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Low affinity channel blocking (uncompetitive) NMDA receptor antagonists as therapeutic agents – toward an understanding of their favorable tolerability

Review Article

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Summary. Studies in experimental models have suggested that NMDA receptor antagonists may have utility in the treatment of a wide variety of neurological and psychiatric disorders. However, clinical trials have not been encouraging largely because the antagonists evaluated to date have exhibited unacceptable neurobehavioral side effects. In animals, therapeutic doses of some low-affinity channel blocking (uncompetitive) NMDA receptor antagonists are associated with less gross neurological impairment and behavioral toxicity than other types of NMDA receptor antagonists. Favorable clinical experiences with several such agents has bolstered confidence in the neurotherapeutic potential of low affinity NMDA antagonists. This article reviews current research attempting to explain the improved tolerability of such antagonists. While no single mechanism appears to account for the reduced toxicity of such agents, kinetic properties, particularly rapid blocking rate, seem to be of key importance. Other factors include partial trapping, reduced agonist-independent (closed channel) block, subunit selectivity (particularly for receptors that do not contain the NR2A subunit), combined block at allosteric (voltage-independent) sites, and synergistic therapeutic effects produced by additional actions at receptor targets apart from NMDA receptors (e.g., weak positive allosteric modulation of $GABA_A$ receptors or state-dependent Na^+ channel block).

Keywords: Amino acids – NMDA-Receptors antagonists

Introduction

Low-affinity uncompetitive NMDA receptor antagonists are a group of structurally diverse compounds that inhibit NMDA receptor-mediated

responses by binding to the cation channel of the NMDA receptor complex (Rogawski, 1993; Harris and Murray, 1996; Parsons et al., 1999b). In comparison with high-affinity ("dissociative anesthetic-like") channel blocking NMDA receptor antagonists, low-affinity uncompetitive antagonists have a far reduced tendency to cause neurobehavioral side effects in animals and man. However, like other NMDA receptor antagonists, they are effective in a wide variety of animal models of neurological disease, including epilepsy, neuropathic pain, Parkinson's disease, spasticity, tardive dyskinesia, and a number of conditions that may be associated with glutamate-induced excitotoxicity including stroke, traumatic brain injury, Huntington's disease, AIDS dementia, and glaucomatous optic neuropathy (Parsons et al., 1998). In addition, low affinity uncompetitive NMDA receptor antagonists may have utility in the treatment of psychiatric conditions including substance abuse, anxiety and depression. Several drugs for which there is considerable human experience are now recognized to act as low affinity NMDA receptor antagonists at clinically relevant doses, including memantine, dextromethorphan, dextrorphan, budipine, orphenadrine, felbamate, and the investigational compounds remacemide, AR-R15896AR and ADCI. There is thus good reason to believe that this class of agent is less likely to exhibit the side effects that have hindered the development of other types of NMDA receptor antagonists (Muir and Lees, 1995; Herrling, 1997).

Recognition of the unique tolerability of low affinity NMDA receptor antagonists

In the early 1980's, as the role of NMDA receptors in seizures and excitotoxicity was being defined, it was recognized that the dissociative anesthetics ketamine and phencyclidine (PCP) were potent and selective NMDA receptor antagonists (Anis et al., 1983). Shortly thereafter, dizocilpine (MK-801), a dibenzocycloalkenimine with behavioral properties similar to those of dissociative anesthetics, was also found to potently block NMDA receptor responses via a channel blocking site common to that of PCP and ketamine (Wong et al., 1986; Kloog et al., 1988ab; Huettner and Bean, 1988). PCP, ketamine and dizocilpine were found to be highly effective as anticonvulsant and neuroprotective agents. However, the possibility of using dissociative anesthetic-like drugs as therapeutic agents was tempered by their high neurobehavioral toxicity (White and Ryan, 1996; Lees, 1997; Abi-Saab et al., 1998). The concept that low affinity NMDA receptor antagonists may be better tolerable originated with studies on the anticonvulsant activity of a series of phenycycloalkylamines structurally related to PCP. Several of the analogs were observed to be protective in anticonvulsant screening models but to have a dramatically reduced propensity to produce neurological impairment (Rogawski et al., 1988; Thurkauf et al., 1990; Blake et al., 1992).

In a similar manner, ADCI {(±)-5-aminocarbonyl-10, 11-dihydro-5H-dibenzo[*a,d*]cyclohepten-5,10-imine} was identified as a dizocilpine analog with anticonvulsant activity and low toxicity. Extensive preclinical studies

were carried out with ADCI leading to dose ranging studies with the more active (+)-enantiomer SGB-017 (WAY-143017) in human volunteers (Bialer et al., 1999). ADCI has a broad spectrum of anticonvulsant activity in animal seizure models (Rogawski et al., 1991). In addition, ADCI is highly effective against ethanol withdrawal seizures (Grant et al., 1992) and cocaine seizures (Seidleck et al., 1994). Like other NMDA receptor antagonists, ADCI retards the development of amygdaloid kindled seizures, indicating that it has antiepileptogenic as well as anticonvulsant properties. ADCI also antagonizes neuroleptic-induced catalepsy suggesting that it could have utility in Parkinson's disease (Bubser et al., 1997). However, compared to its parent dizocilpine and other dissociative anesthetic-like agents, ADCI has dramatically enhanced tolerability in animals and man. Thus, unlike dizocilpine and similar agents, ADCI does not produce hyperlocomotion or stereotypies in rats, an observation that has been interpreted as suggesting that the compound may lack psychotomimetic activity (Bubser et al., 1997). In addition, ADCI failed to substitute for dizocilpine in rats trained to discriminate dizocilpine from saline in an operant procedure (Grant et al., 1977). At doses 10-fold greater than the ED_{50} for protection in the maximal electroshock seizure test, ADCI did not produce deficits in passive avoidance behavior or in the Morris water maze, indicating that the drug lacks amnestic effects (Rajachandran et al., 1993). Finally, ADCI does not cause neuronal vacuolization and necrosis seen with other NMDA receptor antagonists (Fix et al., 1993; J. W. Olney, personal communication).

Voltage-clamp studies in cultured hippocampal neurons demonstrated that ADCI blocks NMDA receptor responses in a use-dependent and voltage-dependent fashion consistent with its action as an uncompetitive (channel blocking) NMDA receptor antagonist (Rogawski et al., 1991). Recent experiments with recombinant NMDA receptors expressed in HEK 293 cells provide further support for a channel blocking mechanism (Harty and Rogawski, 1997).

In Phase I single-dose studies orally administered (+)-ADCI (SGB-017) was generally well tolerated at doses up to 200 mg. The peak plasma concentration (C_{max}) obtained with a 200 mg dose was 2.6 μg/ml (6.4 μM)]. At doses of 300 to 480 mg [C_{max}, 4.9 μg/ml (12 μM)] mild somnolence, paresthesias and visual symptoms were observed in some subjects. At doses of 560 mg and above ($C_{max} > 5.3$ μg/ml), all subjects experienced dizziness and some reported visual symptoms, abnormal thinking and euphoria. The threshold plasma concentration for anticonvulsant activity in the mouse maximal electroshock seizure test is 3 to 5 μg/ml (Rogawski et al., 1995). Consequently, anticonvulsant plasma concentrations are associated with only mild side effects in humans. Although the cerebrospinal fluid and brain concentrations corresponding with these plasma concentrations are not known, it is interesting to note that the *in vitro* IC_{50} for block of recombinant NMDA receptors composed of the NR1a/NR2B subunit is in the same concentration range as those associated with only mild adverse symptoms in humans (7.2 μM; T. P. Harty and M. A. Rogawski, unpublished). Overall, the results with ADCI indicate that a low affinity uncompetitive NMDA receptor

antagonist can be administered to human subjects at doses that block NMDA receptors (and based upon animal studies would likely have therapeutic activity) with only minimal central nervous system toxicity.

During the past decade a wide variety other low affinity channel blocking NMDA receptor antagonists with favorable toxicity characteristics have been identified. For several of these compounds, notably memantine (Parsons et al., 1999b) and remacemide (which is *des*-glycinated *in vivo* to form the moderate affinity channel blocking agent ARL 12495AA; Heyn et al., 1994; Subramaniam et al., 1996), there is extensive clinical experience verifying that the drugs are well tolerated in human subjects. The basis for the favorable toxicity profiles has been a matter of considerable speculation. In the remainder of this article, I consider several hypotheses under investigation. Additional discussion will be found in the reviews by Rogawski (1993), Harris and Murray (1996), Parsons et al. (1999) and the companion articles by Parsons et al. (2000) and Lanthorn et al. (2000).

Use-dependence, voltage-dependence and trapping

A fundamental characteristic of channel blocking NMDA receptor antagonists is that they are "use-dependent" which means that they bind and block agonist gated open channels more rapidly than closed channels. In most cases, channel blockers are cationic (positively charged) at physiological pH. These cationic blockers are thought to bind to the M2 segment of the receptor (a membrane embedded loop) which is believed to be a major component of the lining and selectivity filter of the channel pore (Feffer-Montiel et al., 1998). This region of the channel is within the transmembrane electric field so that the inhibitory activity of such cationic blockers occurs in a voltage-dependent fashion. Block is reduced at positive membrane potentials that tend to neutralize the electrostatic interaction between the blocking drug and its acceptor within the channel pore. Some use-dependent blockers exhibit the property of "trapping" in which agonist dissociation and channel closure can occur with the antagonist still bound within the channel.

It has often been suggested that use-dependence is a desirable property that would enhance the therapeutic utility and safety profile of an NMDA receptor antagonist since NMDA receptors would only be blocked when necessary, for example, during an epileptic seizure or following brain ischemia or trauma (Kemp et al., 1987; Harris and Murray, 1996). Recent evidence in support of this concept comes from studies with a series of permanently charged benzo[*b*]quinolizinium cations that are potent use-dependent NMDA receptor antagonists (Early et al., 1995; Ault et al., 1995). Conventional channel blocking NMDA receptor antagonists are in general highly lipophilic. Thus, while their blocking action is greatly accelerated by channel opening, they do – given sufficient time – have the ability to block closed channels (Javitt and Zukin, 1989). This presumably occurs because such lipophilic agents are able to access their channel blocking site through an alternative hydrophobic pathway (i.e., through the membrane instead

of via the water filled pore). Several of the benzo[*b*]quinolizinium cations, including the prototypical analog WIN 63,480, have open channel blocking potencies that are similar to or even greater than that of dizocilpine. However, because of their charge, these novel antagonists are largely unable to access closed channels. Interestingly, these compounds, in contrast to dizocilpine, did not exhibit dissociative anesthetic-like behavioral side effects (Subramanyam et al., 1995). The basis for the improved toxicity profile is not well understood. Nevertheless, the results do suggest that use-dependence could contribute to reduced behavioral toxicity, perhaps when combined with additional favorable characteristics. The extent to which reduced closed channel access is responsible for the enhanced tolerability of other less behaviorally toxic low affinity NMDA receptor antagonists has not yet been defined.

Reduced binding affinity

Reduced affinity is an obvious characteristic of the less toxic channel blocking compounds that distinguishes them from many of the more behaviorally toxic dissociative anesthetic-like agents. Thus, dissociative anesthetic-like compounds, such as dizocilpine, PCP and aptiganel (CNS 1102) have blocking affinities below 100 nM whereas low affinity channel blockers have affinities that are typically greater than 500 nM (Fig. 1). At equieffective concentrations, lower affinity antagonists would exhibit faster rates of block and unblock. I therefore proposed that the faster effective blocking rates associated with the low affinity antagonists could contribute to their lower toxicity (Rogawski, 1993). However, reduced binding affinity alone cannot completely explain the lower toxicity since there is an imperfect correlation between binding affinity and therapeutic index (Parsons et al., 1995; Fig. 1). This lack of correlation is particularly striking for ketamine which is well recognized to produce profound neurobehavioral toxicity (Abi-Saab et al., 1998; Enarson et al., 1999) yet has a blocking affinity in the range associated with less toxic low affinity channel blocking agents (see e.g., Mealing et al., 1999). Properties other than low affinity would therefore appear to contribute to the more favorable toxicity characteristics of these agents (Table 1). Use-dependence, reduced closed channel block and rapid association kinetics (related to low affinity) have already been discussed. Additional features that have been proposed are rapid intrinsic association kinetics, rapid dissociation kinetics, NMDA receptor subunit specificity, partial trapping, multiple sites of block, and multiple sites of action on targets other than NMDA receptors.

Rapid intrinsic association kinetics

Low affinity channel blockers generally have more rapid effective blocking rates because higher concentrations are required to exert similar degrees of fractional block. These higher concentrations, by the law of mass action, result

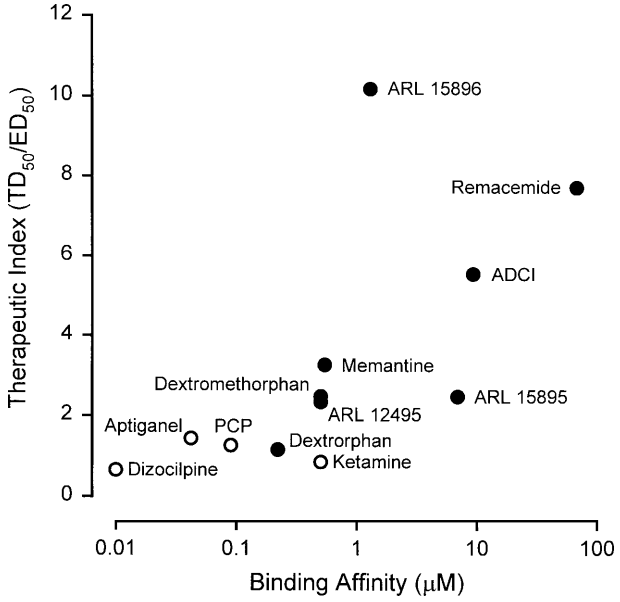

Fig. 1. Relationship between binding affinities (K_i or IC_{50}) of uncompetitive NMDA receptor antagonists and their therapeutic ratios (TD_{50}/ED_{50}), a measure of relative neurological toxicity. Binding affinities are based upon displacement of [^{3}H]dizocilpine. TD_{50} is the dose estimated to produce neurological impairment in 50% of mice. ED_{50} is the dose estimated to protect 50% of mice in the maximal electroshock seizure test. *In vivo* testing utilized oral drug administration except for PCP, dextrorphan, ketamine, dextromethorphan and ADCI which were given intraperitoneally. Data are adapted from Table 1 in Harris and Murray (1996). Open circles indicate dissociative anesthetic-like compounds that have had poor tolerability in human trials

in faster association rates and, assuming the dissociation rates are similar, a correspondingly faster approach to equilibrium block. However, there can be wide variability in the first order forward (association) rate constants (k_1). Compounds with faster intrinsic association rate constants would more rapidly achieve equilibrium block apart from the concentration effect. For example, memantine and ketamine have similar blocking potencies (that is, they have similar equilibrium affinities) yet memantine's forward rate constant (k_1) is ~6-fold greater than that of ketamine (see, Mealing et al., 1999) and possibly as much as ~25-fold greater than that of PCP (Parsons et al., 1995). The reverse (dissociation) rate constant (k_{-1}) of memantine is also correspondingly faster than that of ketamine, thus accounting for the similar equilibrium affinities ($K_d = k_{-1}/k_1$) of the two blockers. The rate of approach to equilibrium is given by the sum of the association and dissociation rates ($k_{eff} = k_1[\text{drug}] + k_{-1}$). Therefore, the effective blocking rate for memantine would be substantially greater than for ketamine even though the two drugs have similar equilibrium affinities, supporting the idea that faster association rate is related to improved tolerability.

Additional evidence in favor of this concept comes from recent studies with a series of novel adamantane derivatives that, like the adamantane

Table 1. Characteristics of low affinity NMDA receptor antagonists that may confer improved tolerability

Property	Comment	Examples
Rapid association rate (related to low affinity)	General feature of many well tolerated uncompetitive antagonists	All low affinity antagonists
Rapid association rate (intrinsic)	Relevant for only selected antagonists	Memantine, IEM-1754
Rapid dissociation rate	Accounts for low toxicity in relation to dizocilpine; otherwise limited importance	Most uncompetitive antagonists other than dizocilpine
Reduced (partial) trapping	Differences in extent of partial trapping small; requires further validation	AR-R15896AR
Reduced agonist-independent (closed channel) block	Requires further validation	WIN 63480
Subunit selectivity (preferential block of NR2B or possibly NR2C)	Differences small but NR2 selective antagonists well recognized to have low toxicity	Felbamate, ADCI
Voltage-independent (allosteric) block	Importance unknown	Remacemide, AR-R15896AR, ADCI
Multiple receptor targets	Common feature of many neurotherapeutic agents	Felbamate, ADCI, remacemide, ARL 12495AA

memantine, act as channel blocking NMDA receptor antagonists (Antonov et al., 1995). One of the analogs (IEM-1754) was found to be 4.5-fold more potent than dizocilpine as an anticonvulsant, yet at equieffective doses failed to produce the behavioral toxicity observed with dizocilpine. The blocking kinetics of IEM-1754 were striking in that this analog had a nearly 10-fold greater microscopic association rate constant than dizocilpine. IEM-1754 also had a much faster dissociation rate and consequently a far lower equilibrium affinity (K_d, 2.8μM). Other adamantane derivatives which had slower association rates but similar dissociation rates as IEM-1754 had relatively low anticonvulsant potencies similar to that of dizocilpine. Thus, the markedly greater blocking rate of IEM-1754 seemed to contribute to its greater anticonvulsant potency. However, since it has an equilibrium affinity less than that of dizocilpine (K_d, ~10nM), at the lower doses that are required for seizure protection by IEM-1754, there would be less tonic block of NMDA receptor and lower toxicity. These results provide a striking example of how kinetic properties – more specifically faster association rate – can result in dramatically improved tolerability.

Rapid dissociation kinetics and voltage-dependence

Is dissociation rate also a factor in the tolerability of uncompetitive antagonists? Dizocilpine is an example of an uncompetitive antagonist that dissociates very slowly from open channels (time constant >3 min *versus* ~3 s for memantine; Parsons et al., 1995; Mealing et al., 1999) and is also nearly fully trapped in closed channels (Blanpied et al., 1997). Therefore, during ordinary ongoing synaptic activation, NMDA receptors would slowly accumulate dizocilpine, resulting in near complete block that would be maintained during synaptic depolarization. In contrast, for antagonists with faster dissociation rates, there can be relief of block during synaptic depolarization. It has been suggested that the partial maintenance of ongoing NMDA receptor-mediated synaptic activity permitted under these conditions is an important factor that accounts for reduced side effects (Parsons et al., 1999a). The high toxicity of dizocilpine may indeed relate to the accumulation of block and the virtual shut down of all NMDA receptor-mediated synaptic transmission. However, dissociation rate *per se* does not provide a basis to identify blockers with low neurobehavioral toxicity. For example, the dissociation rate constant for memantine is only modestly faster than that of ketamine (Mealing et al., 1999). In addition, while remacemide has a high dissociation rate constant ($2.6\,s^{-1}$ at -60 mV; Subramaniam et al., 1996), the dissociation rate constant of its active *des*-glycine metabolite ($0.047\,s^{-1}$) is slower than that of ketamine and in the same range as PCP (Parsons et al., 1995). Moreover, in experiments with recombinant NMDA receptors, the dissociation rate of the low toxicity antagonist ADCI ($0.10\,s^{-1}$ for NR1a/NR2B subunits) was also in the same range as that for ketamine ($0.19\,s^{-1}$).

Rapid channel block can, at least theoretically, produce an additional effect that may bear on the clinical utility of uncompetitive antagonists. NMDA receptor antagonists are well recognized to inhibit many forms of synaptic plasticity and to have detrimental effects on learning. However, Mg^{2+}, an endogenous low affinity channel blocker with rapid kinetics, is required for NMDA receptor-dependent synaptic plasticity, possibly because it blocks tonic low level NMDA receptor activation but unblocks during relevant synaptic activation (Coan et al., 1989). Similarly, use-dependent blockers with appropriate kinetic properties can under some circumstances actually enhance synaptic plasticity (Frankiewicz and Parsons, 1999). Thus, depending upon the details of its kinetic behavior, a channel blocking NMDA receptor antagonist can produce profound behavioral toxicity or, alternatively, may have beneficial behavioral effects.

The depolarization-induced relief of block that occurs with many channel blocking NMDA receptor antagonists might at first glance appear to be undesirable since block would be reduced during situations of excessive NMDA receptor activation, just when such block is needed. However, since depolarization speeds relief of block, the voltage-dependence is essential to the unblocking that allows ongoing synaptic activity to be maintained, thus reducing toxicity. In addition, voltage-dependence is critical to the ability

of low affinity antagonists to enhance synaptic plasticity (Frankiewicz and Parsons, 1999). On balance, some degree of voltage-dependence may therefore be a favorable characteristic. However, as discussed later, some low affinity uncompetitive NMDA receptor antagonists exert a degree of block at additional voltage-independent blocking sites. The portion of tonic block exerted at such sites might be beneficial therapeutically as it would not be overcome during pathological depolarization.

NMDA receptor subunit selectivity

Several lines of converging evidence now suggest that selective blockade of NMDA receptors containing subunits other than NR2A may confer lower toxicity. Thus, in voltage-clamp recordings from HEK 293 cells expressing recombinant NMDA receptor subunit combinations, ADCI was found to have a modestly greater blocking potency for NMDA receptors composed of the NR1a and NR2B subunits (IC_{50}, 7.2μM) than those containing NR1a/NR2A (IC_{50}, 13.9μM) or NR1a/NR2C (IC_{50}, 27.1μM) (T. P. Harty and M. A. Rogawski, unpublished). This contrasts with ketamine which has similar potency as an antagonist of NR1a/NR2A (IC_{50}, 1.3μM) and NR1a/NR2B (IC_{50}, 1.5μM) receptors and is modestly less potent at NR1a/NR2C (IC_{50}, 3.9μM). PCP and dizocilpine are also equieffective as antagonists of NR2A (ε1) and NR2B (ε2) containing receptors and may be somewhat less potent at NR2C (ε2) and NR2D (ε2) (Yamakura et al., 1993; Bresink et al., 1996; Avenet et al., 1997; Monaghan and Larsen, 1997). Similarly, we have observed that the well-tolerated anticonvulsant drug felbamate blocks NMDA receptors composed of the NR1a and NR2B subunits 3- to 4-fold more potently than those composed of NR1a/NR2A or NR1a/NR2C subunits (Harty and Rogawski, 2000; see also, Kleckner et al., 1999). The precise mechanism whereby felbamate blocks NMDA receptors is still incompletely understood (Subramaniam et al., 1995). Nevertheless, felbamate inhibition of NMDA receptor currents does exhibit some biophysical characteristics similar to that of channel blockers although it is a neutral compound and its blocking action is only modestly voltage-dependent.

Other low toxicity channel blocking NMDA receptor antagonists also select against NMDA receptors containing the NR2A subunit (i.e., more potently block NMDA receptors containing NR2B or NR2C in contrast to NR2A), including memantine which preferentially blocks NMDA receptors containing the NR2C and NR2D subunits (Bresink et al., 1996; Parsons et al., 1999b) and AR-R15896AR (a low behaviorally toxic pyridine analog of remacemide's active *des*-glyine metabolite, formerly referred to as ARL 15896AR, that is in clinical development for stroke; Hudzik and Palmer, 1995; Palmer et al., 1999) which preferentially blocks NR2C-containing receptors (Monaghan and Larsen, 1997).

Overall, the selectivity differences among these various compounds is small and the extent to which discrimination among subunit combinations can confer improved toxicity characteristics is still a matter of some speculation. Nevertheless, it is now well recognized that NR2B selective antagonists have

improved tolerability (Chenard and Menniti, 1999), so the NR2B selectivity of certain of the low affinity antagonists cannot be discounted. Moreover, in the adult central nervous system, NR2B and NR2C subunits are less widely distributed than is the ubiquitous NR2A subunit (Mori and Mishina, 1995). The NR2B subunit is expressed in forebrain areas that are vulnerable to ischemic injury and may participate in seizure generation and spread. In addition, NR2B subunits are widely expressed in early development prior to becoming restricted to the forebrain at later developmental stages. This may, in part, underlie the utility of the modestly NR2B-selective antagonist felbamate in the Lennox-Gastaut syndrome, a severe seizure disorder affecting the immature brain (Anon., 1993).

Partial trapping

The concept of partial trapping was originally developed by Blanpied et al. (1997) based upon their observation that a fraction of memantine blocked channels become unblocked even in the absence of agonist (i.e., the blocker can escape from closed channels). In contrast, closed PCP or dizocilpine blocked channels do not release antagonist. Partial trapping guarantees that some channels release blocker between synaptic responses providing a basis for Mealing et al. (1999) to propose that closed channel egress could contribute to the lower toxicity of channel blocking NMDA receptor antagonists with more favorable toxicity profiles. Thus, AR-R15896AR and memantine exhibited less trapping than ketamine. The basis for closed channel egress in partial trapping is not well understood, but does not appear to be related to lipophilicity (Lanthorn et al., 2000). Partial trapping could be a contributing factor in the favorable toxicity profiles of memantine, AR-R15896AR and other low affinity uncompetitive NMDA receptor antagonists. Nevertheless, differences in the degree of untrapping between ketamine and memantine are relatively modest (14% versus 29%, respectively). Moreover, in preliminary experiments ADCI exhibited only 10 to 15% untrapping despite its very favorable toxicity profile (T. P. Harty and M. A. Rogawski, unpublished). Consequently, the extent to which closed channel egress accounts for the good tolerability of low affinity uncompetitive antagonists requires further investigation.

Multiple sites of action on NMDA receptors

An additional characteristic common to many low affinity uncompetitive NMDA receptor antagonists with favorable toxicity profiles is that they often can block NMDA receptors via actions at more than a single site. Thus, along with the conventional pore blocking action, a second component of block may be exerted at a site that is not use-dependent or voltage-dependent. For example, a majority (56%) of the block by remacemide (but not its *des*-glycine metabolite) is exerted via such a voltage-independent allosteric blocking site (Subramaniam, 1996). Similarly, a substantial portion of the block of AR-

R15896AR occurs in such a voltage-independent fashion (Mealing et al., 1997). For ADCI, ~25% of block of recombinant NMDA receptors (NR1a/NR2A and NR1a/NR2B) occurs at voltage-independent sites (T. P. Harty and M. A. Rogawski, unpublished). In other cases, block at largely voltage-independent sites can account for up to 70% of the NMDA receptor inhibition produced by the antagonist, as is the case for the arylalkylamine arthropod toxin philanthotoxin 343 (Donevan and Rogawski, 1996).

The voltage-independent blocking action, when it occurs, is either of the allosteric noncompetitive type (at sites outside the membrane electric field) or may occur through actions at the NMDA or glycine recognition sites. In the case of memantine, block at a lower affinity allosteric site while not use-dependent or trapped, does exhibit some voltage-dependence, albeit weaker than that at the use-dependent channel blocking site (Blanpied et al., 1997). Indeed, uncompetitive blockers can bind to various channel blocking sites, with different affinities and rates of association and dissociation (Grauert et al., 1998; Sobolevsky and Koshelev, 1998).

The consequences of block at a second site are not yet well elaborated. However, block at less voltage-dependent or non-voltage-dependent sites might allow some level of block to persist during strong depolarization, such as would occur with excessive NMDA receptor activation. In addition, the tonic block exerted at such sites would avoid the delays associated with use-dependence. Of course, the importance of block at various sites will depend on the kinetic properties of binding to each site and their relative equilibrium binding affinities.

Restrained drug actions

In traditional drug development, the goal is often to optimize efficacy and affinity at a single relevant target so as to produce maximal therapeutic activity and minimize side effects caused by actions at non-relevant targets. However, when the relevant target is critical to central nervous system function, as is the case for the NMDA receptor, an overly robust pharmacological action will result in unacceptable mechanism-related toxicities. In such situations, only agents that act in a "restrained" or modulatory fashion can be tolerated. Examples of such restrained drug actions include low affinity NMDA receptor antagonism, weak positive allosteric modulation of $GABA_A$ receptors and state-dependent Na^+ channel antagonism (Rogawski, 1996).

Multiple receptor targets

If restrained activity at a single site is desirable, enhanced therapeutic activity might be obtained by a drug that acted in a restrained fashion at more than one relevant site (Fig. 2). Actions at each site would contribute to common therapeutic effects, but the action at each site would be of insufficient magnitude to cause toxicity via the specific mechanism targeted (Rogawski, 1998). For example, in addition to its effects on NMDA receptors, felbamate

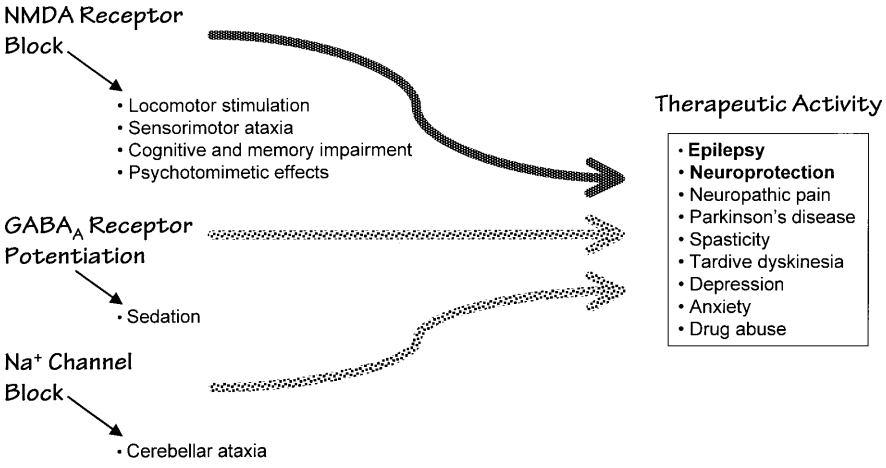

Fig. 2. Actions on multiple receptor targets may produce additive or synergistic therapeutic activities whereas toxicities at each target are distinct. Additional targets of some low affinity NMDA receptor antagonists (e.g. felbamate, ADCI, remacemide) include $GABA_A$ receptors (positive allosteric modulation) and voltage-dependent Na^+ channels (state-dependent block). Primary therapeutic activities believed to be associated with the three mechanisms are shown in bold; other possible therapeutic actions of low affinity NMDA receptor antagonists are also listed. Typical side effects expected from drug action at each site are indicated by thin arrows

is also a low efficacy positive allosteric modulator of $GABA_A$ receptors (Rho et al., 1994) and may also act as a state-dependent antagonist of voltage-sensitive Na^+ channels (Pisani et al., 1995). All of these actions, which occur at clinically relevant concentrations, could contribute to the drug's anticonvulsant activity and possibly also to its neuroprotective properties (Wasterlain et al., 1993; Wallis and Panizzon, 1995; Lyden, 1997). However, NMDA receptor antagonists, $GABA_A$ receptor modulators and state-dependent Na^+ channel blockers have different behavioral toxicities which may not be additive. Thus, a drug with multiple sites of action may achieve the desired therapeutic results at doses that are below the range that produce side effects.

Additional examples of drugs that simultaneously target NMDA receptors and voltage-dependent Na^+ channels are remacemide, its active *des*-glycine metabolite ARL 12495AA and ADCI (Wamil et al., 1996; White, 1994; S. S. Lin, personal communication). The blocking effects of ADCI have been studied on various Na^+ channel types, including human Na^+ channels in NT2 neurons. In common with other anticonvulsants that target Na^+ channels (Rogawski, 1996), ADCI blocked Na^+ channels in a highly voltage-dependent and use-dependent manner. In fact, although ADCI was a relatively weak inhibitor of Na^+ channels at $-80\,mV$ (IC_{50}, $335\,\mu M$), the potency increased dramatically with depolarization to $-60\,mV$ (IC_{50}, $23\,\mu M$) (S. S. Lin, personal communication). At these depolarized potentials, the potency for block of Na^+ channels approached that at which the drug blocks NMDA receptors. Thus, therapeutic concentrations associated with anticonvulsant activity

(Rogawski et al., 1995) would be expected to affect both NMDA receptors and Na^+ channels.

While it is not necessary for a drug that targets multiple sites to be a low affinity agent, at the higher effective concentrations used with such compounds more potentially relevant target sites would tend to be affected, giving greater opportunity for multiple actions. Of course, such promiscuous actions could also easily result in undesirable side effects. Indeed, very low affinity compounds (K_d, $>10\mu M$) may be more likely to have poor tolerability, possibly because of an increased propensity for such nonspecific actions (Harris and Murray, 1996; Parsons et al., 2000).

Conclusion

It is apparent that no single characteristic can fully account for the enhanced tolerability of all low affinity channel blocking NMDA receptor antagonists. Nevertheless, there are a variety properties can be identified that seem to be associated with low toxicity (Table 1). Kinetic factors are undoubtedly of key importance. Among these, rapid association rate related to low affinity is likely to be significant for many well tolerated uncompetitive antagonists. In addition, in specific cases, unusually rapid intrinsic association rate may play a role. Rapid dissociation rate only appears to be important to the extent that dissociation occurs sufficiently fast to avoid accumulation of block; modest differences are not associated with variations in tolerability. Studies demonstrating that improved tolerability is associated with a reduction in agonist-independent closed channel block are intriguing; however, the importance of this factor requires further validation. Subunit specificity is currently of major interest in the design of NMDA receptor antagonists. Whether the small differences in specificity seen among the low affinity channel blocking compounds are sufficient to account for differences in tolerability is currently a matter of speculation. Nevertheless, it does appear that agents with enhanced affinity for receptors containing the NR2B subunit do have improved tolerability. Finally, block at multiple sites on the NMDA receptor and at multiple targets apart from the NMDA receptor may also contribute to the therapeutic activity and improved tolerability of many low affinity uncompetitive NMDA receptor antagonists.

The disappointments attended to recent clinical trials of NMDA receptor antagonists have raised significant doubts about the viability of NMDA receptor blockade as a treatment strategy. Nevertheless, there is still an excellent scientific basis supporting the potential role of NMDA receptor antagonists in the treatment of diverse neurological and psychiatric disorders. Of the possible approaches to blocking NMDA receptors, low affinity uncompetitive NMDA receptor blockade is among the most likely to be successful. Indeed, the favorable human experience with several agents of this type provides a level of confidence not available with other types of antagonists. Knowing the characteristics that confer improved toxicity properties will allow the identification of the most promising compounds for further development.

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