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On the Hypes and Falls in Neuroprotection: Targeting the NMDA Receptor

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Activation of the NMDA (N-methyl-D-aspartate) responsive subclass of glutamate receptors is an important mechanism of excitatory synaptic transmission. Moreover, NMDA receptors are widely involved in many forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD), which are thought to underlie complex tasks, including learning and memory. Dysfunction of these ligand-gated cation channels has been identified as an underlying molecular mechanism in neurological disorders ranging from acute stroke to chronic neurodegeneration in amyotrophic lateral sclerosis. Excessive glutamate levels have been detected following brain trauma and cerebral ischemia, resulting in an unregulated stimulation of NMDA receptors. These conditions are thought to elicit a cascade of excitation-mediated neuronal damage where massive increases in intracellular calcium concentrations finally trigger neuronal damage and apoptosis. Consistent with the hypothesis of NMDA receptors as essential mediators of excitotoxicity, the different functional domains of these ion channels have been identified as potential targets for neuroprotective agents. Following an initial hype on potential NMDA receptor therapeutics, the authors currently see a period of skepticism that, in reverse, appears to neglect the therapeutic potential of this receptor class. This review attempts a reappraisal of this important class of neurotransmitter receptors, with a focus on NMDA receptor heterogeneity, ligand binding domains, and candidate diseases for a potential neuroprotective therapy. *NEUROSCIENTIST* 13(6):594–615, 2007. DOI: 10.1177/1073858406296259

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NMDA Receptors and the Superfamily of Ionotropic Glutamate Receptors

The amino acid L-glutamate acts as a major excitatory neurotransmitter in the vertebrate CNS, acting on both ligand-gated ion channels (ionotropic receptors) and G-protein-coupled (metabotropic) receptors. The group of ionotropic glutamate receptors (iGluRs) comprises three major pharmacological classes, including the AMPA receptors, kainate (KA) receptors, and N-methyl-D-aspartate (NMDA) receptors (Wollmuth and Sobolevsky 2004; Fig. 1). Glutamatergic synapses frequently harbor both AMPA and NMDA receptors. Characteristically, both classes of receptors differ in their response kinetics to the presynaptic release of glutamate. AMPA receptors mediate fast glutamate-gated postsynaptic responses, even at very negative potentials or in the absence of action potentials. The fast desensitization of AMPA receptors leads to short excitatory postsynaptic currents (EPSCs). In contrast, NMDA receptors are blocked by Mg^{2+} ions at

resting potential (Fig. 4). Acting as coincidence detectors of membrane depolarization and ligand-gated channel activation, NMDA receptors require the release of the Mg^{2+} block by an increase in membrane potential to allow cation permeation through the receptor pore (Wollmuth and Sobolevsky 2004).

At the glutamatergic synapse, the presence of both classes of receptors results in a fine-tuned interaction: high-frequency stimulation of AMPA receptors, followed by postsynaptic depolarization and action potentials, abolishes the voltage-dependent Mg^{2+} block and thereby activates NMDA receptors. As a result, NMDA receptor activation is slower and lasts longer, with a much slower desensitization of ion channel conductance. Both components of these glutamatergic synaptic responses can reliably be distinguished by application of subclass-specific blockers—that is, APV (2-amino-5-phosphovalerate) for NMDA receptors and DNQX (6,7-dinitroquinoxaline-2,3-dione) or CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) for non-NMDA receptors (Antzoulatos and Byrne 2004; Fig. 5). As ligand-gated ion channels (LGICs), NMDA receptors respond to binding of glutamate and to an additional depolarization by opening a cation channel pore in the center of the receptor protein complex. Although ionotropic glutamate receptors mediate the flow of Na^+ ions, they fundamentally differ in Ca^{2+} conductance (Garaschuk and others 1996). Generally, Ca^{2+} conductance is a hallmark of NMDA receptors and those AMPA receptor variants carrying a glutamine residue in the ion selectivity filter (Burnashev and

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The superfamily of ionotropic glutamate receptors

Fig. 1. The three major subfamilies of the glutamate receptor superfamily.

others 1995, 1996; Fig. 2). The receptor-mediated flow of cations produces a depolarization of the plasma membrane and generation of electrical currents.

As multimeric assemblies of four subunits, members of the glutamate receptor family share a common protein structure. Each subunit possesses three hydrophobic trans-membrane domains (TMDs A–C) located within the central portion of the sequence and is characterized by an extracellular N-terminus and an intracellularly located C-terminus. The ion pore domain is formed by a hairpin where the polypeptide chain bends into the cytoplasmic membrane from its cytosolic face. This is in contrast to receptors of the nicotinic acetylcholine receptor superfamily (nAChR) characterized by an N-terminal cysteine bridge (i.e., “cys-loop”).

The general architecture of the ion channel displays fundamental structural similarities to that of voltage-gated cation channels (e.g., K^+ channels), where the hairpin is formed by a protein loop accessing the plasma membrane from its extracellular face (Fig. 2). The protein GluR0 is a glutamate-activated potassium-selective channel (Chen and others 1999). GluR0, isolated from the cyanobacterium *Synechocystis* PCC 6803, has been proposed as a common ancestor providing evidence for a missing link between GluRs and K^+ channels. All glutamate receptor subunits presently known carry a ligand binding domain where two intervening sequences, called S1 and S2, form a clamshell-like structure. The long loop between TMD B and TMD C (S2) is exposed to the cell surface and forms part of the binding domain, interacting with the C-terminal half of the N-terminal region S1. These two segments show structural similarities to the sequences of the bacterial periplasmic amino-acid binding protein (LAOBP-like domains) (Wo and Oswald 1995b; Fig. 2).

The NMDA Receptor Family

Among the ligand-gated glutamate receptors, members of the NMDA receptor family carry characteristic features. Thus, this class of glutamate receptors is involved in a variety of physiological and disease-related processes in the brain. Receptor activation requires a simultaneous occupation of an agonistic glutamate site and a glycine binding domain. As pointed out before, NMDA receptors display a voltage-dependent Mg^{2+} block, which is released only after membrane depolarization. In contrast to other glutamate receptors, NMDA receptors show slow gating kinetics and a much higher Ca^{2+} permeability ($pCa_{AMPA}^{2+} \ll pCa_{NMDA}^{2+}$). Although the NMDA receptor is highly permeable to Ca^{2+} , its permeability to monovalent cations such as Na^+ and K^+ still exceeds that for divalent cations ($pNa^+ > pCa^{2+}$) (Garaschuk and others 1996; Ichinose and others 2003). The Ca^{2+} flux mediated by NMDA receptors is thought to trigger downstream intracellular signaling events that initiate various forms of synaptic plasticity, such as LTP and LTD, as well as developmental refinement of synaptic connections or neuronal cell death by excitotoxicity (Sattler and others 1999). Although NMDA receptor variants share many functional characteristics, distinct subtype-specific properties are emerging. In the mammalian brain, functional NMDA receptor channels are heteromultimeric assemblies of an NR1 subunit plus a NR2 or NR3 subunit. Additional diversity may arise from multiple NR2 subunit variants incorporated into the tetrameric complex (Schorge and Colquhoun 2003; Horning and Mayer 2004; Sobolevsky and others 2004; Hatton and Paoletti 2005). Given the high complexity of the subunit permutations possible, little is known about the quaternary structure of NMDA receptors. Although

Fig. 2. Overall topology of a glutamate receptor subunit. Each subunit consists of three transmembrane domains (TMDs) A, B, and C. The far N-terminal domain, called ATD, displays high homology to the amino acid binding protein LIVBP (leucine-isoleucine-valine binding protein) from bacteria. The 3' end of the N-terminus is part of the ligand binding domain that designs a clamshell-like structure by intervening sequences S1 and S2. The second part of the ligand binding pocket is formed by the extracellular loop between TMD B and C. The ligand glutamate is trapped within the S1S2 clamshell. The ion channel domain dips into the membrane from the intracellular site, forming a hairpin-like structure. The pore is permeable to monovalent ions, such as Na^+ and the divalent ion Ca^{++} . The C-terminal domain (CTD) is localized intracellularly.

biochemical studies reveal that NMDA receptors behave as macromolecular membrane protein complexes, elucidation of subunit stoichiometry relies on functional studies (Herkert and others 1998; Banke and Traynelis 2003).

Subfamilies of NR Subunits: Differences in Distribution and Function

NR1-subunit and its variants. The NMDAR1 (NR1) polypeptide was the first NMDA receptor subunit cloned (Moriyoshi and others 1991). The NR1 subunit appears to be a common prerequisite for the expression of functional NMDA receptors, and a glycine binding site is present on all NR1 subunits. In the meantime, several splice variants of the NR1 subunit have been identified that arise from the *Grin1* gene (in humans: *GRIN1*) (Moriyoshi and others 1991; Hollmann and others 1993). Sequence comparisons reveal two sites for splice variation, one each in the N-terminal and the C-terminal regions (Fig. 3). The NR1 subunit is ubiquitously

expressed in the CNS. Incorporation of different NR1 splice variants determines properties such as modulation by Zn^{2+} , polyamines, and protein kinase C, as well as binding to intracellular proteins (Ehlers and others 1996; Wyszynski and others 1997; Lin and others 1998). In oocytes, homomeric expression of NR1 subunits results in functional receptors (Hollmann and others 1993). Thus, it was hypothesized that this structural subunit has retained a low-affinity response to the main receptor agonist (e.g., glutamate). Consistent with this hypothesis, a "unitary glutamate receptor" *XenUI*, which is endogenously expressed in *Xenopus laevis*, was assumed to substitute for NR2 in this species (Soloviev and Barnard 1997). Recently, however, a fragment of a *Xenopus* NR2B subunit was cloned that is highly homologous to the rat NR2B subunit. These data indicate that *Xenopus* uses an endogenous NR2 subunit to form heteromeric complexes with NR1, rather than exclusively relying on the *XenUI* subunit. Thus, the endogenous NR2B may also complement homomeric expression in *Xenopus* oocytes (Schmidt and others 2005).

Fig. 3. Alternative splicing of the NMDA receptor subunit NR1. The eight known splice variants of the NR1 subunit differ in their C-terminal length. All NR1-Xb variants do have an additional exon marked by a triangle (exon 5). At the 5' and 3' end, there are untranslated regions. The coding sequence is shown in white boxes; transmembrane regions are black. The hairpin-like pore domain is localized between transmembrane domains (TMDs) A and B. The signal peptide is marked by a striped box; modified after Hollmann and others 1993. Right columns present the accession numbers for each splice variant isolated either from rat or human according to <http://www.ncbi.nlm.nih.gov/UniGene/>.

The gene family of NR2 subunits. Incorporation of an NR2 subunit is required for an efficient formation of functional NMDA receptors. Indeed, NR2 subunits determine biophysiological ion channel properties such as conductance, mean open time, and sensitivity to the Mg^{2+} block (Monyer and others 1992, 1994; Stern and others 1992). Heteromeric channels of NR1 and NR2 are highly permeable to Ca^{2+} (Burnashev and others 1995; Schneggenburger 1996). This ability to mediate Ca^{2+} fluxes is essential for the special role that NMDA receptors play in synaptic plasticity and neurotoxicity (Malenka and Nicoll 1999; Sattler and others 1999). In contrast to the NR1 subunit, the NR2 subfamily consists of the four subunits NR2A–D, each encoded by a distinct gene. During development, expression of NR2 isoforms is restricted to defined CNS regions (Monyer and others 1994). Although transcripts of the NR2B and NR2D subunits are found prenatally, NR2A and NR2C mRNAs are first detected around birth. Except NR2D, expression of transcripts reaches highest levels around postnatal day 20 (P20). During the first postnatal week, developmental onset of NR2A expression succeeds NR2B expression (Sheng and others 1994). In rat CNS, the NR2B polypeptide undergoes a pronounced redistribution during maturation: in immature neurons, NR2B antigen accumulates in axonal growth cones and varicosities, whereas a punctated distribution pattern with redistribution to somatodendritic spheres is seen in mature cells (Herkert and others 1998). In the telencephalon of the adult rat, subunit NR2B prevails in the CA region of the hippocampus but is also found in

extrasynaptic sites (Charton and others 1999). Outside the CNS, ectopic expression of NR2B in the heart is observed where the NMDA receptor forms molecular complexes with the ryanodine receptor 2 (Seeber and others 2000, 2004), for an as yet unknown function.

Subpopulations of NMDA receptors that differ in their NR2 subtype variant determine the polarity of synaptic plasticity analyzed in the hippocampal CA1 synaptic region (i.e., LTP or LTD is thought to underlie learning and memory). Although the molecular mechanism of these processes is poorly understood, activation of NR2A-containing receptors fosters LTP, whereas activation of NR2B-containing receptors has been associated with LTD, as analyzed with subunit-specific antagonists in rat hippocampal slices. These results demonstrate that the NR2 subunit composition of the NMDA receptor complex is a critical factor for postsynaptic signaling pathways that direct synaptic plasticity (Liu and others 2004). In addition, evidence exists for the formation of NR1/NR2A/NR2B triheteromeric receptors in adult forebrain neurons (Luo and others 1997) and NR1/NR2A/NR2C complexes in cerebellum (Cathala and others 2000; Brickley and others 2003). Although diheteromeric receptors are extensively studied both in recombinant and in vivo systems, evidence for the formation of triheteromeric receptors largely relies on recombinant expression in vitro (Hatton and Paoletti 2005). Little is known about the assembly process of NMDA receptor complexes. By analogy, biogenesis of heteromeric AMPA receptors is highly regulated: the current model of glutamate receptor assembly suggests

that a preliminary step of family-specific subunit dimerization is followed by dimerization of these dimers into the final tetrameric receptor (Tichelaar and others 2004). The X-domain, a structural element important for oligomerization of non-NMDA receptors, overlaps with the amino terminal domain (ATD). So far, the crystal structures of the glutamate binding domain (S1/S2 domains) of the GluR2 and NR1 glutamate receptor subunits resolved by x-ray have been used to model subunit-specific amino acid residues contributing to the formation of the ligand binding pocket in NR2 subunits. Indeed, four subunit-specific amino acid residues located at the edge of the binding pocket were identified, suggesting that large antagonists may be necessary for subtype specificity (A414, T428, R712, and G713; NR2B numbering). Mutational analysis and modeling of these residues suggest that there may be a chance to develop NR2C- and NR2D-selective NMDA receptor antagonists, as residues A414 and T428 may determine subunit variations in agonist affinity (Kinarsky and others 2005).

NR3 subunits. A structurally distinct subfamily is formed by the NR3 polypeptides, which includes the NR3A and NR3B subunits (Ciabarra and others 1995; Sucher and others 1995; Nishi and others 2001; Chatterton and others 2002; Matsuda and others 2002, 2003). The NR3A subunit is developmentally regulated as mRNA and protein expression drop dramatically after the second week postnatally. In contrast, NR3B mRNA expression persists to adulthood in the murine spinal cord, pons, and medulla. NR3B is expressed predominantly in motor neurons, whereas NR3A is more widely distributed (Nishi and others 2001; Chatterton and others 2002). When coexpressed with NR1 and NR2A subunits, NR3A and NR3B suppress the glutamate-induced current in heterologous cells and reduce the Ca^{2+} permeability of NMDA currents (Matsuda and others 2002; Sasaki and others 2002). Interestingly, NR3A and NR3B subunits coassemble with NR1 to form excitatory glycine receptors that are unaffected by glutamate or NMDA and inhibited by D-serine, a coactivator of conventional NMDA receptors (Chatterton and others 2002).

The Nearest Neighbors: The Non-NMDA Types of Glutamate Receptors

Glutamatergic synapses frequently display two main functional components, representing (1) a glutamate-gated neurotransmission mediated by fast AMPA receptors and (2) a slower and longer lasting NMDA receptor-mediated response. Although NMDA and AMPA receptor subunits and protein complexes share the same overall topology and organization of functional domains, both receptor families differ in many aspects, including characteristics of ion channel permeability, conductance, and RNA editing a mechanism contributing to AMPA receptor heterogeneity (Sommer and others 1991). Among the non-NMDA subtypes of glutamate receptors, AMPA and kainate receptors can be distinguished on the basis of defined receptor subtype pharmacology, whereas the family of delta subunits has been identified by mouse genetics. Many of the structural and functional characteristics of NMDA

receptors have become apparent by functional comparison with non-NMDA-type glutamate receptors. These studies suggest that glutamate receptors form an intricate network where functional alterations of NMDA receptors also extend to AMPA and kainate receptor physiology (Wollmuth and Sobolevsky 2004).

AMPA receptors. AMPA receptors mediate the fast excitatory component at glutamatergic synapses. As the first glutamate receptor, GluR1 was identified in 1989 by expression cloning of rat brain cDNA in *X. laevis* oocytes. In the meantime, the AMPA receptor family has grown to four members (GluR1-4), each encoded by a separate gene (Hollmann and Heinemann 1994; Fig. 1). Generally, native AMPA receptor channels are impermeable to Ca^{2+} , except those containing GluR2(Q), a particular variant of the GluR2 subunit. Ca^{2+} permeability of AMPA receptor channels is determined by the posttranscriptional Q/R editing of a single amino acid position located in the ion channel domain of the GluR2 mRNA, changing glutamine (Q) residue encoded in the genome to an arginine (R) (Sommer and others 1991). Although the genomically encoded variant GluR2(Q) is Ca^{2+} permeable, the edited variant GluR2(R) is not. As a result of the Q/R editing, almost all of the GluR2 protein expressed in rodent CNS is in the GluR2(R) form, producing Ca^{2+} -impermeable AMPA receptor channels. In embryonic CNS, however, editing is not complete, generating a small fraction of Ca^{2+} -permeable GluR2 variants (Bennett and others 1996; Pellegrini-Giampietro and others 1997). Studies on knockout mice of the GluR2 subunit demonstrated a ninefold increase in Ca^{2+} permeability in response to kainate application, suggesting one possible mechanism for enhanced LTP. Mutant mice, however, exhibited increased mortality. Surviving mice displayed an impaired motor coordination (Jia and others 1996). All AMPA receptor subunits exist as two splice variants, flip and flop, where an alternative splice cassette is found at the C-terminal end of the extracellular loop between transmembrane domains B and C. Alternative splicing of AMPA receptors results in altered desensitization kinetics (Partin and others 1995), which has been proposed to contribute to synaptic plasticity. Functional interdependence of AMPA and NMDA receptors is becoming apparent from experiments where a transient synaptic activation of NMDA receptors reliably induces an LTP-like phenomenon, associated with an increase in the intensity and number of synaptic AMPA-receptor clusters (Liao and others 2001; Lu and others 2001). Upon induction by high-frequency stimulation, LTP can lead to an NMDA receptor-mediated influx of Ca^{2+} followed by an activation of CaMKII. Indeed, induction of LTP appeared to increase phosphorylation of GluR1 by CaMKII (Barria and others 1997; Barria and Malinow 2005). Recently, it was reported that phosphorylation of the AMPA receptor subunit GluR1 is modulated during LTP and LTD (Castellani and others 2001). This is consistent with murine knock-in mutations where phosphomutant mice show deficits in LTD and LTP as well as memory deficits in spatial learning tasks. These results demonstrate that phosphorylation of GluR1 is critical for LTD and LTP (Lee and others 2003).

Kainate receptors. Kainate receptors form a structurally related and pharmacologically distinct subgroup of glutamate receptors that contributes to postsynaptic responses and modulates presynaptic neurotransmitter release. Kainate receptors form heteromeric assemblies of GluR5-7 and KA-1/2 subunits (Herb and others 1992; Sakimura and others 1992; Fig. 1). Resembling the AMPA receptor subunits, kainate receptors also undergo both splice variation and RNA editing, giving rise to a large number of possible receptors that differ in their functional properties (Chittajallu and others 1999). By domain swapping between AMPA and kainate receptors, a characterization of the pore domains of GluR7 and orphan subunits has become possible on the background of the kainate receptor GluR6 (Villmann and others 1997, 1999). The non-functionality of the high-affinity kainate receptor subunit KA2 has been attributed to an arginine-rich endoplasmatic reticulum (ER) retention/retrieval motif and a di-leucine endocytic sequence located in the C-terminus of the KA2 subunit (Ren and others 2003).

Orphan Members of the Glutamate Receptor Family

Delta subunits. Although NMDA receptors are associated with neuronal dysfunction in glutamate-mediated CNS damage, as yet no spontaneous mouse mutant is known, surprisingly. However, a close relative of the NMDA receptor of obscure physiological function causes neurodegeneration in mice. Delta 1 and delta 2 receptors display significant homologies to other GluR subunits (~26%–30%), but neither delta 1 nor delta 2 form functional ion channels when expressed alone or together with other GluR subunits. Thus, both subunits have remained orphan receptors with unknown natural ligands and channel properties. *Lurcher* is a spontaneous, semidominant neurological mutation of the mouse. Heterozygous mice (*Lc/+*) display ataxia resulting from apoptotic death of cerebellar Purkinje cells during development. Neurodegeneration in *Lurcher* mice results from a constitutive activation of delta 2. Positional cloning identified *Lurcher* as G-to-A transitions that change a highly conserved alanine to a threonine residue in the TMD B of the mouse delta 2 glutamate receptor gene (Zuo and others 1997; Wollmuth and others 2000; Kohda and others 2003; Yuzaki 2004).

Kainate binding proteins (KBPs). These proteins form a family of structurally related ionotropic glutamate receptors isolated only from nonmammalian species (frog, goldfish, *xenopus*, duck, and chick). This protein family was first identified on Bergmann glial processes. The Bergmann glial kainate receptors have been proposed to modulate the efficacy of the glutamatergic synapses between parallel fibers and Purkinje cell spines, thereby forming a glial machinery responsible for plastic changes in synaptic transmission (Somogyi and others 1990; Ortega and others 1991). Although they share transmembrane structures with other glutamate receptors, kainate binding proteins represent some exceptions to this rule. The overall

homology with other GluRs is ca. 38%. They carry a functional ligand binding domain and bind kainate with very high affinity (Wo and Oswald 1995a), but ligand binding fails to induce channel opening. Transplantation of the ion channel domain of these receptors into a functional GluR6 environment revealed that, indeed, kainate binding proteins (KBPs) do have a functional ion channel domain (Villmann and others 1997). The physiological role of these KBPs, however, remains still enigmatic.

Topology of the NMDA Receptor Proteins: Functional Domains as Target Sites of Ligand and Drug Action

Structural concepts for the NMDA receptor were derived from paradigmatic studies on crystal structures of the extracellular domains of non-NMDA receptors of the GluR family, which, as pointed out before, share extensive homology with primary structures of NMDA receptor subunits. Evolutionarily, the GluR appears as a mosaic of domains derived from various other proteins (Wo and Oswald 1995b), where each domain is responsible for a distinct functional aspect, and all puzzle pieces together determine the pharmacological profile of a receptor subtype (Fig. 2). The large extracellular domains (i.e., the N-terminus and the loop between TMD B and C), share high homology to the amino acid binding proteins from bacteria, as LIVBP (leucine-isoleucine-valine binding protein) and LAOBP (leucine-arginine-ornithine binding protein). The LAOBP-like domain is divided into two subdomains, S1 and S2, which form the ligand binding core. The crystal structures of these bilobed S1S2 domains were solved from the GluR2, GluR5/6, and NR1 subunits (Armstrong and Gouaux 2000; Furukawa and Gouaux 2003; Mayer 2005; Naur and others 2005; Furukawa and others, 2005). Common to all members of the glutamate receptor family is the reentrant loop between TMDs A and B, which forms the ion pore proper (Fig. 2).

Ligand Binding Domain

Classical NMDA receptors differ from other classical neurotransmitter receptors in their strict dependence on two amino acid agonists, where glycine serves as an indispensable coagonist that sets the stage for the final fast-acting agonist glutamate (Nakanishi and Masu 1994; Dingledine and others 1999). Glutamate and its structurally related agonists bind to a domain in the cleft of a clamshell formed by S1 and S2 lobes (Kuusinen and others 1995; Ivanovic and others 1998). Agonist binding is thought to trigger a motion of the clamshell that directly or indirectly gates the cation channel. Glutamate is thought to stabilize a closed conformation of the two lobes (Madden 2002; Qian and Johnson 2002; McFeeters and Oswald 2004; Fig. 2). As a consequence, factors that modulate clamshell domain closure also affect activation and desensitization of the receptor and ion channel (Armstrong and others 1998; Armstrong and Gouaux 2000). When the x-ray structure of the ligand binding domain of NR1 was solved, it became apparent that the

Fig. 4. Schematic cross section of the glutamate receptor. NMDA receptors are highly permeable for the divalent ion Ca^{++} but are blocked by Mg^{++} ions at resting potential. The binding sites for the agonists (glutamate and glycine) and the antagonists (ifenprodil, spermidine, APV) are extracellularly located. The ion channel pore can be blocked by either the high-affinity blocker dizocilpine (MK-801) or the low-affinity blocker dextrometorphane. 7-Chlorokynurenate, a synthetic analog of the endogenous metabolite kynurenic acid, is a potent and specific blocker at the glycine binding site.

architecture of the coagonist binding site closely resembles the agonist domain (Furukawa and Gouaux 2003; Inanobe and others 2005).

Agonist and coagonist domains. Ionotropic glutamate receptor variants characteristically differ in agonist binding profiles, permitting a pharmacological classification of their subtypes. In the presence of glycine, heteromeric NMDA receptors show agonist responses in the order of GLU (glutamate) > NMDA > QA (quisqualate) but are not responsive to KA or AMPA (Figs. 4, 5). As a coagonist, glycine may be replaced by D-serine (Shleper and others 2005), an important endogenous coagonist in the telencephalon and cerebellum (Mothet and others 2000). Agonist and coagonist domains appear to be distributed over distinct subunits, NR1 comprises the glycine binding subunit, and NR2 subunits harbor the binding site for the agonists. The general architecture of the ligand binding domain of the NR1 subunit was derived by analogy from AMPA receptor subunit crystallization of the ligand binding core (Armstrong and others 1998; Armstrong and Gouaux 2000). Recently, direct evidence for a similar organization of the ligand binding domain was obtained by solving the x-ray structure of the ligand binding core of NR1 (Furukawa and Gouaux 2003; Furukawa and others 2005; Inanobe and others 2005). Full agonists (AMPA and glutamate) and partial agonists (kainate) induce different extents of domain closure of the AMPA receptor subunit GluR2. Studies on the NR1 subunit showed that the agonists, glycine and D-serine, as well as the partial agonist D-cycloserine favor the same degree of domain closure of the S1S2 clamshell. These findings provide a paradigm for partial agonist action that is distinct from that reported for the related GluR2 (Armstrong and Gouaux 2000). The recent increase in knowledge about structural determinants of the ligand binding core of NMDA receptors

should allow the design of novel therapeutics in particular at the modulatory glycine binding site.

Glycine binding site. NMDA receptor ion channel activation depends on the action of glycine on the NR1 coagonist binding site, sometimes also referred to as the “glycine_B” site (Fig. 4). In contrast to inhibitory glycine receptors, binding of glycine to this site is strychnine insensitive and of higher affinity. In physiological concentrations, it reduces one form of the rapid NMDA receptor desensitization. Depending on the NR1 splice variant incorporated, NMDA receptor subtypes differ in their glycine affinities. Experimentally, however, this property is difficult to assess, as homomeric NR1 subunits fail to form functional ion channels. In contrast to the NMDA receptor variants containing NR2 subunits, glycine alone suffices to trigger a burst of firing mediated by receptor complexes composed of NR1 and NR3 subunits. These data from cerebrocortical neurons indicate that NR3 subunits indeed constitute an excitatory glycine receptor (Chatterton and others 2002).

Redox agents and pH regulation of NMDA receptor function. Alterations in brain homeostasis, as encountered in ischemic conditions, exert pronounced effects on neuronal activity. Neuronal responses mediated by NMDA receptors are effectively altered by the redox state of the extracellular space. Although disulfide-reducing agents, including dithiothreitol (DTT), potentiate NMDA-gated currents, sulfhydryl oxidants such as 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), lipoic acid, and pyrroloquinoline quinone reverse DTT action (Tang and Aizenman 1993; Brimecombe and others 1997). As analyzed in heteromeric receptors composed of the NR1 polypeptide and either NR2A, B, C, or D subunits, this effect is mediated by defined amino acid positions. Receptors are rendered insensitive to reducing agents

Agonists

Antagonists

Fig. 5. Chemical structure of the major agonists and antagonist of the NMDA receptor.

when cysteines 744 and 798 of the rat NR1 subunit are mutated (Sullivan and others 1994). These cysteines reside in a strategic position at the hinge of the ligand binding cleft of the NR polypeptide. The oxidation state of the disulfide bond determines the flexibility of this hinge (Furukawa and Gouaux 2003). NMDA receptor-mediated currents are also inhibited by protons with an IC_{50} that corresponds to physiological pH (Traynelis and Cull-Candy 1990). Likewise, even small changes in extracellular pH exert pronounced effects on NMDA receptor function. A scanning mutagenesis of the NR1 subunit revealed a cluster of amino acids at the extracellular end of TMD B and its adjacent linker to the S2 portion of the glycine binding domain to act as a determinant of proton sensitivity. Because the TMD B-S2 linker controls channel gating, it is hypothesized that proton sensor and the receptor gate may be functionally integrated (Jones and others 2002; Low and others 2003).

Exploring the Unresolved Territories: The ATD-LIVBP-Like Domain

The far N-terminus of the NMDA receptor subunits carries a domain homologous to the bacterial binding protein for LIVBP. Structurally, the LIVBP-like domain or ATD is composed of the first 400 amino acids of the mature protein (Wo and Oswald 1995b; Sutcliffe and others 1996; Fig. 2). LIVBP-like domains are also found in other membrane receptors, including the metabotropic glutamate and GABA_B receptors. Crystallographic studies on LIVBP-like domains reveal a two-lobed structure with a central cleft that opens or closes upon agonist binding (Kunishima and others 2000). As evident from recombinant expression experiments, the LIVBP-like domain is important for subunit assembly of glutamate receptors during biogenesis

(Kuusinen and others 1999; Leuschner and Hoch 1999; Ayalon and Stern-Bach 2001). In NR2 subunits, this domain modulates ion channel function and desensitization behavior (Madden 2002; Herin and Aizenman 2004). In a detailed study on the assembly of chimeric AMPA receptor subunits, it was shown that the LIVBP-like domain requires additional regions of the receptor to interact with to form stable oligomers (McIlhinney and others 2003).

Homology modeling of the LIVBP structure modeled the ATD of the NR2A subunits as two globular domains separated by a central cleft. In this model, both of the globular lobes are composed of β -sheets surrounded by α -helices. Two clusters of zinc-binding residues were determined facing each other across the central cleft. Upon zinc binding, both lobes close tightly around the divalent cation. Thus, the ATDs of the NR2 subunits appear to bind modulatory compounds in the cleft of a clamshell-like structure (Paoletti and others 2000).

Antagonists. Under physiological conditions, NMDA receptors are modulated by a variety of endogenous agents (McBain and Mayer 1994). The ATD of NMDA receptors is targeted by these modulatory agents, including zinc, polyamines, sulfhydryl-reducing agents, oxidizing agents, and protons (Aizenman 1994; Traynelis and others 1995; Wo and Oswald 1995b; Sutcliffe and others 1996; Brimecombe and others 1997; Paoletti and others 1997; Williams 1997; Zheng and others 2001; Fig. 4).

Ifenprodil and derivatives. Ifenprodil is a polyamine that exerts a pronounced inhibitory effect on NMDA receptors, depending on the subunit composition of the NMDA receptor isoforms. The action of ifenprodil is incomplete and noncompetitive and dependent on pH (Mott and others 1998). A similar effect has been reported for

cyanide (Williams 1993; Arden and others 1998; Masuko and others 1999). In particular, ifenprodil and other phenylethanamines inhibit the responses of NMDA receptor isoforms containing the NR2B subunit that carries the binding site (Brimecombe and others 1998; Masuko and others 1999; Paoletti and others 2000; Perin-Dureau and others 2002). Swapping the ATD from the ifenprodil-sensitive NR2B to the ifenprodil-resistant NR2A subunit generated NR1/NR2A isoforms, which display the same ifenprodil binding properties as found in NR1/NR2B receptors. Recently, it was proposed that the desensitization of NR1/NR2B receptors by ifenprodil could be due to an allosteric interaction between the ATD and the ligand binding domain of the NR2B subunit similar to the desensitization of NR1/NR2A receptors induced by zinc (Zheng and others 2001). Resembling the model of Zn^{2+} binding, ifenprodil is thought to act via binding sites located in a cleft between two lobes of the respective NR2B amino terminal domains ("Venus fly-trap"), thereby promoting cleft closure (Hatton and Paoletti 2005). Triheteromeric NR1/NR2A/NR2B receptors retain ifenprodil sensitivity, but the degree of inhibition depends on the occupancy of the Zn^{2+} binding site in the NR2A neighborhood and therefore on endogenous Zn^{2+} . These findings are important for the therapeutic potential of ifenprodil in vivo, given the neuroprotective effect of this substance (Kemp and McKernan 2002). In addition, the effect of ifenprodil is strongly modulated by the NR1 polypeptide. Masuko and others (1999) identified residues in NR1 that exert a 50-fold effect on the sensitivity of NR1/NR2B receptors to ifenprodil. Possibly, the NR1 subunit is important for stabilizing the binding site of the NR2B polypeptide through steric interactions.

Zinc. NMDA receptors display a dual mechanism of inhibition by Zn^{2+} . Depending on the subunit composition of the receptor complex, both a voltage-independent and a voltage-dependent inhibition are observed. Zinc inhibition is involved in the fast desensitization of NR1/NR2A receptors due to the high-affinity zinc binding site in the ATD of NR2A (Chen and Lipton 1997; Chen and others 1997; Krupp and others 1998; Zheng and others 2001). All of the NMDA receptor variants analyzed display a voltage-dependent Zn^{2+} inhibition that is dictated to amino acid residues situated in the pore-forming domain. The affinity of voltage-independent Zn^{2+} inhibition is largely determined by the NR2 subunit variant analyzed. In recombinant expression experiments, the affinity for Zn^{2+} was about 50-fold higher with NR1/NR2A receptor complexes than with NR1/NR2B heteromers (McBain and Mayer 1994; Chen and others 1997; Paoletti and others 1997). When high-affinity Zn^{2+} inhibition of NR1/NR2A receptors was analyzed, a voltage-independent block was observed, amounting to 40% block of the peak current. To define the structural basis of Zn^{2+} dependency of fast desensitization, potential Zn^{2+} binding residues located in the amino terminal domain of NR2A were mutated and found to eliminate this process. Mutations of histidine residues in the NR2A subunits NR2A(H44G) and NR2A(H128A) not only decrease the affinity of NR1/NR2A receptors for Zn^{2+} but also reduce the

degree of desensitization (Zheng and others 2001). A cysteine scan (i.e., the substitution of amino acids situated in the amino terminal domain of NR2A by cysteines) demonstrated that the presence of Zn^{2+} protects these cysteines introduced within the cleft against chemical modification by the cysteine-modifying agent 2-trimethylammonioethylmethane thiosulfate (MTSET). This observation assigns the determinant of Zn^{2+} binding to the ATD (Paoletti and others 2000). Previous work suggested that the fast desensitization of NR1/NR2A ion channel complexes results from a positive allosteric interaction between an extracellular Zn^{2+} binding amino terminal domain and the glutamate binding domain of the NR2A subunit. In a recent model proposed by Paoletti and others (2000), this allosteric coupling was attributed to interactions between two modulatory lobes of the NR2A subunit, forming a tandem of Venus flytrap domains. Accordingly, the high-affinity Zn^{2+} site is formed by two lobes connected by a hinge with the Zn^{2+} binding residues facing each other across the central cleft (Paoletti and others 2000). The affinity of Zn^{2+} for its binding site appears to be regulated by glutamate but not by glycine binding. Likewise, channel pore opening appears to be unaffected by Zn^{2+} (Erreger and Traynelis 2005). Moreover, Zn^{2+} inhibition of NR1/NR2A heteromers is proton dependent (Choi and Lipton 1999; Zheng and others 2001). This is consistent with the tandem flytrap mechanism, as those amino acid substitutions in the cleft of the ATD that have the most effect on Zn^{2+} affinity also effectively modulate proton dependency of Zn^{2+} binding. This suggests that a Zn^{2+} binding residue is either protonated or that Zn^{2+} binding domains interact functionally with proton-sensitive elements (Low and others 2003).

Although most functional studies were performed on diheteromeric NMDA receptor complexes, little is known about the potential formation and function of triheteromeric complexes. Taking advantage of a combination of point mutations in the N-terminal domains of the Zn^{2+} -sensitive NR2A and the ifenprodil-sensitive NR2B subunits, the sensitivity of triheteromeric NMDA receptors to allosteric modulators was defined. Compared to their diheteromeric counterparts, triheteromeric receptors show a reduced inhibition by modulators, be it zinc or ifenprodil. Receptors with one open and one closed NR2-ATD have only partial access to the downstream inhibitory machinery (Hatton and Paoletti 2005).

Polyamines. Spermine and some other polyamines exert multiple modulatory effects on NMDA receptors (Fig. 4). Spermine potentiates NMDA currents in the presence of saturating glycine concentrations (glycine-independent stimulation) by increasing the frequency of channel opening and decreasing the desensitization. In contrast, glycine-dependent stimulation involves an increase in glycine affinity of NMDA receptors. When present in the extracellular space, spermine mediates an inhibitory effect that is strongly voltage dependent and may be due to a fast open-channel block (Williams 1997).

Stimulation by spermine is dependent on the NR1 splice variant of the NMDA receptor complex. The *GRIN1* gene,

encoding the NR1 receptor subunit, gives rise to numerous splice variants, which respond differently to spermine. Those splice variants containing the amino acid sequences encoded by exon 5, such as the NR1b subunit, are not potentiated by spermine, whereas NR1a variants show glycine-independent stimulation. The magnitude of currents mediated by NMDA receptor channels containing either NR1a or NR1b splice variants does not significantly differ, implying that an unliganded exon 5, as contained in NR1b, mimics the action of spermine. Indeed, the amino acids encoded by exon 5 are predicted to form a surface loop that acts as a tethered modulator of receptor function via binding near the polyamine recognition site, thereby potentiating receptor function (Traynelis and others 1995).

The Ion Channel Pore

In the NMDA receptor isoforms composed of NR1 and NR2 subunit variants, simultaneous occupation of the glutamate and glycine binding domains by agonists is required for ion channel opening. In contrast, glycine binding suffices for ion channel activation of NMDA receptor isoforms containing NR1/NR3 polypeptides (Chatterton and others 2002). In the polymeric NR1/NR2 receptor complex, occupation of a glutamate binding site in one subunit results in a subunit-specific gating event. As occupation of each binding site is dependent on the others, this mechanism is consistent with a cooperative effect in full gating of the channel. Individual NMDA receptor variants are characterized by ion channel properties: gating is associated with a fast and a slow kinetic component, where the fast component has been related to the efficacy of glycine site (NR1) agonists. In contrast, the slower component is affected by the respective NR2 subunit variant that carries the glutamate binding site (Banke and Traynelis 2003; Gibb 2004).

In heteromultimeric NMDA receptors, NR1 and NR2 polypeptides contribute differently to the structure of the ion channel. The narrow constriction in the NMDA receptor channel is formed by a nonhomologous asparagine residue in NR1, the so-called N site of the NR1 polypeptide. This site interacts with two asparagine residues located in the homologous N and N + 1 positions of the adjacent NR2 subunits. Like the other glutamate receptors, NMDA receptors are permeable to the monovalent cations K^+ and Na^+ (Fig. 2). The asymmetry of the ion pore has been associated with the ion channel selectivity for divalent cations (i.e., NMDA receptors are permeable to Ca^{2+} but not Mg^{2+} ions). This selectivity has important functional consequences. The lack of permeability of the NMDA receptor channel to Mg^{2+} is essential for the voltage-dependent Mg^{2+} block (Qian and Johnson 2002; Wollmuth and Sobolevsky 2004). The Mg^{2+} block also depends on interaction of the monovalent ion K^+ and Na^+ with the pore (Zhu and Auerbach 2001).

NR3 subunit variants are efficient modulators of ion channel properties. As compared to the coexpression of NR1 or NR2 subunits, the trimeric coexpression of NR1/NR2/NR3A subunits decreases both single channel conductance and Ca^{2+} permeability of the NMDA receptors

generated. In particular, the channel conductance shows a fivefold decrease in NR1/NR2A/NR3A heterotrimers as compared to NR1/NR2A alone, associated with longer open times (Das and others 1998; Perez-Otano and others 2001). A similar reduction in Ca^{2+} permeability was observed when NR3B was exogenously transfected into hippocampal neurons that endogenously express NR1 and NR2A subunits (Matsuda and others 2003).

Dizocilpine, memantine, and related agents. The cation pore of the NMDA receptor is also sensitive to blockage by noncompetitive antagonists, including MK801 (dizocilpine), memantine, and ketamine (Figs. 4, 5). MK801 acts as an open-channel blocker of NMDA receptors, exerting an irreversible mode of action (Fig. 5). In contrast, native AMPA/KA receptors are resistant to MK801. In a position corresponding to the N site of NMDA receptor subunits, AMPA/KA receptors carry a glutamine or arginine residue. Conversion of the N site of the NR1 subunit into a corresponding glutamine or arginine yields MK801-resistant NMDA receptor channels (Sakurada and others 1993), indicating that MK801 blocks the narrowest part of the ion channel, its selectivity filter. Memantine is an uncompetitive antagonist at glutamatergic NMDA receptors that produces a low-affinity voltage-dependent block. As it binds to the NMDA receptor with a higher affinity than Mg^{2+} ions, memantine is able to inhibit the prolonged influx of Ca^{2+} ions, which underlies neuronal excitotoxicity. Memantine and ketamine are in clinical use for symptomatic therapy of Alzheimer's disease (Lipton 2005).

Ethanol. Ethanol rapidly and reversibly inhibits the NMDA-evoked currents on heteromeric NMDA receptors. Inhibition by ethanol is independent of the glycine concentration used (1–100 μM). As ethanol was without significant effect on the EC_{50} for glycine, it does not act as a competitor at the glycine site. Action of ethanol is voltage independent and does not significantly alter the magnesium block. However, the sensitivity of the NMDA receptor to ethanol is reduced when the NR1 subunit is mutated into N616R or N616Q at the ion-selective N site (Burnashev and others 1992). Various NMDA receptor subunit combinations differ in ethanol sensitivity, as demonstrated for NR1/NR2C heteromers that are less sensitive to ethanol than NR1/NR2A or NR1/NR2B complexes (Gonzales and Woodward 1990; Dildy-Mayfield and Leslie 1991; Woodward 2000).

The C-terminus and the Intracellular Anchoring of the Postsynaptic Receptor Complex

Transmembrane domain C and the intracellular C-terminus of NMDA receptor subunits mediate synaptic protein-protein interaction and serve as determinants of receptor trafficking and turnover (Scott and others 2001; Fig. 2). The C-terminal domain undergoes phosphorylation by various kinases, such as CaMKII and casein kinase 2 (CK2) (Bayer and others 2001; Chung and others 2004).

In many brain regions, Ca^{2+} entry through postsynaptic NMDA receptors and the subsequent activation of CaMKII trigger synaptic plasticity. Active CaMKII binds to NR2 subunit polypeptides with different affinities, where the binding site of the catalytic domain of CaMKII has been mapped to the far C-terminus (Bayer and others 2001). However, the physiological role of this interaction is not well understood. Recent studies on CaMKII suggest that the switch from NR2B to NR2A in synaptic NMDA receptors observed in many brain regions contributes to reduced plasticity by controlling the binding of the active enzyme (Barria and Malinow 2005; Merrill and others 2005). Contradictory results exist for NMDA receptor subtypes responsible for inducing LTP and LTD (Liu and others 2004; Berberich and others 2005).

Membrane insertion and postsynaptic targeting of NMDA receptors during biogenesis is primarily mediated by interactions between transmembrane region C, the C-termini of NMDA receptors, and the PDZ family proteins such as PSD95/SAP90 and SAP102 (Chung and others 2004). In contrast to the N-terminus of NMDA receptors, C-terminal sequences do not contribute to subunit oligomerization (Meddows and others 2001; McIlhinney and others 2003). The ER retention of NR1 subunits in heterologous cells and neurons depends on the alternative splicing of the C-terminus, as shown by dividing the C-termini of the four NR1 splice variants into cassettes C0, C1, C2, and C2'. The C1 cassette of NR1 was found to harbor an ER retention signal (RRR) resembling the RXR motif present in adenosine triphosphate (ATP)-sensitive K^+ channels. Here, the retention of single subunits is masked by an interaction between subunits that, in turn, allows the receptor complex to exit from the ER (Zerangue and others 1999). If the C2' cassette harboring a PDZ binding domain is present in NMDA receptor subunits, the receptor complex is able to reach the cell surface during biogenesis. Apparently, the C2' cassette overrides the C1 retention signal. Accordingly, a mutation deleting the PDZ binding domain (ΔVSTVV) from C2' prevents surface expression of receptors (Standley and others 2000; Holmes and others 2002). The ER retention of NR2 subunits does not appear to depend on a single motif. In addition to three putative ER retention signals (KRRK, KKR, and RRR), present in the amino acid segment from Tyr-1070 to His-1119 (numbers refer to NR2B), a conserved segment immediately following TMD C (HLFY) was identified as an element responsible for the release of the assembled receptor from the ER (Hawkins and others 2004). Although the NR3A and NR3B subunits considerably differ in their C-termini, the regions between amino acid positions 952 to 985 share similar sequence homology and contain RXR motifs responsible for ER retention. The presence of an NR1 subunit appears to be required for targeting NR3 subunits to the plasma membrane (Perez-Otano and others 2001; Matsuda and others 2003). These data show that splice variation of NMDA receptor subunits is an important regulator of intracellular interactions, including those with PDZ binding proteins.

Pathogenesis of CNS Disorders and the Potential Benefit of NMDA Receptor Modulation

Cerebral ischemia, brain trauma, and inflammation have been associated with increased extracellular glutamate levels, resulting in an excessive activation of NMDA receptors (Leker and Shohami 2002; Bramlett and Dietrich 2004). This condition of NMDA receptor activation is thought to result in excitotoxicity, where high concentrations of the physiological neurotransmitter elicit neuronal dysfunction or even cell death. Excitotoxicity has initially been worked out in primary neuronal cell cultures, where exposure to glutamate produces key features of in vivo excitotoxicity, including swelling of the cells due to Na^+ entry and the final cell death dependent on Ca^{2+} entry into the cell. In the model of glutamate as an inducer of apoptotic cell death, mitochondria play an important role by mediating multiple steps of excitotoxicity, including an initial bioenergetic collapse that triggers the ischemic release of glutamate. This event is followed by Ca^{2+} entering through NMDA receptor channels, generating reactive oxygen species (ROS) and thereby triggering the apoptotic signal cascade (Nicholls and Budd 2000). Following stroke or brain trauma, neurons residing in the damaged core region are thought to release glutamate, which then overactivates surrounding neurons in an area called the penumbra (Fig. 6). Overactivity of excitatory pathways is also observed in other disorders, including epilepsy and neuropathic pain. Likewise, excess of glutamate has been proposed to contribute to neurodegenerative diseases such as Alzheimer's (AD), Parkinson's, or Huntington's diseases, playing a secondary role by sensitizing neurons to excitotoxic damage. Based on the pathogenic concept of an overactivation of the excitatory pathways, NMDA receptors had been a longstanding therapeutic target for rational drug design (Parsons and others 1998; Kemp and McKernan 2002). Following an initial excitement, however, skepticism prevails. Many of the newly generated NMDA receptor antagonists that efficiently block the excitatory pathways in experimental models failed to elicit significant therapeutic effects in humans (Ben-Abraham and Weinbroum 2000; Temkin and others 2001; Ikonomidou and Turski 2002).

Ischemia, Trauma, and Epilepsy: Conditions of Acute Cell Loss

Ischemic stroke. Ischemic stroke is among the leading causes of death and disability worldwide. Ischemia results from an interruption of the cerebral blood supply, due to an occlusion of extra- and intracerebral blood vessels, cardiac arrest, or other causes affecting brain perfusion. Ischemic brain damage is a result of hypoxia (i.e., an insufficient supply of oxygen) and hypoglycemia (i.e., a depletion of glucose levels). Under these conditions of impaired energy supply, affected neurons depolarize, releasing glutamate and other neurotransmitters (Palmer 2001). The excessive release of glutamate, in turn, leads to an overactivation of NMDA receptors in adjacent neurons, resulting in a

Fig. 6. Cascade of interacting responses leading to excitotoxic cell death after ischemia. Cerebral ischemia has been associated with increased extracellular glutamate levels, resulting in an excessive activation of NMDA receptors. Following primary lesions, the ischemic process continuously spreads to the periphery, also affecting the surrounding tissue. Glutamate, the essential mediator of neuronal cell death in this model, leads to an overactivation of surrounding neurons in an area called the penumbra. Specific receptor blockers of the NMDA receptor are thought to selectively interrupt the excitotoxic process after ischemia.

massive increase in intracellular Ca^{2+} concentrations. Ischemia triggers a cascade of interacting responses that finally lead to excitotoxic cell death. Starting from its initial focus, the ischemic process continuously spreads to the periphery, also affecting the surrounding tissue in an area called the penumbra (Fig. 6). As glutamate is an essential mediator of neuronal cell death in this model, specific receptor blockers are thought to selectively interrupt postischemic excitotoxicity. As a consequence, high hopes had been associated with the design of novel NMDA receptor antagonists, including selfotel, aptiganel, and gavestinel (Omae and others 1996; Wood and Hawkinson 1997; Farin and Marshall 2004; Wood 2005). The efficient chemical design of these antagonists yielded excellent ligand and binding properties at the glycine site of the NMDA receptor. Even more, these compounds proved to be highly effective neuroprotectants under experimental conditions. In the hippocampus model of the rat, administration of these and other NMDA receptor antagonists prevents death of neurons induced by ischemia. Under conditions of global ischemia, neuronal death primarily affects pyramidal cells of the dorsal CA1 area. Within this area, a significant decrease of NR2A/NR2B receptor complexes, but not NR1 transcripts, was observed (Gass and others 1993; Zhang and others 1997). In animal stroke models induced by the occlusion of the middle cerebral artery, several antagonists proved to be effective in reducing neuronal cell death, consistent with the distribution of NMDA receptors. In particular, the NMDA receptor antagonist MK801 and the AMPA receptor antagonist NBQX are able to reduce the extent of cerebral damage (Table 1). When administered immediately after arterial occlusion, effectiveness was highest, and it was less effective in aged than in young animals (Suzuki and others 2003).

In contrast to the promising situation in animal models, however, clinical trials have been disappointing. Discouraging observations started to accumulate as, one by one, the clinical trials on these drugs failed due to adverse CNS effects (Albers and others 1999; Morris and others 1999; Davis and others 2000; Ikonomidou and Turski 2002; Kemp and McKernan 2002). The discrepancy between advantageous experimental and clinical studies has been attributed to both a poor relevance of the animal models to the human clinical situation and an inappropriate design of the clinical trials (Hoyte and others 2004). Most of these studies attempted to modulate the early events of ischemic brain damage, including the initial phase of excessive glutamate receptor activation. Although being beneficial for the initial phase of brain damage, however, antagonizing NMDA receptors may also exert adverse effects during brain regeneration. As a consequence, high-affinity antagonists may have hindered endogenous mechanisms supporting neuronal survival and regeneration (Ikonomidou and Turski 2002). Indeed, a delayed application of NMDA antagonists appears to suppress neurogenesis, as triggered by focal ischemia in the hippocampus (Arvidsson and others 2001). Apparently, the difficult task in neuroprotective therapy lies in the short window of opportunity for therapeutic intervention after ischemia. Yet, new signs of hope appear over the horizon: novel strategies take advantage of combination therapies covering the time course of a stroke—for example, by combining glutamate receptor modulators with antioxidants as well as antiapoptotic and anti-inflammatory agents (Gladstone and others 2002).

Trauma. A rapid increase in extracellular glutamate is also observed following traumatic brain injury (Ikonomidou and

Table 1. Agonists and Antagonists of the Three Major Groups of Glutamate Receptors

NMDA Agonist	Antagonist	AMPA Agonist	Antagonist	Kainate Agonist	Antagonist	www.ncbi.nlm.nih.gov/ PubChem Compound	References
NMDA						CID: 4376	Hollmann and Heinemann (1994)
				KA		CID: 10255	Hollmann and Heinemann (1994)
GLU		GLU		GLU		CID: 611	Hollmann and Heinemann (1994)
		AMPA				CID: 1221	Hollmann and Heinemann (1994)
Glycine				Domoate		CID: 5353596	Hollmann and Heinemann (1994)
D-serine						CID: 750	Hollmann and Heinemann (1994)
						CID: 71077	Mothet and others (2000); Shleper and others (2005)
D-cycloserine						CID: 401	Goff and others (1996); Javitt (1999)
			CNQX			CID: 3721046	Antzoulatos and Byrne (2004); Hollmann and Heinemann (1994)
			DNQX			CID: 3140	Antzoulatos and Byrne (2004); Hollmann and Heinemann (1994)
			NBQX			CID: 3272524	Hollmann and Heinemann (1994); Suzuki and others (2003)
	Ifenprodil					CID: 3689	Mott and others (1998); Brimecombe and others (1998); Masuko and others (1999); Paoletti and others (2000); Perin-Dureau and others (2002)
	MK801					CID: 180081	Sakurada and others (1993)
	Memantine					CID: 4054	Chen and Lipton (1997); Scarpini and others (2003); Lipton (2004, 2005); Chen and Lipton (2005)

Ketamine	CID: 3821	Luby and others (1959); Itil and others (1967); Lipton (2005)
Selfotel	CID: 68736	Omae and others (1996); Wood and Hawkinson (1997); Farin and Marshall (2004); Wood (2005)
Aptiganel	CID: 60839	Omae and others (1996); Wood and Hawkinson (1997); Farin and Marshall (2004); Wood (2005)
Gavestinel	CID: 3086670	Omae and others (1996); Wood and Hawkinson (1997); Farin and Marshall (2004); Wood (2005)
Phencyclidine	CID: 6468	Luby and others (1959); Itil and others (1967)
Felbamate	CID: 3331	Palmer (2001)
Remacemide	CID: 60511	Palmer (2001)
Dextrometorphan	CID: 5360696	Palmer (2001)
Flupirtinmaleat	CID: 53275	Muller and others (1996); Osborne and others (1998); Seyfried and others (2000); Dorr and others (2005)
GV196771A		Quartaroli and others (1999, 2001); Wallace and others (2002)
Ro63-1908		Chazot and others (2002); Gill and others (2002)
CP101,606		Chazot and others (2002); Gill and others (2002)

Summary of the agonist and antagonists of NMDA, AMPA, and kainate receptors according to <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>, search: PubChem Compound. The compound identity numbers are listed in the second right column (CID: XX). References for detailed information about the effects of a single compound on the appropriate receptor subtype are summarized in the right column.

others 2000). Partially resembling the situation in the ischemic brain, trauma is also associated with serious perturbations of energy homeostasis and a significant rise in extracellular glutamate levels. Following trauma, persistent elevation in the extracellular glutamate concentration has been observed to be neurotoxic, offering an opportunity for delayed therapy with NMDA antagonists (Ikonomidou and others 2000). In animal studies on traumatic brain injury, pretreatment with NMDA channel blockers, but not by competitive glutamate antagonists, and AMPA receptor antagonists is neuroprotective. Cortical damage in the infant rat was reduced by pretreatment with MK801 (Ikonomidou and Turski 1996). Disappointingly, however, all clinical trials employing NMDA antagonists had been considered unsuccessful by 2001. This conclusion has, however, been reconsidered as the fact that glutamate could also preserve endangered neurons in the long term was ignored (Clausen and Bullock 2001). Moreover, NMDA antagonists, when given prior to a traumatic injury, effectively prevented neuronal death (Ikonomidou and others 2000).

Epilepsy. Despite their clinical and etiological heterogeneity, epileptic seizures share an underlying process of uncontrolled neuronal excitability, particularly affecting the hippocampus and temporal lobes. Epileptic seizures result from an imbalance of neuronal excitation, primarily mediated by glutamate, and inhibition, predominantly mediated by GABA receptors or voltage-gated potassium channels. Given the critical role of ionotropic glutamate receptors during seizures, antagonists of this receptor class are powerful anticonvulsants. Thus, blockade of NMDA receptors may reduce damage from prolonged seizures, resulting in partial neuroprotection (Brandt and others 2003). Considering the role of NMDA receptors in long-term plasticity, NMDA receptors may also contribute to the initiation or maintenance of the epileptic state. Studies using the kindling model of partial complex seizures in rats demonstrated the inhibition of seizure development by NMDA receptor antagonists (Honack and Loscher 1995). Although MK801, phencyclidine, aptiganel, ketamine, dextrorphan, felbamate, and remacemide exhibit anticonvulsant effects, none of these agents promises to be superior to established anticonvulsants. Established anticonvulsant drugs include phenobarbital, phenytoin, dilantin, and carbamazepine, none of which has been associated with glutamatergic mechanisms. On the other hand, NMDA receptor antagonists promise a potential neuroprotective effect, although clinical trials have been unsuccessful to date, possibly due to their narrow windows and side effects.

Neurodegeneration: Alzheimer's Disease, Amyotrophic Lateral Sclerosis, and Glaucoma

Neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's diseases, as well as amyotrophic lateral sclerosis (ALS) are characterized by a prodromal decay of neurons, resulting in loss of memory and/or motility. Although each of these diseases has

its own characteristics, they predominantly affect persons of middle or older age, gradually progressing and finally resulting in premature death. Although resulting in blindness, glaucoma shares many pathological characteristics of a neuropathy. Neuronal death begins long before a patient experiences symptoms of the disease. The mechanisms triggering neuronal death are diverse but share the final pathways of cell death. Different factors may contribute to cell death: (1) excitotoxicity, (2) cellular calcium overload, (3) mitochondrial dysfunction/metabolic failure, or (4) oxidative stress.

Alzheimer's disease. Starting from the entorhinal cortex and hippocampus, pathological alterations spread to cortical regions, coinciding with a progressive and age-related deficiency in learning and memory. In these CNS regions, glutamatergic neurons are primarily affected by the disease (Francis 2003). In addition to the pathological hallmarks of Alzheimer's disease, including neurofibrillary tangles and senile plaques associated with β -amyloid, neuronal cell loss is a prominent feature of this disorder. Recently, a new mechanism was reported where β -amyloid induces the endocytosis of NMDA receptors (Snyder and others 2005). The composition of the NMDA receptor protein complexes appears to differ between unaffected persons and Alzheimer disease cases. In contrast to the NR2A isoform, NR1 and NR2B levels are decreased in human postmortem brains in Alzheimer's disease (Mishizen-Eberz and others 2004). In rodents, studies also show an age-related decline of glutamate receptor mRNA levels. Among these, NMDA receptors are more prominently affected than AMPA receptor subunits (Mishizen and Ikonomovic 2001). In particular, changes in NMDA receptor mRNA levels show a steeper decline with age than NMDA receptor ligand binding at both the glutamate and the glycine sites. Loss of cholinergic inhibition can be partially compensated by therapy with acetylcholine esterase inhibitors (Munoz-Ruiz and others 2005). By analogy, enhancing glutamatergic transmission was even discussed as a therapeutic option in Alzheimer's disease. Key to this approach is the delicate balance between underactivation of NMDA receptors, associated with cognitive dysfunction, and glutamatergic overactivation resulting in excitotoxic cell death. Given the key role of NMDA receptors for learning and memory, strategies for therapeutic enhancement of glutamatergic transmission were developed. Compounds acting at the glycine binding site, including D-cycloserine, however, failed to detectably improve cognitive function in Alzheimer's patients (Falk and others 2002; Laake and Oeksengaard 2002).

Conversely, slowing down the neuronal degeneration associated with Alzheimer's disease may offer an alternative therapeutic approach. In Alzheimer's disease, lack of transmitter inactivation due to a reduction of glutamate transporters has been proposed to aggravate the disease (Lynch and Guttman 2002; Fiskum and others 2003). The impairment of glutamate transporters may increase the risk of excitotoxic injury, resulting in an overactivation of

NMDA receptors and an unregulated influx of Ca^{2+} into the cell. Contrasting this hypothesis, however, NMDA antagonists, including dizocilpine and cerestat (Table 1), failed to slow the progression of the disease. Rather, these drugs displayed unwanted actions, including cognitive and psychological dysfunction. As a representative of a new class of Alzheimer's disease drugs, memantine acts as an NMDA receptor ion channel blocker of moderate affinity (Scarpini and others 2003). It interacts rapidly and in a voltage-dependent manner with the ion channel (Chen and Lipton 1997, 2005; Lipton 2004). In experimental systems, memantine inhibits the prolonged influx of Ca^{2+} ions underlying neuronal excitotoxic mechanisms. Treatment results in an improvement in the cognitive, psychological, and motor impairments associated with the disease (Reisberg and others 2006).

Amyotrophic lateral sclerosis. This rapidly progressive, invariably fatal disease is characterized by a loss of cortical neurons involved in the control of voluntary muscles (McGeer and McGeer 2005). Mutations in the gene of superoxide dismutase (SOD1) are associated with variants of familial ALS. This enzyme is a powerful antioxidant that protects the body from damage caused by free radicals. As another risk factor, glutamate has been discussed as ALS patients show elevated serum and spinal fluid levels of glutamate. In mouse studies, neurons begin to die off when exposed to excessive amounts of glutamate over longer periods. Although there is no cure for ALS, riluzole (Rilutek®) has been approved as the first drug for treatment of this disease. Riluzole is thought to reduce damage to motor neurons by decreasing the presynaptic release of glutamate. Clinical trials showed that riluzole prolongs survival of ALS patients by a few months, mainly in the bulbar variant associated with a difficulty in swallowing. However, riluzole fails to reverse the damage to the affected motor neurons. Besides riluzole, rasagiline is an antiapoptotic compound with neuroprotective potential. In several mouse studies on ALS in mice, neuroprotection was demonstrated by a combination of rasagiline, which inhibits the monoaminoxidase type B, with the glutamate release blocker riluzole and other drugs (Kriz and others 2003; Waibel and others 2004). Thus, a therapy that combines drugs with different mechanisms may be promising for the neuroprotective strategies in ALS.

Glaucoma. Glaucoma is characterized by a chronic loss of retinal ganglion cells resulting from an apoptotic cell death of unknown cause (Kerrigan and others 1997; Quigley 1998; Halpern and Grosskreutz 2002). Potential glaucoma pathogens include an increased intraocular pressure associated with an impairment of microcirculation. However, damage of retinal ganglion cells may as well be secondary to toxic factors accumulating in the glaucomatous eye, including a synaptic accumulation of the excitotoxic transmitter glutamate (Martin and others 2002). Along this line, release of glutamate may reflect an ischemic condition associated with a reduced perfusion of retinal vessels due to an increase in intraocular pressure. Experimental ischemia indeed induces enhanced release of

neurotransmitters, such as glutamate, in the retina (Osborne and others 1995, 2006). The increase in synaptic glutamate may lead to an activation of NMDA receptors, followed by a signal cascade of increased Ca^{2+} influx, formation of reactive oxygen species, and, finally, neuronal apoptosis (Sucher and others 1997; Becker 1999). The initial hypothesis of a mere correlation between the density of NMDA receptors expressed by a cell type and its vulnerability was not confirmed for retinal ganglion cells in glaucoma (Kalloniatis and others 2004). Moreover, some researchers failed to correlate the distribution of glutamate receptor subunits NR1 and GluR2 in the retina of macaques with neurons vulnerable to experimental glaucoma (Hof and others 1998). As an alternative model, a coupling of retinal cell death to a loss of distinct NMDA receptor subunits was postulated but not yet confirmed (Colonnese and others 2005). Neuroprotective strategies, such as the application of NMDA receptor antagonists, prevent the loss of retinal ganglion cells in experimental paradigms of glaucoma (Weber and others 1995; Sucher and others 1997). Effective drugs include NMDA antagonists, such as the channel blockers MK801 and memantine, as well as the nonopioid analgesic flupirtine (Osborne and others 1998). Although these findings offer intriguing promises for an effective treatment of the glaucomatous ganglion cell loss, the experimental paradigms used do hardly reflect the chronic course of this blinding disease. Still, therapies protecting neurons against glutamate toxicity may finally prove useful in the management of glaucoma (Osborne and others 2006).

Pain. Painful sensation frequently results from a stimulation of C-fiber afferents to the spinal cord, evoking the release of excitatory amino acids and neuropeptides (Ossipov and others 2000). Acute noxious stimuli are relayed through the spinal pathways and give an accurate report of pain sensation to higher centers. This process is primarily mediated by fast-acting AMPA receptors. Continued or more intense stimulation increases the activity in the primary afferent pathways, resulting in the release of pain peptides such as substance P (Ossipov and others 2000). The release of peptide transmitters onto spinal neurons evokes postsynaptic potentials sufficient to relieve the Mg^{2+} block of the NMDA receptor channel. The resultant influx of Ca^{2+} ions leads to a state of hyperexcitation that, in turn, produces an increased response to further stimuli. This process, called the "NMDA receptor windup," was demonstrated in an in vivo rat model, where it underlies central hypersensitivity and transformation of acute into chronic pain (Herrero and others 2000; Palmer 2001). One of the compounds, which initially appeared promising to reduce the "windup" state, was the NMDA receptor antagonist dextromethorphan. Although animal studies demonstrated that dextromethorphan exerts fewer side effects than other NMDA antagonists, this drug was of limited efficiency in humans (Wong and others 1999). Recent work has defined novel targets for the treatment of neuropathic pain: NMDA receptor ligand binding domains, such as the glycine and glutamate NR2B binding sites (Parsons 2001; LoGrasso and McKelvy 2003).

Preclinical data were encouraging as some NR2B and glycine site antagonists showed efficacy in animal models. For the glycine site antagonist GV196771A, an anti-hyperalgesic activity was predicted as this compound exhibited an elevated affinity for the NMDA glycine binding site and suppressed the spinal windup activity (Quartaroli and others 1999, 2001). Yet, the preclinical promise of GV196771A did not hold in the clinical treatment of neuropathic pain (Wallace and others 2002).

Several studies in mice demonstrated the potential of NR2B subunits as antinociceptive drug targets. Studies on heterozygous NR2B^{+/-} mice demonstrated exaggerated nociceptive responses in the hot plate and tail-flick tests, indicating an important role of the NR2B subunit in the regulation of acute nociceptive responses (Wainai and others 2001). Using genetic manipulation of forebrain NMDA receptors, overexpression of NR2B in pain-related forebrain areas did, however, not affect acute nociception, as evident from tail-flick response latency. These transgenic mice showed an enhanced behavioral response to hindpaw inflammation, suggesting a role of NR2B subunits in prolonged models of peripheral inflammation (Wei and others 2001; Miki and others 2002). The *in vivo* antinociceptive action of selective NR2B antagonism was demonstrated by two compounds, Ro63-1908 (benzyl-piperidiny-4-ol) and CP101,606 (an ifenprodil analog) (Chazot and others 2002; Gill and others 2002). Furthermore, flupirtinmaleate (Katadolon®) was discussed as an NMDA receptor antagonist (Muller and others 1996; Seyfried and others 2000), and studies on human brain slices also showed that flupirtinmaleate is neuroprotective. Although the mechanism underlying this effect is not well understood (Dorr and others 2005), this agent is thought to antagonize the outward flow of K⁺ ions. As an indirect NMDA receptor antagonist, it stabilizes the membrane potential and, therefore, the Mg²⁺ block of NMDA receptors. Yet, new hopes come from the deep of the sea: conotoxins are obtained from the venom ducts of predatory snails of the genus *Conus*, with the venom of each containing between 50 and 200 peptide components. Among these, the conantokines G and T, antagonizing NR2A and NR2B receptors, display potent antinociceptive effects in models of injury-induced pain (Malmberg and others 2003).

Schizophrenia. The predominant hypothesis attempting to explain the pathophysiology of schizophrenia is the dopamine hypothesis. This hypothesis is based on the clinical observation that dopamine-releasing stimulants promote psychotic states, whereas antipsychotics act by blocking D₂ dopamine receptors (Snyder and Largent 1989; Melzer and others 1997; Winterer and Weinberger 2004; Kellendonk and others 2006). Dissociative anesthetics such as phencyclidine (PCP) and ketamine produce syndromes that clinically resemble schizophrenia (Luby and others 1959; Itil and others 1967). Both of these agents bind to NMDA receptors and act as noncompetitive, use-dependent antagonists, linking the drug-induced psychotic state to glutamatergic dysfunction (Javitt and Zukin 1989, 1991). Ketamine has been proposed to antagonize an NMDA receptor-dependent inhibitory system

that is partially compromised in schizophrenia. Along this line, reduction of inhibition by ketamine is thought to increase glutamate release, producing a hypermetabolic state in the frontal and cingulate cortical regions. In contrast, loss of inhibition and increased glutamate release have been associated with distorted thoughts and diminished cognitive abilities (Holcomb and others 2005).

In addition, NMDA receptor hypofunction has been proposed to paradoxically cause an increase in glutamate release and hypermetabolism in corticolimbic regions. Recently, the hypothesis of an NMDA receptor hypofunction in schizophrenia received further support (Goff and Coyle 2001), as treatment with glycine or D-cycloserine produced a significant reduction in negative, cognitive, and positive symptoms (Goff and others 1996; Javitt 1999). Improvement was also reported for sarcosine (N-methyl glycine), a potent inhibitor of the glycine transporter 1 (GlyT1), the subtype associated with the NMDA receptor (Tsai and others 2004). Summarizing, the glycine modulatory site of NMDA receptors emerges as a potential site for therapeutic intervention in schizophrenia.

Outlook

Recapitulating, is this the time to give up on the therapeutic concept of excitotoxicity? Certainly not—on the positive side, the past decade has seen a wealth of discoveries, including the resolution of the x-ray structure of the ligand and binding domain (Armstrong and Gouaux 2000; Furukawa and Gouaux 2003), permitting deep insights into the molecular mechanisms of NMDA receptor ligand binding and channel gating (Jones and others 2002; Banke and Traynelis 2003). Insights into how the composition of heterotrimeric NMDA receptors (Hatton and Paoletti 2005) determines the physiology and pharmacological selectivity of these ligand-gated cation channels should enable medicinal chemistry to generate selective antagonists. New therapeutic options may arise from a specific modulation of NMDA receptor subtypes rather than blocking the whole NMDA receptor machinery.

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