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COMMENTARY

STRUCTURAL REQUIREMENTS FOR THE DEVELOPMENT OF POTENT *N*-METHYL-D-ASPARTIC ACID (NMDA) RECEPTOR ANTAGONISTS

CHRISTOPHER F. BIGGE*
Department of Chemistry, Parke-Davis Pharmaceutical Research, Warner-Lambert Co., Ann Arbor, MI 48105, U.S.A.

Dramatic advances have been made recently in our understanding of excitatory amino acid (EAA)† mediated neurotransmission. Postreceptor signal transduction and receptor molecular biology have provided tools to help define aspects of EAA neurotransmission that when defective may result in disease. Increases in synaptic concentrations of glutamate (the primary EAA), or increased vulnerability to glutamate, can precipitate neuronal injury or death. Interference with EAA pathways during normal development may cause abnormal neural connectivity or selective neuronal loss. Primary disturbances of EAA transmission may be responsible for seizure disorders, and modest functional abnormalities may alter learning, memory, perception or even personality.

Ionotropic glutamate receptors may be classified in three pharmacologically distinct receptor groups: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and *N*-methyl-D-aspartic acid (NMDA) receptors. A G-protein coupled glutamate receptor (metabotropic glutamate receptor) is selectively activated by *trans*-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD). Classification of the glutamate receptors is based upon pharmacological responses to these specific agonists, and the evolutionary branches of sequence similarity are distinguished by molecular biology. NMDA receptors received the most attention early in the study of glutamate receptors because of the availability of selective antagonists, and the importance of NMDA receptors in brain function and dysfunction. Recent advances in the molecular biology of the NMDA receptor have returned it to prominence. NMDA receptors are intimately involved in the phenomenon of excitotoxicity, which may be a critical determinant of outcome following acute ischemic events such as stroke or cerebral trauma [1, 2], and may be a significant causal factor in chronic neurodegenerative disorders such as Alzheimer's disease [3] and Huntington's Disease [4]. NMDA receptors have also been implicated in the etiology of epilepsy [5]. Because NMDA receptors may be responsible for long-term potentiation (LTP), an event associated with memory formation, knowledge of NMDA receptor function may allow the molecular details of cognitive function to be understood. NMDA receptors are also involved in synaptogenesis and neuronal connectivity, processes that occur both during development of the brain and from learning [6].

A great deal of experimental evidence *in vitro* and *in vivo* supports the hypothesis that antagonists of NMDA receptor function will be clinically useful for the indications mentioned above, but because NMDA receptor function is involved in basic physiological processes and development, it is quite likely that antagonists will have some side effects. Further, there are multiple pathways that may lead to neuronal cell injury, and it is unlikely that NMDA antagonists by themselves will be a panacea. Despite the multifactorial causes of neuronal injury and cell death, if an NMDA antagonist were available to clinicians, it is highly likely that it would at the least be an effective adjunctive therapy for stroke, cerebral trauma, the chronic neurodegenerative diseases, and/or epilepsy. Imagination and purposefulness will be required to shepherd these agents through clinical trials. It is the intent of this commentary to describe briefly some of what is known about the NMDA receptor and its modulatory sites, and how that information is being used to develop both pharmacological tools, and clinically useful NMDA antagonists.

NMDA receptor structure and basic pharmacology

The NMDA receptor is a ligand-gated/voltage-

* Correspondence: Dr. Christopher F. Bigge, Department of Chemistry, Parke-Davis Pharmaceutical Research, Warner-Lambert Co., 2800 Plymouth Road, Ann Arbor, MI 48105, U.S.A. Tel. (313) 996-7136; FAX (313) 996-5165.

† Abbreviations: ACPD, *trans*-1-amino-1-3-cyclopentanedicarboxylic acid; ADCI, 5-aminocarbonyl-10,11-dihydro-5*H*,dibenzo[*a*,*d*]cyclohepten-5-10-imine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP5, 2-amino-5-phosphonovaleric acid; AP7, 2-amino-7-phosphonoheptanoic acid; 7-ClKA, 7-chlorokynurenic acid; DCQX, 6,7-dichloroquinoxaline-2,3-dione; 6,7-diClHQC, 6,7 - dichloro - 2 - hydroxyquinoxaline - 3 - carboxylic acid; EAA, excitatory amino acid; HA-966, 3-amino-1-hydroxypyrrolid-2-one; LTP, long-term potentiation; MNQX, 5,7-dinitroquinoxaline-2-3-dione; NMDA, *N*-methyl-D-aspartic acid; PCP, phencyclidine or *N*-(1-phenylcyclohexyl)piperidine; PKC, protein kinase C; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; and SDZ EAB 515, (*S*)-α-amino-5-(phosphonomethyl)-[1,1′-biphenyl]-3-propanoic acid.

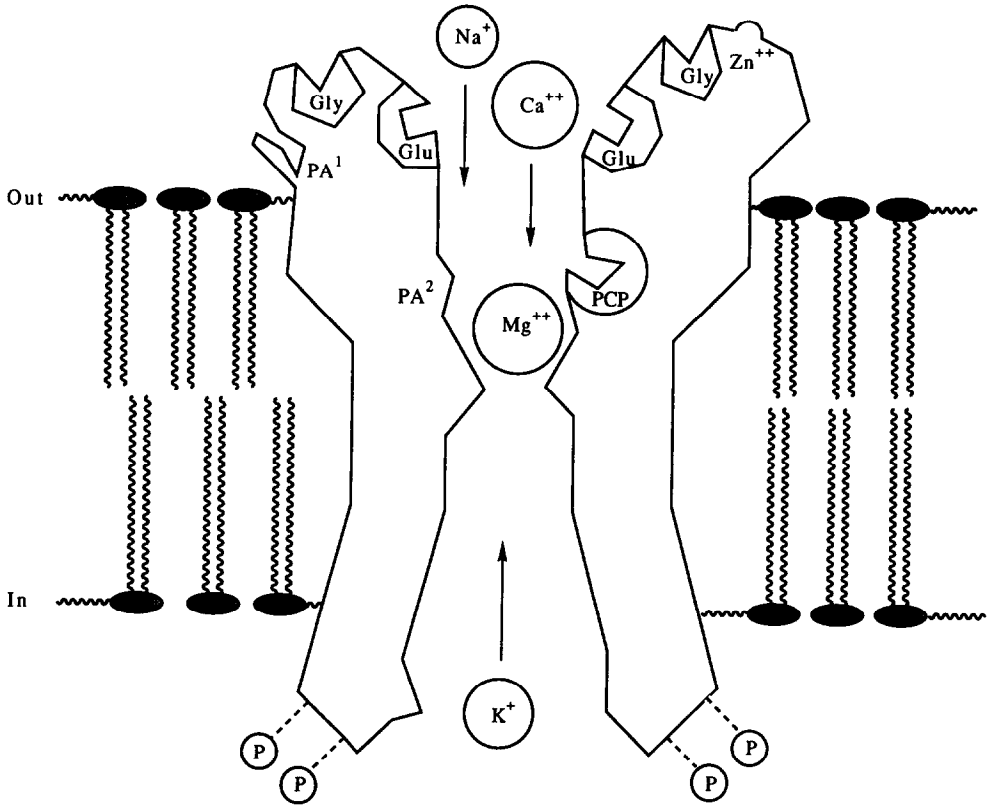

Fig. 1. Schematic representation of the NMDA receptor and its modulatory sites. The NMDA receptor allows passage of Na^+, Ca^{2+} into the cell and K^+ out of the cell through the ion channel. Mg^{2+} acts as a voltage-sensitive channel blocker, and may either coordinate or overlap with the same site as the noncompetitive ion channel blockers (PCP site) within the ion channel. Glutamate (Glu) and glycine (Gly) are coagonists of receptor function, and stoichiometry indicates that two molecules of each agonist are required for activation. Polyamines (PA) are positive modulators of receptor function at low concentrations (PA^1 site), but may inhibit receptor function at higher concentrations by binding within the vestibule of the ion channel (PA^2 site). Zinc (Zn^{2+}) acts as a non-voltage-sensitive inhibitory modulator of receptor function. Phosphorylation (P) on the intracellular domain of the receptor may help regulate/amplify the receptor signal.

sensitive ion channel (Fig. 1). In addition to a glutamate recognition site, it contains a separate agonist recognition site for glycine, which is required for receptor activation [7]. The recent cloning of a functional NMDA receptor has demonstrated unambiguously that both of these recognition sites reside on the same protein [8]. *In vivo*, glycine is present in the extracellular fluids at sufficient concentration to ensure a high degree of occupancy at its binding site. Thus, synaptic activation of NMDA receptors can be triggered solely by the release of glutamate. The unusual combination of dual ligand-gating and voltage-sensitivity is the soul of NMDA receptor function. The voltage-dependent block of ion channel gating by physiological concentrations of Mg^{2+} in the extracellular solution is thought to be due to binding of Mg^{2+} at a site that lies within the ion channel pore of the NMDA receptor. Association and dissociation rate constants for binding of Mg^{2+} change with membrane potential. As a result, responses to NMDA are strongly attenuated close to the resting potential, but with depolarization the blocking action of Mg^{2+} becomes progressively weaker. This interaction between the nerve cell membrane potential, and the block of NMDA receptors by extracellular Mg^{2+}, has the following consequence: NMDA receptor currents behave like classical voltage-dependent channels, which increase their activity with depolarization. Because NMDA receptor activation requires two types of input (binding of agonist such as L-glutamate, and depolarization to reduce Mg^{2+} block), the synaptic activation of NMDA receptors shows associative properties. This allows NMDA receptors to act as Hebbian switches which detect excitatory activity from other synapses, and this behavior is thought to underlie the associative basis for LTP [9].

Further modulation via receptor kinetics and second messenger systems allows for exquisite regulation and/or amplification of NMDA receptor signals, which ultimately regulate processes such as LTP. Phosphorylation of glutamate receptors is an example of this [10]. In hippocampal neurons, basal

phosphorylation may be necessary to prevent the rundown of both kainate [11, 12] and NMDA evoked currents [13]. NMDA gated currents in trigeminal neurons are modulated by protein kinase C (PKC) which acts by reducing the voltage-dependent Mg^{2+} block of the receptor channel [14]. In addition, current responses of a recombinant NMDA receptor in *Xenopus* oocytes are enhanced by phorbol ester, possibly acting through PKC [15].

The NMDA receptor was an elusive target for molecular biologists and biochemists to clone. However, recent disclosures show that the NMDA receptor is a family of receptors with heteromeric subunits, which adds a new dimension to the complexity of NMDA receptor pharmacology. Expression of the NMDAR1 (ζ1) subunit as well as the ζ1–2 subunit can produce NMDA receptor channels in *Xenopus* oocytes. But highly active NMDA receptor channels are formed only when both the ε (1–3, or NR2 A–C) and ζ (or NR1) subunits are coexpressed [16,17]. Thus, the ε and ζ subfamilies represent the necessary subunits of the NMDA receptor channel. The ε1 and ζ1 subunit mRNAs are widely distributed in neural cells. The ε2 subunit mRNA is expressed selectively in the forebrain, and the ε3 subunit mRNA is found predominantly in the cerebellum. The ε2/ζ1 and ε3/ζ1 channels exhibit higher apparent affinities for L-glutamate and glycine than the ε1/ζ1 channel. The ε1/ζ1 channel is most sensitive to 2-amino-5-phosphonovaleric acid (AP5), whereas the ε3/ζ1 channel is most sensitive to 7-chlorokynurenic acid (7-ClKA) and is resistant to Mg^{2+} block. The ε2/ζ1 channel was less sensitive to 7-ClKA. These data suggest that at least three distinct populations of NMDA receptors exist with different pharmacological characteristics and regional distributions in the brain. Beyond that, it may help to explain why the heteroaromatic bicyclic glycine antagonists have not demonstrated neuroprotection in the sensitive forebrain regions following permanent cerebral occlusion (see later discussion). In addition, the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) enhances the channel activity of ε1/ζ1 and ε2/ζ1 but not ε3/ζ1. As mentioned, NMDA receptors may be positively modulated by protein kinases (through G-coupled proteins) to establish the threshold of induction of LTP [17, 18]. Evidence from recent site-directed mutagenesis experiments have demonstrated that replacement of the conserved asparagine residue in the transmembrane segment M2 with glutamine of either the ε2 or ζ1 subunits abolishes the block of the heteromeric receptor channel by Mg^{2+}. The same substitution reduces the sensitivity of the heteromeric channel to MK-801 suggesting that the phencyclidine (PCP) site and the Mg^{2+} site overlap, or are the same [19].

By analogy with other ligand gated receptors, a pentameric structure of subunits is plausible, raising the possibility that up to five glutamate and five glycine binding sites could be present at each receptor channel complex since both glutamate and glycine binding sites are present on the subunits. However, the rate of onset of block and the rate of recovery from the action of competitive antagonists seem best fit by a two-site model, consistent with the stoichiometry of activation determined from the limiting slope of the NMDA receptor agonist dose–response curve [20]. Both glycine sites must be occupied for activation of the NMDA receptor gating. Although it is too early to know how these multiple NMDA receptors will translate into the discovery and development of NMDA antagonists as neuroprotective agents, knowledge of the NMDA receptor structure and function at the molecular level may be useful to refine drug design if methods for mass screening and crystallography of membrane bound receptors are utilized.

Glycine site antagonists

The glycine site is potentially important as a locus for therapeutic agents since inhibition should be able to diminish the pulse of excessive NMDA tone during the cycle of glutamate excitotoxicity. However, negative regulation of the glycine site must overcome high concentrations of endogenous glycine that are believed to be present at the synaptic cleft. From a different perspective, competing with high glycine concentrations may induce modulation of NMDA receptor activity and thus provide a more physiological inhibition than complete receptor blockade (compare with channel blockers).

The structural requirements for agonist activity of the glycine site have been described [21]. The agonists depend on an unhindered amino acid functionality and activity is stereoselective as demonstrated by the 30-fold enhancement of affinity of D-serine relative to L-serine. The ω-terminal hydroxy group of D-serine apparently provides a critically positioned hydrogen bonding interaction (proton acceptor). Complementary molecular modeling studies and electrophysiological investigation suggest that the glycine recognition site is a small pocket with three-point attachment [22].

Glycine site antagonists may be divided into two distinct classes of compounds structurally and functionally (see Fig. 2). The first class may be represented by the partial agonists 3-amino-1-hydroxypyrrolid-2-one (HA-966, first identified as a glutamate antagonist by Fletcher and Lodge [23]) and cycloserine [24], both of which are amino acid like structures of relatively small size. Both of these compounds fit in with the small pocket theory of glycine binding. The second class can be described as bicyclic heteroaromatic derivatives and is more structurally diverse (discussion to follow). A prototype is 5,7-dichlorokynurenic acid, **3** [25]. Subtle structural modifications result in dramatic differences in receptor affinity, and are responsible for better understanding of receptor requirements.

HA-966 and derivatives: Partial agonists at the glycine site. Several structural and pharmacological features are worth mentioning to illustrate the activity of HA-966 [26]. First, the small cyclic structure is compact. The 3-amino group and the N-hydroxy function (hydroxamic acid) form the requisite α-amino acid moiety. The only additional structure of the molecule consists of the compact 4-methylene unit tied into the ring. Resolution of the (*R*)- and (*S*)-enantiomers of HA-966 demonstrated the stereoselectivity of the glycine site, and the profound differences in the pharmacological activity

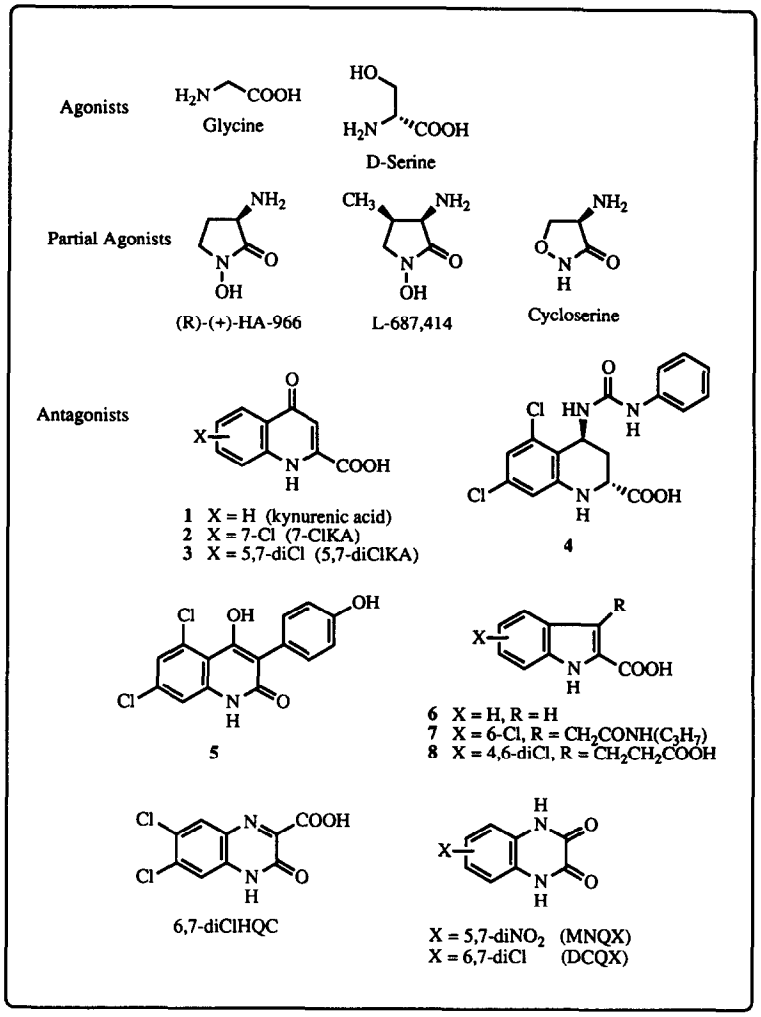

Fig. 2. Glycine site agonists, partial agonists and antagonists.

of the stereoisomers. The (*R*)-(+)-enantiomer is a selective and relatively potent glycine site antagonist (IC_{50} = 12.5 μM in [^{3}H]Gly binding), whereas the (*S*)-(−)-enantiomer has extremely weak affinity (IC_{50} = 340 μM). More importantly, the (*R*)(+)-enantiomer antagonized sound (ED_{50} = 53 mg/kg, i.p.) and NMDA (ED_{50} = 900 mg/kg)-induced seizures in mice. These effects could be reversed by the coadministration of D-serine. In addition, the adverse sedative/ataxic effects were attributable mostly to the (*S*)-enantiomer, which was 25-fold more potent than the (*R*)-enantiomer [26]. HA-966 was found to reduce NMDA-mediated brain damage in a dose-dependent manner in rat pups [27].

The receptor site configuration of HA-966 was explored by preparation of 4-methyl derivatives. Among the 4-methyl derivatives, glycine site activity was found to demand stereospecificity and resides in the 3*R*-amino, 4*R*-methyl derivative (L-687,414, IC_{50} = 1.4 μM in [^{3}H]Gly binding), and is 5- to 10-fold more potent than (*R*)-(+)-HA-966 at the glycine site [28]. Comparison of molecular mechanics calculations and glycine site activity have led the authors to suggest that an energetically less favorable axial conformation may be required for receptor recognition. This conformation also provides that the α-amino group is in a totally unhindered position. L-687,414 has been demonstrated to be neuroprotective in models of focal ischemia, and is being investigated as a potential development candidate for use in stroke. At present, except for L-687,414 and HA-966, glycine antagonists have failed to fulfill the promise of *in vivo* active NMDA antagonists.

Bicyclic heteroaromatic glycine antagonists. A second class of bicyclic heteroaromatic glycine site antagonists does not fit the small pocket hypothesis and has some steric hindrance around the α-amino functionality. In addition, they have a fused planar aromatic functionality which defines the volume parameters of receptor binding. The additional structural diversity and greater glycine site affinity provided by this class of compounds have disclosed a wide range of design features that can be

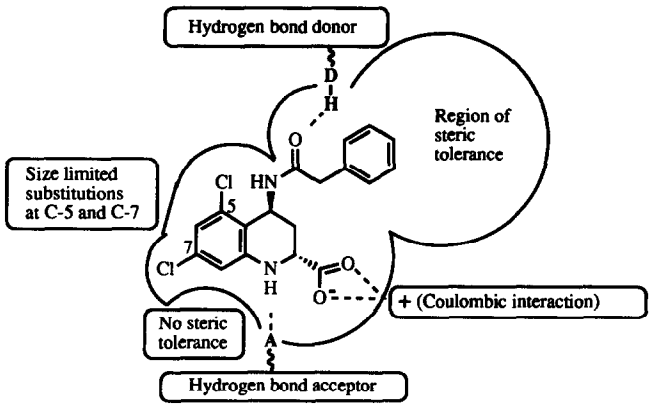

Fig. 3. Schematic representation of the glycine site of the NMDA receptor with L-688,385 ([^{3}H]Gly IC_{50} = 14 nM).

incorporated. As suggested by the molecular biology of the NMDA family of receptors, certain NMDA receptors, such as $\varepsilon 3/\zeta 1$ concentrated in the cerebellum, may have greater intrinsic affinity for the heterocyclic glycine antagonists than other NMDA receptors, for example $\varepsilon 2/\zeta 1$ concentrated in forebrain. Such differences in neuronal distribution and affinity for glycine antagonists could account for the poor *in vivo* activity of this class of compounds. Alternatively, their lack of *in vivo* activity may have much to do with their physicochemical properties. Although these heteroaromatic compounds behave as competitive antagonists of glycine binding and are reversed by added glycine, it seems unlikely that they bind directly to the same site as glycine. It may be more likely that they bind reciprocally with glycine, and when bound, produce an inactivated receptor state. This hypothesis is in contrast to the ambitious, unifying model proposed by Manallack *et al.* [29] which overlays several glycine agonists and antagonists including glycine, D-serine, 7-ClKA, HA-966, and 6,7-dichloro-3-hydroxyquinoxaline-2-carboxylate [29].

The major structural templates of glycine antagonists are represented by kynurenates, quinoxalines, indoles, and quinolones. The kynurenates offer the best example of the strides that have been made to improve affinity at the glycine site. Early studies showed that kynurenic acid could antagonize NMDA responses in a manner partially reversible by the addition of excess glycine, but its selectivity for the glycine site compared to the NMDA site was limited [30]. Chlorine substitution of the aromatic ring at C-7 to give 7-ClKA acid provided a potent and selective glycine site antagonist ([^{3}H]Gly binding $IC_{50} = 0.56\,\mu M$ vs $41\,\mu M$ for kynurenic acid) [31]. Introduction of 5-, 7-, and 5,7-disubstitution led to selective glycine site antagonists, and examination of tautomeric forms provided a model for glycine receptor binding [25]. This preliminary model (Fig. 3), which can also account for the binding of quinoxalinediones and quinoxalic acids, suggests that (a) there is a size-limited hydrophobic binding of the aromatic ring, (b) the 4-oxo tautomer acts as a hydrogen bond acceptor, (c) the 1-amino group acts as a hydrogen bond donor, and (d) the 2-carboxylate provides a Coulombic interaction with the receptor.

Elegant structural studies have helped refine the stereochemical and conformational requirements and produced glycine site antagonists with low nanomolar binding affinity. A series of tetrahydroquinolines, represented by *trans*-2-carboxy-5,7 - dichloro - 4-[[(phenylamino)carbonyl]amino] - 1,2,3,4-tetrahydroquinoline ($IC_{50} = 0.0074\,\mu M$ in [^{3}H]Gly binding), defines the α-amino acid center as *R*, in common with the glycine site partial agonists. A new region of bulk tolerance in the "northeast quadrant" was identified by these compounds [32]. Further elaboration at the C-3 position reinforces this finding [33], and a series of 3-phenyl-4-hydroxy-2-quinolones (**5**) demonstrate the importance of both the hydrogen bond acceptor at C-4 and the added bulk tolerance at C-3 [34].

Comparison of the parent kynurenates and the tetrahydroquinolines described shows that the partial saturation of the aromatic system causes a substantial reduction in melting point, which might also translate into improvement of solubility and/or other physical properties. The dramatic increase in glycine site binding affinity and potentially important changes in physical properties demonstrated by the tetrahydroquinoline may be critical to drug delivery and CNS bioavailability. There is some evidence that the kynurenates may be actively removed from the brain via a transport system. Synthetic efforts are underway whose aim is to utilize amino acid transport systems to improve CNS penetration, or to design water-soluble prodrugs that will improve formulation and help deliver the parent compound into the CNS.

A few years ago, indole-2-carboxylic acid was reported to antagonize completely glycine potentiation of NMDA receptor responses [35]. The structure–activity relationships of the indole-2-carboxylic acids were explored by two separate groups. Gray *et al.* [36] found that *N*-propyl-2-carboxy-6-chloro-3-indoleacetamide was the most potent compound in their series ($K_i = 0.47\,\mu M$ in

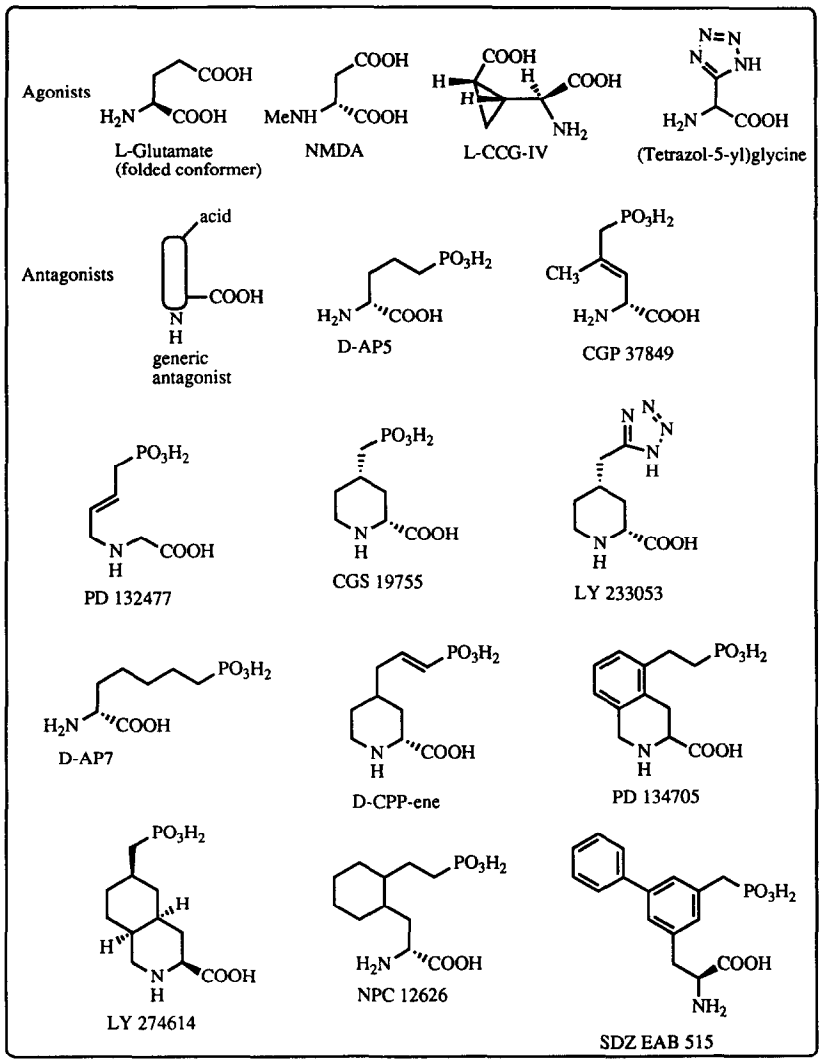

Fig. 4. Competitive NMDA site agonists and antagonists.

[^{3}H]Gly binding). Parallel to the kynurenate series, 4,6-dichloro substitution of 3-(2-carboxyindol-3-yl)propionic acid resulted in enhanced glycine site affinity ($IC_{50} = 0.17\ \mu M$) and increased selectivity relative to the glutamate binding site (>2500-fold) [37, 38]. Results from these studies have confirmed the importance of the aromatic substitution pattern (size, lipophilicity and electronic effects) on affinity and selectivity, the absolute requirement for a proton on the indole nitrogen (or kynurenate), and the suggestion of a larger binding pocket for the extended side chain off of C-3.

Both the quinoxalinediones and 2-hydroxy-3-quinoxalinecarboxylic acids have been shown to be excitatory amino acid antagonists [39]. Generally, this class of compounds have affinity for both NMDA and non-NMDA receptors. Among these compounds are 5,7-dinitroquinoxaline-2,3-dione (MNQX), 6,7-dichloroquinoxaline-2,3-dione (DCQX) and 6,7-dichloro-2-hydroxyquinoxaline-3-carboxylic acid (6,7-diClHQC). All of these compounds have good activity in *in vitro* systems, but none has shown acceptable *in vivo* activity. If problems with bioavailability can be solved, this type of mixed antagonist may be expected to have greater neuroprotective potential since it has been postulated that inhibition of both NMDA and non-NMDA receptors is required for acceptable neuroprotective effects [40].

Competitive NMDA antagonists

Several competitive NMDA antagonists are being developed as clinical candidates for epilepsy (anticonvulsants) or as neuroprotective agents for acute use following stroke. Selective agonists have been useful to help understand the configuration of glutamate required to produce NMDA receptor activation, and ultimately, in the design of antagonists. Examples of both NMDA site agonists and antagonists are shown in Fig. 4. NMDA itself

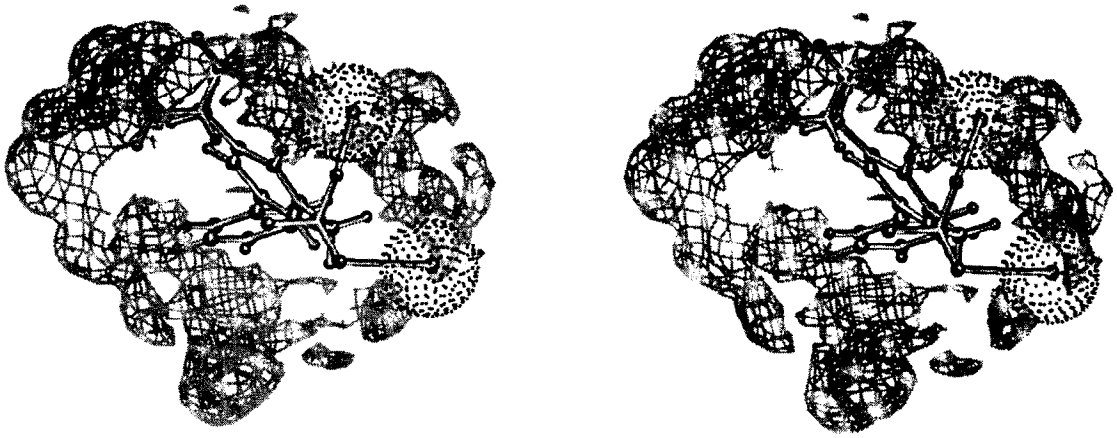

Fig. 5. Stereoview of (*S*)-α-amino-5-(phosphonomethyl)-[1,1′-biphenyl]-3-propanoic acid (SDZ EAB 515) docked in the competitive NMDA receptor pharmacophore model.

presaged the ability, and perhaps preferred fit, of the receptor to accommodate the *R*-stereochemistry of the acidic amino acid antagonists. A series of stereocontrolled 2-carboxycyclopropylglycine derivatives has been most useful in determining the absolute configuration of agonist binding: the 2*S*,3*R*,4*S*-derivative (L-CCG-IV) is a very potent and selective NMDA agonist, and strongly suggests that the active conformer of glutamate at the NMDA receptor is the folded form [41]. This is in agreement with the agonist pharmacophore model that we have described [42]. A recent report provides evidence that (tetrazol-5-yl)glycine gives a full agonist effect, and is selective and more potent than glutamate or L-CCG-IV at the NMDA site [43]. The ability to delocalize the ionic charge around the tetrazole ring may allow this compound to adapt to the glutamate site more facilely than a simple carboxylic acid. Both L-CCG-IV and the tetrazolylglycine provide design elements that are featured in antagonists.

A great deal of work has been done with rigid analogs of both agonists and antagonists which has been applied to understanding the topography of the competitive NMDA site. Pharmacophore models for both agonists (not shown) and antagonists (Fig. 5) are now available which provide a great deal of detail of the primary receptor binding points, the volume consumption of active compounds, and stereoelectronic considerations [42, 44, 45]. Ortwine *et al.* [42] have hypothesized that the NMDA agonists and antagonists can indeed occupy the same site. For the antagonists, there are three primary ionic interactions which provide the binding energy. These are from the α-amino group, the α-carboxylic acid group and the terminal ω-acidic group. It is postulated that phosphonic acids have an additional hydrogen bonding or ionic interaction (secondary binding site) that is not available to carboxylic acid moieties, and may explain their enhanced affinity (up to 10-fold) relative to other terminal acidic functionalities. Antagonists generally occupy additional volume compared to agonists. Although there are exceptions, antagonists generally prefer the *R*-stereoisomer at the α-carbon of the amino acid portion for high affinity. Our model was built by superimposing the basic amine, its lone pair, and the α-carboxylic acid. Using a distance map approach, a primary receptor interaction point and a secondary receptor interaction point were defined that explain the geometry and interaction of the terminal acidic group with the receptor. Similar to the agonist model, the competitive antagonist pharmacophore model entails a folded conformation and contains two specific interaction points off of the distal phosphonic acid. Both points are roughly in the plane of the piperazine ring of CPP and one is positioned such that a receptor moiety could simultaneously hydrogen bond to oxygens on the phosphonate and carboxylate groups. This point is also in proximity to the distal acidic hydroxyl of the agonist model. Strict volume requirements are found near the basic amine, α to the phosphonic acid, and surrounding the receptor interaction sites, presumably reflecting critical receptor interactions occurring in these areas. Some volume tolerance is noted in the receptor. Perhaps this is demonstrated best by the fit of the biaryl derivative, (*S*)-α-amino-5-(phosphonomethyl)-[1,1′-biphenyl]-3-propanoic acid (SDZ EAB 515) [46], in our pharmacophore model (Fig. 5). For this compound, the (*S*)-enantiomer is a selective and potent NMDA antagonist. In our model, the biphenyl ring slips neatly through a hole in the volume map of the receptor, whereas the biphenyl ring of the (*R*)-enantiomer encountered disallowed space (not shown). The biphenyl ring system must provide additional hydrophobic interactions at the receptor surface that stabilize binding since it is greater than 10-fold more potent than the parent aryl spaced derivative. This compound represents a leap in the design of NMDA competitive antagonists in that the (*S*)-configuration may allow this compound to utilize an amino acid transport system and thus to increase CNS accessibility. Although this hypothesis has not been confirmed, such a feature may be prominent in future design strategies.

The majority of recently developed glutamate site

antagonists are cyclic derivatives of either AP5 [47] or 2-amino-7-phosphonoheptanoic acid (AP7) [48], and can be represented by the generic antagonist structure in Fig. 4 where the principal functional groups are attached to a structural template. All that is required of the template is to provide the appropriate geometry and fit stereoelectronic and volume requirements. NMDA antagonists of this class demonstrate a periodicity determined by chain length, i.e. the butyric and hexanoic acid analogs have dramatically less affinity for the NMDA receptor. The AP5-like compounds are superbly represented by CGS 19755 [49] and CGP 37849 [50, 51]. The geometric constraints imposed by the piperidine ring of CGS 19755 and by the *trans* double bond of CGP 37849 hold these compounds in a receptor preferred configuration, and have provided rigid templates for modeling receptor interactions. CGP 37849, one of the most potent competitive NMDA antagonists known, represents the ultimate in design efficiency, with the minimum structure necessary to provide the receptor preferred configuration of its functional groups. By extending the 4-methyl to a 4-propyl group (CGP 39653, used in its [^{3}H]-form as a receptor binding ligand) [52], an even more potent antagonist is created, reminding us of the importance of hydrophobic interactions. Entropy plays an important role in determining the binding affinity as demonstrated by a series of *N*-(phosphonoalkenyl)glycine derivatives, represented by PD 132477 [53]. In these examples, rather than the typical substitution off of the α-methylene group of the amino acid, the phosphonate side chain emanates from the α-amine. If as hypothesized, the α-carboxylic group and the ω-terminal acidic group need to be in close proximity for NMDA receptor binding, N-substitution necessarily increases the entropy of the system, and it was found that only those compounds with features that constrain the geometry in an allowable conformation show activity, and features that disrupt the proximity of the two acidic groups produce inactive compounds. The ω-tetrazole derivative, LY 233053, is a short acting derivative of CGS 19755 that demonstrates the utility of replacing the terminal phosphonic acid with a tetrazole [54].

CPP was the first constrained AP7-like derivative [55]. This compound was later improved further by the addition of an olefin in the side chain to give D-CPP-ene [56]. The AP7 framework has been incorporated into more rigid analogs, such as the *cis*-1,2-cyclohexyl derivative NPC 12626 [57], and even more rigidly into the isoquinolinecarboxylic acid derivatives represented by PD 134705 [42]. Such modifications not only provide increased structural rigidity, but also additional lipophilicity that was expected to improve the CNS penetration of these highly polar compounds. The decahydroisoquinoline, LY 274614, represents the ultimate in terms of receptor stereoselectivity with four stereocenters, which provides further structural information for the refinement of the computer pharmacophore models [58]. These competitive antagonists are illustrated in Fig. 4.

Currently, some of the competitive antagonists thought to be in, or approaching, clinical development are: CGP 37849, which demonstrated potent oral anticonvulsant activity [50]; LY 274614, which was effective systemically against NMDA-induced toxicity and convulsions [59]; D-CPP-ene, which showed consistent results in focal ischemia models [60]; and perhaps most intriguing, SDZ EAB 515 [46], which due to its (*S*)-configuration and large hydrophobic biphenyl moiety, may be able to piggyback the large neutral amino acid transporter into the brain.

Noncompetitive NMDA antagonists

Noncompetitive NMDA antagonists are "use-dependent" ion channel blockers that bind to a specific site in the ion channel pore called the PCP site (named for phencyclidine). Access to the PCP site is agonist-dependent, suggesting that it is not accessible from the extracellular fluid when the channel is closed. The dissociative anesthetic ketamine, PCP, and the anticonvulsant, MK-801 (dizocilpine), were among the first compounds identified which produce a voltage-dependent ion channel block similar to that induced by Mg^{2+} [61, 62]. In fact, recent evidence from site mutagenesis studies in heteromeric receptors suggests that the Mg^{2+} blocking site and the PCP site overlap, and may even be the same [19]. Compounds from this class have demonstrated remarkable neuroprotective effects in a number of *in vitro* and *in vivo* pharmacological models. Because they are dependent on agonist for their antagonist activity, it has been speculated that they would be most effective during events which are most likely to lead to excitotoxicity. For this reason there is great interest in developing agents that are PCP site ligands. Other advantages of this class of compound entail their ability to penetrate rapidly into the central nervous system, the ease with which they can be administered i.v. for acute treatment, and their excellent neuroprotective effects demonstrated in stroke models. Unfortunately, the positive neuroprotective effects of this class of compound have not been separated from a side-effect profile that includes hyperactivity at low doses, sedation/ataxia at higher doses, and psychotomimetic effects.

A basic pharmacophore model of PCP site ligands has been described [63, 64]. Potent ligands generally contain an aromatic ring and a basic amine separated by areas of lipophilic bulk. The basic model has been refined to include both essential active site volume, excluded volume, and electrostatic potentials [65]. A primary receptor interaction point on the receptor wall forms an ionic interaction with the protonated amine, a region of vertical hydrophobicity orthogonal to the planar aromatic ring fills the pore region, and a secondary specific hydrogen bonding interaction exists on a distal wall of the ion channel (Fig. 6).

A great number of structural types have been discovered that fit the PCP site (Fig. 7). Modifications of PCP itself have been fruitful in defining the active site conformation and have led to more potent agents [66, 67]. However, MK-801 represents the ideal structure for modeling to define the PCP site. The molecule is held in a rigid configuration that defines the planes of both aromatic rings and the position

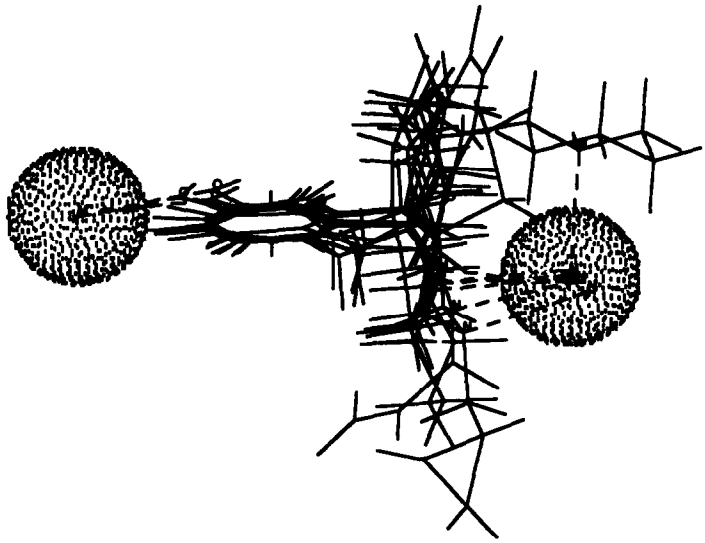

Fig. 6. An edge-on view of an initial set of noncompetitive NMDA antagonists within the PCP site. The location of the primary and secondary receptor interaction points are illustrated with balls. The primary interaction site (right) binds to the amine portion of the antagonist presumably via an ionic interaction. The secondary interaction site (left) provides additional affinity at the PCP site for ligands that contain a hydrogen bond acceptor (OH or OMe, for example) emanating from the appropriate position of the planar aryl group.

of the basic amine. Even the C-5 bridgehead methyl group of MK-801 was found to be critical for structure and function. In comparison with analogs without the C-5 methyl, the C-5 methyl group imparts a 10-fold increase in receptor affinity. Substitution of the aromatic ring has provided additional structural information, showing that halogenation at C-3 or hydroxylation at C-7 or C-8 can provide compounds with higher intrinsic activity than the parent [68], and may identify a secondary binding site within the ion channel pore. Generally, there is a 5- to 10-fold difference in receptor affinity between the (+)- and (−)-optical antipodes at the PCP site. The modest activity of the (−)-enantiomers is not too surprising because of the near symmetry of the molecule. Indeed both molecules can be fit into the receptor models. Further studies have demonstrated that the aromatic rings are not strictly required; hydrogenation of either one or both of the aromatic rings of MK-801 gave compounds that retained receptor affinity [69]. This shows that if the primary interaction with the amine is maintained, hydrophobic groups can be designed to occupy critical space. A hybrid structure of MK-801 and carbamazepine, 5-aminocarbonyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (ADCI), illustrates the sensitivity to substitution at the C-5 position [70]. ADCI contains a carboxamide functionality in place of the C-5 methyl. This compound is about 3000-fold less potent in ligand binding studies at the PCP site than MK-801, but is extolled because of its lack of behavioral side effects at anticonvulsant doses. The authors assume that association rates for the PCP site are similar for ADCI and PCP-like compounds, but that the dissociation rate of ADCI is much more rapid, and thus has an improved toxicity profile. A kinetic argument has been proposed to explain these differences: for weak PCP site ligands such as ADCI, the channel block can be relieved more easily. These fast-acting compounds become filters of NMDA channel activity. From this argument, there should exist an optimal kinetic profile to effectively block NMDA channel hyperactivity while having little effect on normal receptor function. Structural dissection has led to the synthesis or identification of numerous open chain analogs related to MK-801, including the anticonvulsant remacemide [71, 72]. Glycine is removed metabolically from remacemide *in vivo* to provide a more potent NMDA channel blocker. Apparently, the combination of requiring metabolism to an active compound and fast dissociation kinetics from the PCP site minimizes its potential for adverse effects. Remacemide has demonstrated anticonvulsant and neuroprotective effects following ischemia, and is being developed for a stroke indication. It is unclear that ADCI and remacemide are neuroprotective due to their NMDA effects; some of their anticonvulsant activity may be due to interaction at sodium channels.

A second class of rigid and potent compounds, represented by the fluorenamine (PD 137889) and phenanthrenamine (PD 138289), that has been useful for refining the PCP site pharmacophore was developed in our laboratories [65]. (Kozikowski and Pang made important contributions in this area as well [73].) A vast range of affinity for the PCP site is accessible through modification of substituents on either the A, B or C rings, by altering the amine substitution, by altering the stereochemistry, or by

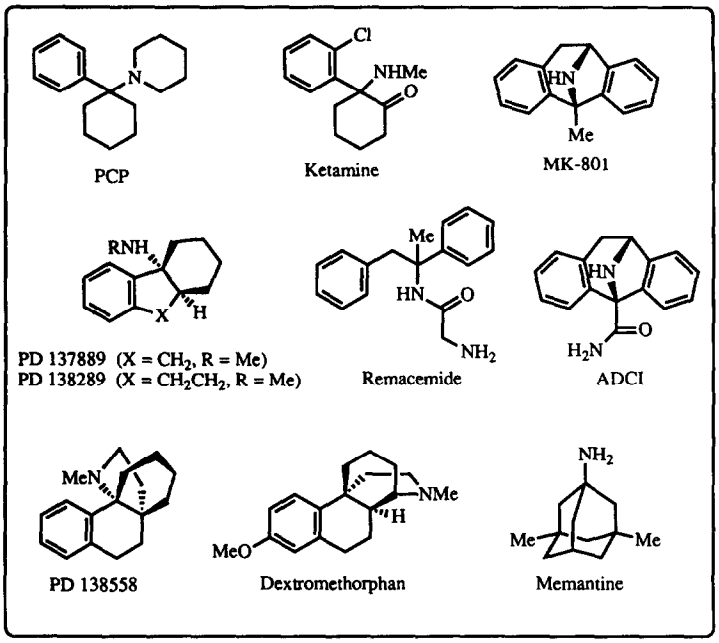

Fig. 7. Noncompetitive NMDA antagonists (ion channel blockers).

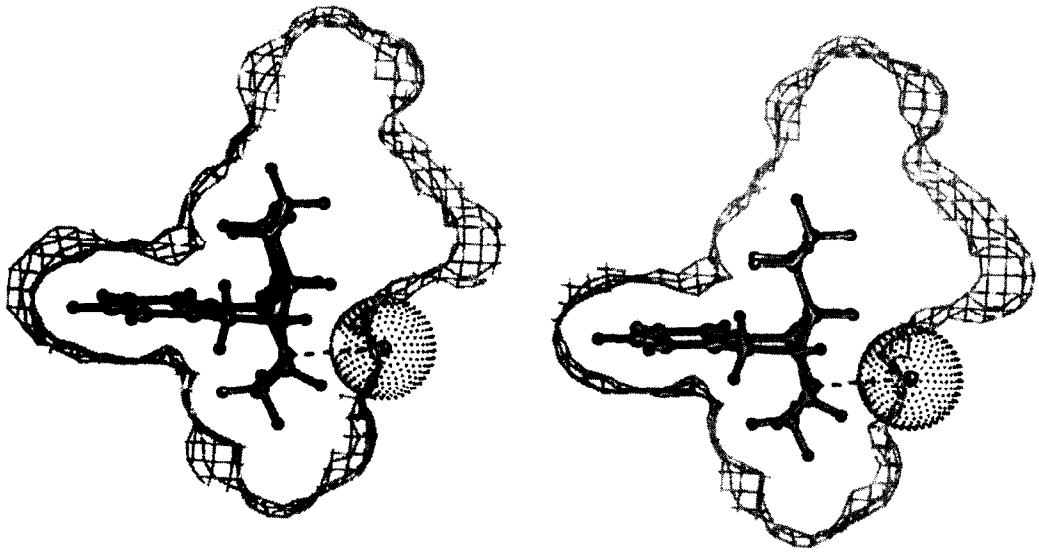

Fig. 8. Stereoview of the fit of the rigid tetracycle, PD 138558, in the PCP site pharmacophore model with cutaway of volume map.

changing the degree of saturation. In this series of compounds the PCP site discriminates for a single enantiomer, but similar to MK-801, the other enantiomer shows about 10-fold reduction in receptor affinity. Although we examined those compounds with high affinity for the PCP site most thoroughly, compounds are available that may have PCP site kinetics related to the fast dissociation mentioned for ADCI. As a class, these compounds can be simply viewed as rigid analogs of PCP wherein either a methylene or ethylene group is used to link the C-2 position of the cyclohexyl ring with the ortho position of the aromatic ring. Heteroatoms, such as S or O, can be included in this bridge unit as well. Unlike PCP where the piperidine ring satisfies both the amine and a steric requirement, in these compounds

an additional hydrophobic component is not needed to fit the receptor and smaller substitutions on the angular amine provide more potent compounds: NHMe > NH_2 > NHEt ≫ NC_5H_{10}. Extrapolation from literature data where the meta-hydroxy derivative of PCP had higher affinity than PCP itself suggested that substitution at C-6 or C-7 on the aromatic ring could identify a second hydrogen bonding site. Modeling predicted correctly that substitution at C-6 would lead to higher potency compounds and that substitution at C-7 would diminish activity. Unfortunately, toxicity was increased in compounds with C-6 hydroxy (or methoxy) substitution in the fluorenamines and phenanthrenamines, although it is uncertain that the toxicity is directly related to high affinity for the PCP site. The molecular modeling approach led to the synthesis of two tetracyclic series wherein the amine is more conformationally immobile and further constraints were placed on the limits of volume tolerance. One of these compounds, PD 138558, shown for illustrative purposes in the (*R*)-configuration in Fig. 7, fits snugly into the ion channel pore of the PCP site pharmacophore model (Fig. 8) [74].

Interestingly, other drugs have been identified that have affinity for the PCP site and are being evaluated in the clinic for their neuroprotective properties. Among these are the cough suppressant dextromethorphan [75], and the anti-Parkinson drug memantine [76, 77]. Dextromethorphan does not show the same dependence on glutamate and magnesium as typical PCP site ligands such as MK-801, and may be binding to a low affinity state of the PCP site. Dextromethorphan has also been shown to protect against hypoxic ischemia. Memantine acts as an NMDA channel blocker and thus protects neurons during excitotoxic conditions. Memantine may have secondary effects on reuptake or release of glutamate to maintain excitatory tone. Both of these compounds may have the rapid dissociation kinetics from the receptor that differ significantly from the slow on/off rate of MK-801 and other potent PCP ligands. This difference in kinetic profile is used to explain the positive neuroprotective effects of NMDA receptor modulation and the reduced side-effect profile. However, the interrelationships of the receptor kinetics, side-effect profile, and neuroprotective effects of compounds such as ADCI, remacemide, dextromethorphan and memantine are not fully understood. They are potentially useful NMDA antagonists and are tools with which to study more subtle interactions than straight channel block of the NMDA receptor complex.

Zinc and polyamine sites

The NMDA receptor also has binding sites for zinc and polyamines. The major effect of zinc is to reduce the frequency at which the NMDA receptor channels open, and thus reduce mean channel open time. The zinc binding site is on the extracellular side of the membrane, and is not within the membrane electric field. Zinc provides essentially a voltage-independent block of NMDA receptor responses, and thus is strikingly different than the action of Mg^{2+}. However, zinc also binds to the Mg^{2+} site, but with much lower affinity than at the extracellular zinc binding site [78]. Synaptic vesicles in the hippocampal mossy fiber system contain high concentrations of zinc and it has been suggested that during synaptic transmission zinc may be released into the extracellular space. Zinc is also present in many other areas of the CNS, but its physiological function in the hippocampus, as well as at other sites, has yet to be clearly identified. It has been suggested that imipramine and other tricyclic antidepressants modulate the activity of glutamate receptors in a manner similar to zinc [79]. But it remains unclear whether they have actions similar to dextromethorphan and block the ion channel pore, or if they indeed act at the zinc site [75].

The polyamines spermine and spermidine may act as allosteric regulators of the action of glycine. Consistent with an allosteric effect on the action of glycine, Mayer *et al.* [9] found that in embryonic hippocampal neurons spermine reduces glycine-sensitive desensitization in response to NMDA, effectively enhancing the receptor signal. However, the stimulatory action of spermine and spermidine on responses to NMDA through the glycine site appears to be variable, perhaps due to a second voltage-dependent interaction at the vestibule of the NMDA ion channel pore [80]. Clearly, the mechanism of action of polyamines is far from resolved, and their physiological action in the intact CNS has yet to be addressed. The phenylethanolamines, ifenprodil and SL 82.0715 (currently in clinical trials for stroke, Fig. 9), are reported to be potent antagonists of the polyamine modulatory site of the NMDA receptor [81]. Others have reported that the pharmacology of action of drugs such as ifenprodil is complex, and may involve activity at several sites [82, 83]. Recent structural studies on the phenylethanolamine structure have demonstrated that the greatest NMDA potency resides in the levorotatory threo enantiomer of ifenprodil. α_1-Adrenergic effects were partially separated from NMDA activity in this series, and a minimum pharmacophore was identified that may be embellished to enhance NMDA antagonist activity and selectivity [84].

Perspective

The potential therapeutic activity for drugs acting at NMDA receptors in a variety of disease states is enormous. NMDA receptor antagonists are undergoing clinical investigation for the treatment of epilepsy and stroke. Indications for neurodegenerative diseases, such as Parkinsonism and Alzheimer's disease, may result if the appropriate agent is discovered. Despite widespread evidence of neuroprotection in ischemia models, questions remain whether an NMDA receptor antagonist will prove to be effective as a single clinical entity in stroke. It is quite likely, however, that the profound neuroprotective effects of this class of compound will be extraordinarily valuable as an adjunctive therapy for the acute treatment of stroke or cerebral trauma. The first step in developing agents that modulate the function of the NMDA receptor is to grasp the complex physiology and pharmacology associated with the receptor. Although significant

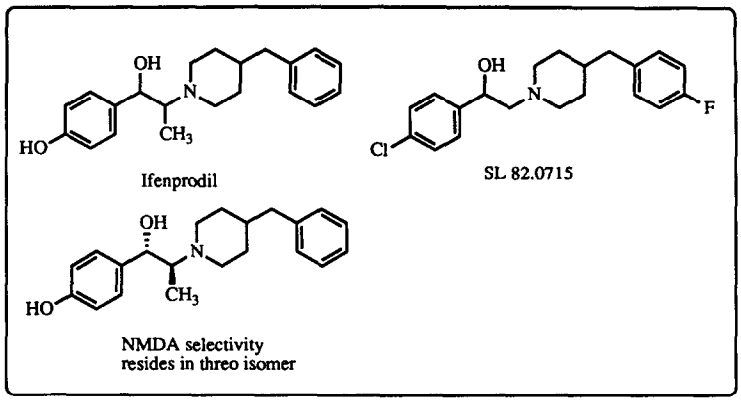

Fig. 9. Phenethanolamines (polyamine antagonists?).

progress has been made in understanding the molecular mechanisms by which NMDA receptor activity is regulated, the intricacies of receptor regulation and questions evolving around the molecular biology, receptor subtypes and distribution of those subtypes continues to provide fertile ground for investigation. Solving these puzzles will affect our ability to develop and use drugs with a novel mechanism of action.

Multiple targets for drug action exist on the NMDA receptor complex and include the ion channel pore, both the glutamate and glycine binding sites, and the modulatory sites for Mg^{2+}, Zn^{2+}, and polyamines. Molecular modeling of the PCP site, the competitive antagonist site and the glycine site have provided tools to aid in the design of novel agents, and have provided templates to understand the molecular interactions of the endogenous ligands with their receptors. Multiple criteria, including appropriate physicochemical properties and receptor fit, must be met when targeting an agent for use in the CNS. The PCP site ligands generally are able to access the CNS easily, but present clear evidence that behavioral side effects may accompany the abrupt blockade of the NMDA receptor. The competitive antagonists have been shown to have difficulty crossing the blood–brain barrier due to their highly ionic character, and yet have demonstrated profound anticonvulsant and neuroprotective effects *in vivo*. Recent advances in this area suggest that compounds can be designed to take advantage of amino acid transport systems or prodrug approaches to help deliver these powerful agents to the CNS. *In vivo* studies with glycine site antagonists have intimated that agents of this type will be able to modulate NMDA receptor activity without notorious behavioral side effects. Although their mechanism of action at the allosteric polyamine site on the NMDA receptor is controversial, the same can be said for agents such as ifenprodil. They have demonstrated neuroprotection in several *in vivo* models with a limited side-effect profile. Whichever type of NMDA antagonist or modulator is selected for development, it will be necessary to obtain proof of clinical effect whilst minimizing its effect on normal physiological events associated with receptor function. This is one of the greater challenges of the 1990s, the decade of the brain.

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