2A receptors than receptors composed of NR1/NR2B, NR1/NR2C, and NR1/NR2D (ranked in order of increasing potency; refs. *158,164,208–211*).

1-Amino-1-carboxycyclopropane (ACPC) is a selective agonist of the glycine binding site with an intrinsic activity of 92% *(212)*. ACPC has a structure that is similar to that of the amino acid agonists, while being incorporated into a cyclopropyl ring. Expanding the cyclopropyl ring of ACPC to a cyclobutyl ring results in 1-aminocarboxycyclobutane (ACBC), a partial agonist with low efficacy *(213)*. Increasing the size of the ring structure of ACBC to cyclopentane results in the amino acid derivative cycloleucine, a full antagonist of the NMDA glycine-binding site with weak potency *(214)*. HA-966 *(215)* and D-cycloserine *(216)* are also glycine site partial agonists with roughly 15% and 50% intrinsic activity, respectively. In contrast to NR1/NR2A and NR1/NR2B, D-cycloserine, at NR1/NR2C receptors, has higher efficacy than glycine itself *(217)*.

### *3.3.4. NMDA Receptor: Glycine Site Antagonists*

One of the first glycine-binding site antagonists to be identified was kynurenic acid *(218)*. Kynurenic acid is a weak nonselective excitatory amino acid antagonist, the first selective glycine site antagonists are variants of this compound. They include 7-chlorokynurenic acid *(219)*, 5-7-dichlorokynurenic acid *(220)*, and 7-chloro-5-iodokynurenic acid (L-683,344) *(221)*. Other high-affinity antagonists include: (E)-3-(2-phenyl-2-carboxyethenyl)-4,6-dichloro-1H-indole-2-carboxylic acid (MDL 105,519; ref. *222*), 7-chloro-4-hydroxy-3(3-phenoxy)phenyl-2(H)quinolone (L-701,324; ref. *223*), and (+/−)-4-(*trans*)-2-carboxy-5,7-dichloro-4-phenylamino-carbonylamino-1,2,3,4-tetrahydroquinoline (L-689,560, ref. *224*).

### *3.3.5. NMDA Receptor Channel Blockers*

More than 20 yr ago, Lodge and colleagues discovered that ketamine and phencyclidine (PCP) can block NMDA receptor-mediated responses *(225)*. Since then, many compounds have been identified that block NMDA receptor action in an uncompetitive manner by binding to a site(s) within the open ion channel. NMDA receptor channel blockers are typified by the high-affinity compounds MK-801 (dizocilpine maleate), PCP, and TCP (1-[1-(2-thienyl)-cyclohexyl] piperidine). Each of these compounds display use-dependent and voltage-dependent blockade of the receptor complex. In both electrophysiological *(226)* and radioligand-binding *(227)* studies, channel blockade (or radiolabeled channel blocker binding) is dependent upon the activation of the receptor complex by agonist binding at both the glutamate- and glycine-binding sites.

### *3.3.6 Allosteric Modulatory Sites on the NMDA Receptor*

#### 3.3.6.1. Polyamines

Polyamines (e.g., putrescine, spermidine, and spermine) can modulate NMDA receptor activity. These compounds are found throughout the brain *(228)* and can be released following neuronal depolarization *(229)*. Polyamines have three effects on NMDA receptor activity: (a) glycine-dependent stimulation characterized by a polyamine-stimulated

increase in glycine affinity for its binding site, (b) glycine-independent stimulated increase in the maximal amplitude of NMDA receptor responses, and (c) voltage-dependent inhibition of NMDA receptor responses (for a review, *see* ref. *230*).

Polyamine sensitivity is subunit-dependent. Glycine-independent stimulation by spermine in recombinant receptors is inhibited by the N-terminal insert (exon 5) of the NR1 subunit *(231,232)*. In addition, the NR1 amino acid residue E342, is necessary for glycine-independent spermine stimulation *(233)* but has no effect on polyamine glycine-dependent potentiation or voltage-dependent channel block. Mutations at equivalent positions in NR2A and NR2B subunits had no effect on spermine stimulation.

The extracellular loop region between TM3 and TM4 of the NR1 subunit also participates in glycine-independent spermine stimulation as well as voltage-dependent channel block. Mutations in this region reduce glycine-independent polyamine potentiation and mutations of specific negatively charged amino acids in this sa me region on both NR1a and NR2B subunits reduced the voltage-dependent block by spermine *(234)*. Additionally, amino acids in a portion of the transmembrane-spanning regions of the NR1 subunits (TM1,2,3) are involved in spermine stimulation probably through allosteric effects or changes in gating processes *(235,236)*.

In addition to the NR1 subunit, the NR2 subunit also contributes to both the stimulatory and inhibitory effects of polyamines at NMDA receptors *(232,237,238)*. Polyamines cause glycine-independent stimulation at NR1a/NR2B receptors but not at NR1a/NR2A, NR1a/NR2C, or NR1a/NR2D receptors. However, glycine-dependent stimulation *(237)* and voltage-dependent inhibition *(239)* were seen at both NR1a/NR2A and NR1a/NR2B receptors. Taken together these data suggest that there are at least three distinct polyamine-binding sites on NMDA receptors.

#### 3.3.6.2. Ifenprodil and Other NR2B Selective Compounds

A variety of other pharmacological agents bind and modulate NMDA receptor activity with a selectivity similar to the polyamines. Ifenprodil is an NMDA receptor antagonist *(240)* at a site separate from that of glutamate and glycine. Ifenprodil exhibits greater than a 100-fold selectivity for NR2B over NR2A-containing receptors *(165,241)* and very low affinity at NR2C- and NR2D-containing receptors *(238)*. A variety of other related compounds show NR2B selectivity; these include haloperidol *(242)*, CP-101,606 *(243)*, Ro 8-4304 *(244)*, and Ro 25-6981 *(245)*. Site-directed mutagenesis studies show that spermidine, haloperidol, and ifenprodil all have overlapping binding sites but that the specific molecular determinants required for high-affinity binding differ between each of these compounds *(194,241,242)*. These compounds have been useful for defining the actions of NR2B-containing receptors in brain.

#### 3.3.6.3. Proton Inhibition

At low pH, NMDA receptor responses are inhibited *(246,247)*. Increased external protons suppress NMDA receptor currents by decreasing channel open probability. The proton site appears independent of agonist binding sites because proton blockade was noncompetitive with NMDA and glycine. Proton inhibition may represent an intrinsic mechanism to protect neurons from NMDA receptor excitotoxicity during pathological acidosis. The absence of the N-terminal insert of the NR1 subunit is required, like that of glycine-independent stimulation by spermine, for proton inhibition. Thus the presence of exon 5, and more specifically K211 in exon 5, potentiates NMDA receptor function through relief of

the tonic proton inhibition that is present at physiological pH *(248)*. Additionally, polyamine stimulation may be linked to the relief of tonic inhibition by protons suggesting that polyamines and protons share common molecular-binding determinants *(249)*, particularly within NR2B-containing receptors for which both are most selective.

#### 3.3.6.4. Zinc

Zinc displays subunit-specific actions at recombinant NMDA receptors. At low concentrations, zinc (1 μ*M*) enhances homomeric $NR1_{OXX}$ (NR1 lacking the N-terminal insert) receptor responses while having no effect on homomeric receptors containing $NR1_{1XX}$ subunits *(155,250)*. At higher concentrations zinc inhibits both NR1 subunits with and without the N-terminal insert. Both of these phenomena occur without a change in the affinity for glutamate or glycine. The NR2 subunits also contribute to zinc's actions on NMDA receptors. Zinc displays a voltage-dependent inhibition of NMDA receptor responses in heteromeric NR1/NR2A and NR1/NR2B receptors and, at lower zinc concentrations, a voltage-independent inhibition of NR1/NR2A receptors *(251–253)*. This appears to account for the observation that the addition of heavy-metal chelators to buffer solutions significantly potentiates NR1a/NR2A, but not NR1a/NR2B, receptor responses *(252)*.

### *3.4. NMDA Receptor Radioligands*

#### *3.4.1. Glutamate Site Ligands*

Radioligands represent a straightforward method of quantifying receptor density and distribution. Many different radioligands have been developed for the study of the glutamate-binding site of NMDA receptors: L-[$^3$H]glutamate, D-[$^3$H]AP5, [$^3$H]CPP, [$^3$H]CGS19755 *(12,19,254)*, and, of highest affinity, [$^3$H]CGP39653 *(255)*. Of these radioligands, only L-[$^3$H]glutamate labels all four NR2 subunits of native and recombinant NMDA receptors *(163,256)*, the other compounds (which are all antagonists) selectively label NR2A- and, to varying degrees, NR2B-containing receptors *(256)*. The agonist [$^3$H]homoquinolinate labels predominantly NR2B-containing NMDA receptors in rat brain *(257)*. Thus, there is still a need for subunit-selective radioligands for NR2C- and NR2D-containing receptors.

#### *3.4.2. Glycine Site Ligands*

The glycine-binding site on NMDA receptors can be labeled with a variety of radioligands. [$^3$H]Glycine itself labels NMDA receptors *(258)* as well as the antagonists [$^3$H]MDL 105,519 *(259)*, [$^3$H]5,7 dichlorokynurenic acid *(260,261)*, [$^3$H]L-689,568 *(262)*, and others. The glycine site antagonist ([$^3$H]CGP 61594) has been shown to display a high-affinity selectively for NR2B-containing receptors *(263)*. Glycine binding to the inhibitory glycine receptor, localized in the lower brainstem and spinal cord, can be distinguished from glycine binding to the NMDA receptor by using the inhibitory glycine receptor antagonist strychnine.

#### *3.4.3. Channel Blocker and Polyamine Site Ligands*

Many studies have used the radioligand [$^3$H]-MK801 to characterize the ion channel of NMDA receptors *(264)*. This agent has high affinity and is highly specific. [$^3$H]-TCP and [$^3$H]-PCP can also be used to label the NMDA receptor ion channel (though PCP is less specific, ref. *265*). An important factor to consider in using channel blocker radioligands is that these are usually slowly accessible to the closed NMDA receptor ion channel.

Thus the time required to achieve equilibrium reflects the degree of channel activation. This property can be useful, because channel blocker ligands can be used to measure channel activation/inhibition at short, nonequilibrium conditions or used under fully activated, equilibrium conditions to measure NMDA receptor density *(227)*.

Multiple radioligands have been described that can be used to examine the ifenprodil/polyamine binding site. These include [$^3$H]ifenprodil *(266,267)*, [$^3$H]Ro-25-6981 *(245)*, and [$^3$H]CP-101,606 *(268)*. These agents are selective for NR2B subunits.

### *3.5. NMDA Receptor Function: Information From Transgenic Studies*

#### *3.5.1. NR1 Knockouts*

Each of the NMDA receptor subunits have been knocked out in mice. Additionally, some subunits have been over expressed or replaced with point mutations. The NR1 knockout is lethal neonatally *(269)*. In these animals, the brainstem barrel fields representing the whisker somatosensory map fail to develop *(141)*. The development of the cortical whisker representations were also found to be dependent upon NR1 when studied with the conditional NR1 knockout mouse in which NR1 is missing from excitatory cortical neurons *(142)*. Interestingly, mice expressing very low levels of NR1 display behavior consistent with schizophrenia and these behaviors are treatable with antipsychotics *(270)*. Consistent with extensive pharmacological evaluation, conditional knockout of CA1 hippocampal NR1 subunits blocks LTP in the hippocampus and blocks spatial learning *(271)*.

#### *3.5.2. NR2 Knockouts*

In the NR2A knockout, there is a reduction in hippocampal LTP and spatial learning *(272)* and a reduction in the conditioned eyeblink response *(273)*. Of the NR2 knockouts, only NR2B –/– mice do not survive, in part because of a loss of the suckling response *(274)*. These mice show a loss of LTD and NMDA receptor-mediated responses in the hippocampus and have impaired development of the barrel fields. NR2C knockouts show few effects *(275)*. NR2C subunits are predominantly found in the cerebellum where they are coexpressed with NR2A subunits *(160)*. When both NR2A and NR2C are knocked out there is a deficit in motor coordination *(276)*. As with NR2C, NR2D knockout effects are relatively subtle. NR2D knockout mice display reduced spontaneous activity *(277)*, reduced sensitivity to stress *(278)*, and a block of pain in a specific model of allodynia *(279)*.

#### *3.5.3. NR3 Knockouts*

Consistent with NR3 coexpression studies where NR3 expression inhibits NMDA receptor currents, NR3 knockout mice display an increase in NMDA-induced currents. The NR3–/– mice also display increased spine density, increased spine head length, and increased spine neck length in cortical neurons *(151)*.

## 4. CONCLUSIONS

The glutamate-gated ion channels underlie most of the fast excitatory synaptic transmission in the vertebrate CNS. The identification and cloning of these receptors have revealed extensive diversity in molecular structure owing to multiple subunits, alternative splicing, and RNA editing. With these diverse structures, glutamate receptors display a wide variety of channel kinetic properties, desensitization mechanisms, cellular localization mechanisms, and biochemical signal transduction mechanisms. Thus, instead of a simple fast-on, fast-off depolarizing signal, the ionotropic glutamate receptors display an array of highly specialized

physiological properties that can give complex and distinctive qualities to signaling in specific synapses. Furthermore, these specific signaling properties can be regulated by a variety of mechanisms. Consequently, in various CNS disease states, there is considerable potential for seemingly subtle alterations in receptor function that may have profound clinical implications.

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