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Memantine and the amino-alkyl-cyclohexane MRZ 2/579 are moderate affinity uncompetitive NMDA receptor antagonists – *in vitro* characterisation

Review Article

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Summary. There is general agreement that moderate affinity uncompetitive NMDA receptor antagonists combine good efficacy and tolerability in animal models of disturbances in glutamatergic transmission. There are several theories on which properties are important for this profile including 1, rapid access to the channel at the start of pathological overactivity 2, rapid, voltage-dependent relief of blockade during physiological synaptic activation and 3, partial untrapping. Merz has developed a series of novel uncompetitive NMDA receptor antagonists based on the cyclohexane structure. In cultured hippocampal neurones MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexane) shows similar blocking kinetics to memantine (K_{on} 10.7 * $10^4 M^{-1} sec^{-1}$, K_{off} $0.20 sec^{-1}$ at -70 mV) and binds at the same depth in the NMDA receptor channel ($\delta = 0.8$). The potency of MRZ 2/579 assessed as Kd $= K_{off}/K_{on} = 1.87 \mu M$ agrees well with the IC_{50} of $1.29 \mu M$ against steady-state currents in cultured hippocampal neurones (at -70 mV) and with the Ki in [^{3}H]-MK-801 binding of $0.65 \mu M$. MRZ 2/579 protected cultured cortical neurones against glutamate toxicity with an IC_{50} of $2.16 \mu M$ and was also effective in protecting hippocampal slices against hypoxia / hypoglycaemia-induced reduction of fEPSP amplitude in CA1 with an EC_{50} of $7.01 \mu M$. MRZ 2/579 has similar potency and bio-availability to memantine *in vivo* assessed using microdialysis, microiontophoresis and MES-induced seizures. Initial characterization in animal models provides strong support for the assumption that MRZ 2/579 could be a useful therapeutic in morphine/alcohol dependence, inhibition of morphine tolerance, chronic pain and as a neuroprotective agent.

Keywords: Amino acids – N-Methyl-D-aspartate – Uncompetitive antagonist – Memantine – MRZ 2/579

Introduction

Although N-methyl-D-aspartate (NMDA) receptor antagonists have therapeutic potential in a wide range of neurological and psychiatric disorders several high affinity compounds have been abandoned in the last few years due to serious CNS side effects and concern about negative effects seen in animal models such as PCP like psychotomimetic activity, memory impairment, ataxia / myorelaxation and neuronal toxicity in the cingulate / retrosplenial cortices in female rats (Danysz et al., 1995; Parsons et al., 1998a). The only class of compound that has been proven to be clinically effective at doses devoid of side effects are moderate to low affinity uncompetitive antagonists i.e. open channel blockers (Danysz et al., 1997; Parsons et al., 1999b). Amantadine, memantine, dextromethorphan, felbamate, budipine, mepyramine, desipramine, biperidine, orphenadrine and carbamazepine have been in the clinic for several years and are safe, well tolerated drugs which exert at least part of their effects via NMDA receptor channel blockade. Other agents such as remacemide, ARL-15896AR, HU-211, ADCI and NPS-1506 are at various stages of clinical development and seem to confirm the good tolerability of low to moderate affinity uncompetitive NMDA receptor antagonists.

There is general agreement that the good efficacy and tolerability of these agents is directly related to their low to moderate affinity and associated rapid blocking / unblocking kinetics (Chen et al., 1992; Parsons et al., 1993, 1995, 1999b; Rogawski, 1993; Kornhuber et al., 1995; Black et al., 1996; Blanpied et al., 1997; Danysz et al., 1997; Mealing et al., 1997). Offset kinetics are inversely related to affinity with lower affinity compounds showing the fastest relief of blockade upon removal of antagonist (Parsons et al., 1995). Although onset kinetics assessed as K_{on} values are not correlated with affinity, blocking kinetics at IC_{50} concentrations are also inversely related to affinity with lower affinity compounds showing the fastest onset of blockade at therapeutically effective concentrations (Parsons et al., 1995).

There are several hypotheses as to why such properties allow these compounds to differentiate between pathological and physiological activation of NMDA receptors and it is possible that multiple factors are of relevance (Lipton, 1993; Rogawski, 1993; Porter and Greenamyre, 1995; Blanpied et al., 1997; Parsons et al., 1999b). Moreover, it is important to stress that there seems to be an optimum range of affinity of between 0.5 to 10μM. High affinity compounds like phencyclidine (PCP), Cerestat (CNS-1102) and Dizocilpine ((+)MK-801) have very slow blocking kinetics and cause numerous side effects. Some very low affinity compounds also cause side effects that are probably related to lack of selectivity i.e. actions at other receptor channels. However, for some very low affinity compounds such as amantadine and ADCI these other actions can also be positive via e.g. additional synergistic actions at neuronal nicotinic receptors or voltage-activated Na^+ channels (Rogawski et al., 1991; Danysz et al., 1997).

Some authors have proposed that rapid receptor block by moderate affinity uncompetitive NMDA receptor antagonists is important for their

good therapeutic profile as anti-epileptics and in stroke as these compounds gain rapid access to the channel at the start of pathological overactivity and thereby terminate it's propagation (Chen et al., 1992; Rogawski, 1993). However, this factor alone cannot explain their therapeutic safety as receptors blocked during overactivity would then be unavailable for subsequent physiological activation. In other words, there must be a mechanism which allows subsequent relief of channel blockade under therapeutic conditions i.e. in the continuing presence of antagonist.

We were the first to propose that the rapid offset kinetics and the strong voltage-dependency of moderate to low affinity channel blockers is an important factor (Parsons et al., 1993, 1995, 1999b). This may underlie the ability of memantine to block tonic low level pathological activation of NMDA receptors by μM concentrations of glutamate and mild membrane depolarization in chronic neurodegenerative diseases but allow their physiological activation following synaptic release of mM concentrations of glutamate which causes transient, pronounced membrane depolarization. Indeed, memantine seems to act like a somewhat more potent surrogate for Mg^{2+} and, in contrast to (+)MK-801, therapeutically relevant concentrations of memantine reverse deficits in LTP in hippocampal slices due to increased synaptic "noise" following reduction of Mg^{2+} concentration (Frankiewicz and Parsons, 1999b see also Zajaczkowski et al., 1997).

Another recent theory to explain how these agents can leave the NMDA receptor channel in the absence of glutamate is partial trapping (Blanpied et al., 1997; Sobolevsky et al., 1998; Mealing et al., 1999). Thus, whilst high affinity compounds such as PCP and (+)MK-801 are completely trapped in the channel following removal of agonist i.e. show 100% non-sequential block, the proportion of channels blocked by low to moderate affinity antagonists decreases upon agonist removal. The degree of this partial trapping may also be related to affinity and unblocking kinetics as the proportion of channels releasing antagonist shows the following rank order amantadine > ARL-15896AR > memantine > ketamine > PCP = (+)MK-801. The mechanism underlying this partial trapping is still unclear as the simplest models of sequential block would predict the presence of tail currents upon agonist removal as seen for 9-aminoacridine (Benveniste and Mayer, 1995) i.e. channels would have to pass through a conducting open state to release the antagonist. However, the facts that partial trapping is unrelated to lipophylicity (Lanthorn et al., 1999), occurs in the continuous presence of antagonist (Parsons et al., 1999b) and does seem to depend on unblocking kinetics provides strong evidence against a lipophyllic, closed channel pathway for this effect.

It seems likely that most moderate to low affinity uncompetitive antagonists block at two or more different channel sites with different affinities and different depths. This is reflected in clear double exponential blocking and unblocking kinetics (Bresink et al., 1996; Frankiewicz et al., 1996; Sobolevsky and Koshelev, 1998; Spielmanns et al., 1999). It is also interesting to note that the magnitude of the faster component increases with depolarization (Bresink et al., 1996). It is tempting to speculate that the faster

component reflects binding to a more superficial channel site and that antagonist unbinding from this site underlies partial untrapping. If this were true, then one might predict that the phenomenon of partial untrapping would be more pronounced at depolarized potentials. This has not yet been tested, but if this theory turns out to be true then it would serve to partially unify the voltage-dependency and partial trapping hypotheses.

Another common characteristic for well tolerated low to moderate affinity uncompetitive NMDA receptor antagonists is their relatively weak effects at NR2A containing receptors. Most moderate affinity compounds characterized to date show highest binding affinity in the cerebellum, a region known to express high levels of NR2C receptors (Bresink et al., 1995; Porter and Greenamyre, 1995; Greene et al., 1996). We investigated this further by testing the effects of memantine and amantadine on NMDA receptor subtypes expressed in *Xenopus* oocytes (rat NR1a co-injected with NR2A, 2B, 2C or 2D). The IC_{50}s at −70 mV against glutamate 1 mM (glycine 10 μM) were 0.9, 0.4, 0.3 and 0.3 μM for memantine and 26, 18, 10 and 10 μM for amantadine (Spielmanns et al., 1998). The relatively weak (3 fold) subtype selectivity of memantine was increased (to 5 fold) under more physiological conditions i.e. at −30 mV in the presence of 1 mM Mg^{2+}: IC_{50}s of 10.3, 10.9, 2.0 and 1.9 μM at NR2A, 2B, 2C and 2D respectively. This is a reflection of both the weaker block of NR2C and NR2D receptors by Mg^{2+} and the similar voltage-dependency of memantine at all four receptor subunits (= 0.72–0.82). On the other hand, felbamate has recently been reported to be three times more potent at NR2B than NR2A or NR2C receptors (Harty and Rogawski, 1998; Kleckner et al., 1999). It is still not clear whether NMDA receptor subtype selectivity is an important factor for the therapeutic safety of these compounds because 1, the selectivity is a best very small and 2, it is unclear why more pronounced effects in the cerebellum for NR2C selective compounds should produce less side effect such as ataxia.

One aspect that has not yet been addressed is subtype selectivity for the eight splice variants of NR1 subunits (see Danysz and Parsons, 1998; Parsons et al., 1998a). These splice variants also show a heterogeneous distribution and temporal expression profile in the CNS and selective actions at specific receptors could also underlie therapeutic safety. Certainly, differences in the channel domain can have dramatic effects on the potency of some channel blockers whilst having much less effects on others. For example, recent data from our laboratory indicate that the potency of Mg^{2+} at NR1a(N598G) mutants expressed with native NR2A in *Xenopus* oocytes was reduced by over three orders of magnitude whereas the potency of memantine was similar to that seen in native NR1a/NR2A receptors (Spielmanns et al., 1998; Parsons et al., 1999c). The dramatic reduction in the potency of Mg^{2+} may be due to a change in the dimensions of the channel pore which is enough to allow the permeation of Mg^{2+} but not memantine. This would indicate that these agents do not bind to a specific deep recognition site in the channel but rather are trapped in a funnel like domain.

Mg^{2+} is known to gain access to the NMDA receptor channel from both the extra- and intracellular compartments (e.g. Kupper et al., 1998).

Memantine shows lysosomal accumulation which leads to intracellular concentrations (30 μM) much higher than therapeutic extracellular concentrations (1 μM) (Honegger et al., 1993). As such we were interested to know if memantine can also block the NMDA receptor channel from the intracellular compartment. Patch clamp recordings from *Xenopus* oocytes (pipette glutamate 1 mM, Mg^{2+} 1 mM) expressing NR1a/NR2A receptors revealed rectification at positive potentials in cell attached mode which disappeared after pulling an inside-out patch (Parsons et al., 1999c). Application of Mg^{2+} 1 mM to the intracellular side of the receptor reintroduced the rectification seen in cell attached mode and Mg^{2+} 5 mM produced much more pronounced rectification. In contrast, memantine 30 μM was completely unable to block the NMDA receptor from the intracellular compartment. As such, intracellular block of the NMDA receptor is not of significance for the therapeutic effects of memantine.

NMDA receptor antagonism by amino-alkyl-cyclohexanes

In view of the above mentioned evidence and considerable experience with memantine and amantadine, Merz has developed a series of novel uncompetitive NMDA receptor antagonists based on the cyclohexane structure (Parsons et al., 1999a). Nineteen of these amino-alkyl-cyclohexanes displaced [^{3}H](+)-MK-801 binding to rat cortical membranes with Kis between 0.65 and 12.7 μM and antagonized current responses of cultured hippocampal neurones to NMDA with a similar range of potencies (IC_{50}s of 1.3–21 μM, at −70 mV) in a use- and strongly voltage-dependent manner (= 0.55 to 0.87). In agreement with our previous data with well characterised uncompetitive NMDA receptor antagonists, the offset kinetics of NMDA receptor blockade by the amino-alkyl-cyclohexane derivatives was strongly correlated with their affinity at equilibrium (corr. coeff. 0.86, $p < 0.0001$). Most compounds were protective against glutamate toxicity in cultured cortical neurones (IC_{50}s of 0.53–19 μM). These amino-alkyl-cyclohexanes inhibited MES-induced convulsions in mice with ED_{50}s ranging from 3.6 to 53 mg/kg i.p. Six of these compounds had affinities, kinetics and voltage-dependency most similar to those of memantine and had good therapeutic indices (3 to 5) against MES-induced convulsions.

NMDA receptor antagonism by MRZ 2/579

Merz is presently pursuing the development of MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexane). MRZ 2/579 had similar blocking kinetics to those previously reported for memantine (K_{on} 10.7 and 7.5 * $10^4 M^{-1} sec^{-1}$, K_{off} 0.20 and 0.21 sec^{-1} at −70 mV for MRZ 2/579 and memantine respectively) (Parsons et al., 1998b). The potency of MRZ 2/579 assessed as $Kd = K_{off} / K_{on}$ = 1.87 μM agreed well with the IC_{50} of 1.29 μM against steady-state currents in cultured hippocampal neurones (at −70 mV) and with the Ki in [^{3}H]-MK-801 binding of 0.65 μM. The potency of both compounds against NMDA-induced

currents in freshly dissociated hippocampal neurones was slightly greater (IC_{50}s at −70 mV of 0.60 ± 0.05 μM and 0.66 + 0.03 μM for memantine and MRZ 2/579 respectively) (Tsintsadze et al., 1999).

The onset and offset kinetics of blockade following concentration jumps with memantine and MRZ 2/579 (both 10 μM at −70 mV) showed similar, double exponential kinetics: τ_{on}fast 86.9 ± 6.3 ms (64.7%) and 154.3 ± 21.3 ms (41.2%) respectively; τ_{on}slow 1,383 ± 122 ms and 1,352 ± 102 ms respectively; τ_{off}fast 834 ± 321 ms (22.9%) and 2,306 ± 974 ms (25.0%) respectively; τ_{off}slow 4,795 ± 921 ms and 7,616 ± 2,140 ms respectively (Parsons et al., 1998b). Voltage-dependency experiments indicated that MRZ 2/579 and memantine bind to the same NMDA receptor channel site (δ = 0.8) in cultured hippocampal neurones (Parsons et al., 1998b). In the continuous presence of MRZ 2/579 or memantine (10 μM) voltage-steps from −70 mV to +70 mV caused near complete relief of blockade with similar rapid, double exponential kinetics (MRZ 2/579: τ_{off1} = 121 ± 33 (39%), τ_{off2} = 953 ± 69 ms; Memantine: τ_{off1} = 99 ± 38 (44%), τ_{off2} = 725 ± 122 ms) (Parsons et al., 1998b). MRZ 2/579 and memantine showed a similar small degree of partial untrapping i.e. around 10 to 15% (Parsons et al., 1999a, 1999b).

In contrast to memantine and amantadine, MRZ 2/579 showed no selectivity between NMDA receptor subtypes expressed in *Xenopus* oocytes – IC_{50}s of 0.49 ± 0.11, 0.56 ± 0.01 μM, 0.42 ± 0.04 and 0.49 ± 0.06 μM on NR1a/NR2A, 2B, 2C and 2D respectively (Parsons et al., 1999a, 1999b). Memantine, MRZ 2/579 and amantadine all showed strong voltage-dependant block of NR1a/NR2A receptors with delta values of 0.74, 0.72 and 0.76 and predicted IC_{50}s at 0 mV of 9.76, 9.44 and 319 μM respectively. The voltage-dependency of all three compounds was also similar for NR1a/NR2B, NR2C and NR2D receptors (Spielmanns et al., 1998).

It might be predicted that moderate affinity uncompetitive NMDA receptor antagonists also block other Ca^{2+}-permeable channels. We therefore investigated the effects of MRZ 2/579 on non-differentiated voltage-activated Ca^{2+} channels (VACC) in cultured hippocampal neurones (Hesselink et al., 1999). Maximal inward Ca^{2+} currents following steps from −70 mV to −10 mV were antagonized by very high concentrations of MRZ 2/579 (IC_{50} = 340 ± 14 μM). Memantine and MRZ 2/579 were also tested against L- and N-type VACCs in freshly dissociated hippocampal neurones and P-type VACCs in freshly dissociated cerebellar Purkinje neurones and were both very weak antagonists (all IC_{50}s > 180 μM) (Tsintsadze et al., 1999). Memantine and MRZ 2/579 were also very weak antagonists of voltage-activated Na^+ channels in freshly dissociated DRG neurones (TTX sensitive and TTX-resistant, IC_{50}s > 100 μM for memantine and MRZ 2/579) (Tsintsadze et al., 1999).

Higher concentrations of memantine also block neuronal nicotinic receptors in cultured hippocampal neurones with an IC_{50} of 12.3 μM (Parsons et al., unpublished). Under similar conditions (Ach 1 mM in the presence of 1 μM atropine), MRZ 2/579 was even weaker as a neuronal nicotinic receptor antagonist with 10 μM only producing moderate block of peak responses (72.3 ± 3% of control) and no effect on steady-state responses (Hesselink et al.,

1999). MRZ 2/579 was also tested for antagonistic effects at AMPA and $GABA_A$ receptors (Hesselink et al., 1999). As previously reported for memantine (Chen et al., 1992; Parsons et al., 1993), MRZ 2/579 (10 to 100 μM) had no effect on inward currents to GABA (10 μM) or AMPA (100 μM).

MRZ 2/579 protected cultured cortical neurones against glutamate toxicity with an IC_{50} of 2.16 μM (Parsons et al., 1999a). MRZ 2/579 was also effective in protecting hippocampal slices against a 7 min. hypoxia / hypoglycaemia-induced reduction of fEPSP amplitude in CA1 with an EC_{50} of 7.01 μM (Parsons et al., 1999a). The potency of MRZ 2/579 was two fold higher than memantine (IC_{50} = 14.8 μM) but 20 fold lower than that of MK-801 (0.5 μM) in the same model (Frankiewicz et al., 1999). Comparison of the potencies of these three antagonists in this severe model of acute ischaemia to their potencies in inhibiting LTP in the same preparation, confirmed that memantine and MRZ 2/579 are 3 to 5 times superior to (+)MK-801 in differentiating between pathological and physiological activation of NMDA receptors (Frankiewicz et al., 1996, 1999; Frankiewicz and Parsons, 1999a). However, it should be noted that the absolute therapeutic indices of memantine and MRZ 2/579 were also quite low, although certainly not nearly as low as that of (+)MK-801, which reflects the severity of this *in vitro* model of severe ischaemic stroke.

MRZ 2/579 was also a selective, systemically-active NMDA antagonist *in vivo* and blocked responses of single spinal neurones to microelectrophoretic NMDA with an ID_{50} of 4.1 mg/kg i.v. whilst having far less effect on responses to AMPA (ID_{50} > 30 mg/kg i.v.) (Headley, P.M. pers. comm.). Memantine shows similar potency and selectivity in the same model under similar conditions i.e. against control NMDA responses of moderate intensity (see Parsons et al., 1999b). MRZ 2/579 inhibited MES-induced convulsions in mice with an ED_{50} of 3.6 mg/kg i.p. It was slightly less potent after s.c. administration (ED_{50} = 4.6 mg/kg) and more potent after i.v. injection (ED_{50} = 2.5 mg/kg) (Parsons et al., 1999a). Following p.o. treatment it was less active i.e. ED_{50} = 13.6 mg/kg (Parsons et al., 1999a).

MRZ 2/579 administered at 10 mg/kg resulted in peak plasma concentrations of 5.3 and 1.4 μM following i.v. and p.o. administration respectively, which then declined with a half life of around 170–210 min (Parsons et al., 1999a). Analysis of A.U.C. concentrations indicates a p.o./i.v. bio-availability ratio for MRZ 2/579 of 60%. MRZ 2/579 injected i.p. at a dose of 5 mg/kg resulted in peak ECF concentrations of 0.70 μM as measured by brain microdialysis, while plasma levels were c.a. 2 times higher (Hesselink et al., 1999). In summary, MRZ 2/579 has similar potency and bio-availability to memantine *in vivo*. However, a slightly higher acute dose of 10 mg/kg i.p. seems to be the maximal relevant dose for use in rats due to the somewhat shorter half life.

Initial behavioural characterization of MRZ 2/579 in animal models of diseases involving disturbances of the glutamatergic system provide strong support for the assumption that MRZ 2/579 could be a useful therapeutic in morphine/alcohol dependence, inhibition of morphine tolerance, chronic pain and as a neuroprotective agent (see Danysz and Parsons, 1999).

In conclusion, MRZ 2/579 was initially selected for further characterisation on the basis of it's biophysical properties which are very similar to those of memantine. The validity of this choice was confirmed in several *in vivo* models of disturbances in glutamatergic transmission where MRZ 2/579 has a very similar promising profile to memantine. MRZ 2/579 (40 mg p.o.) also showed very good tolerability in initial phase I trials in healthy individuals and Merz is pursuing the development of this compound. We predict that MRZ 2/579 could be a useful therapeutic in a wide range of CNS disorders proposed to involve disturbances of glutamatergic transmission. If this prediction finds verification, then it will provide further support for the concept that moderate affinity uncompetitive NMDA receptor antagonists represent a promising class of potential therapeutics for numerous CNS disorders.

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