

A novel class of amino-alkylcyclohexanes as uncompetitive, fast, voltage-dependent, N-methyl-D-aspartate (NMDA) receptor antagonists – *in vitro* characterization

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Summary. The fact that potent NMDA receptor channel blockers produce phencyclidine-like psychotropic symptoms in man and rodents implies that uncompetitive antagonism of NMDA receptors may not be a promising therapeutic approach. However, recent data indicate that agents with moderate affinity such as memantine and neramexane (MRZ 2/579) are useful therapeutics due to their strong voltage-dependency and rapid unblocking kinetics. Merz has developed a series of novel uncompetitive NMDA receptor antagonists based on an amino-alkylcyclohexane structure. These compounds displaced [³H]-MK-801 binding to rat cortical membranes with K_i values between 1 and 100 μM and inward current responses of cultured hippocampal neurons to NMDA were antagonized in a strongly voltage-dependent manner with rapid blocking/unblocking kinetics. Three of these compounds, with similar biophysical properties to memantine, were chosen for development. MRZ 2/759 (1-ethenyl-3,3,5,5-tetramethyl-cyclohexylamine), 2/1010 (1,3,3,5-tetramethyl-6-azabicyclo[3.2.1]octane) and 2/1013 (8,8,10,10-tetramethyl-1-azaspiro[5.5]undecane) displaced [³H]-MK-801 binding with K_i values of 1.18, 2.59 and 3.64 μM , respectively. They were similarly potent against NMDA-induced currents in hippocampal neurons – IC_{50} values of 1.51, 3.06 and 2.20 μM , respectively. In line with their moderate affinity, all were voltage-dependent ($\delta = 0.86, 0.96$ and 0.89 , respectively) and fast, open-channel blockers (k_{on} 7.90, 1.70 and $2.60 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$, k_{off} 0.13, 0.12 and 0.24 sec^{-1} , respectively). These compounds are also NMDA receptor antagonists in the CNS following systemic administration and have good therapeutic indices in a variety of *in vivo* behavioural models where glutamate is known to play a pivotal role. In view of their relatively low affinity and associated rapid kinetics, they should prove to be useful therapeutics in a wide range of CNS disorders.

Keywords: Memantine; neramexane; N-methyl-D-aspartate NMDA; patch clamp; potency; kinetics; voltage-dependency; selectivity; hippocampal neurons

Introduction

N-methyl-D-aspartate (NMDA) receptors are one class of ionotropic glutamate receptors that have been extensively studied over the past two decades (Danysz and Parsons 1998; Parsons et al. 1998; Dingledine et al. 1999; Gardoni and Di Luca 2006). Animal models indicate that antagonists of these receptors could find therapeutic utility in numerous neurological and psychiatric diseases (Danysz and Parsons 1998; Parsons et al. 1998). However, the fact that potent NMDA receptor channel blockers and competitive NMDA receptor antagonists produce phencyclidine-like psychotropic symptoms in man and rodents has lead some to assume that such uncompetitive antagonism of NMDA receptors is not a promising therapeutic approach (Leppik et al. 1988; Sveinbjornsdottir et al. 1993). Attempts have been made to circumvent these side effects by addressing different modulatory sites of the NMDA receptor such as the strychnine-insensitive glycine (glycine_B) (Danysz and Parsons 1998; Parsons 2001; Tuominen et al. 2005) and NR2B selective polyamine sites (Parsons 2001; Danysz and Parsons 2002; Loftis and Janowsky 2003; Chizh and Headley 2005; Gardoni and Di Luca 2006). However, recent data indicate that uncompetitive antagonists with moderate affinity such as memantine (1-amino-3,5-dimethyl-adamantane) and neramexane (1-amino-1,3,3,5,5-pentamethyl-cyclohexane, MRZ 2/579) could be useful therapeutics due to their strong voltage-dependency and rapid unblocking kinetics (Parsons et al. 1999a, b; Danysz et al. 2002; Danysz and Parsons 2003; Rogawski

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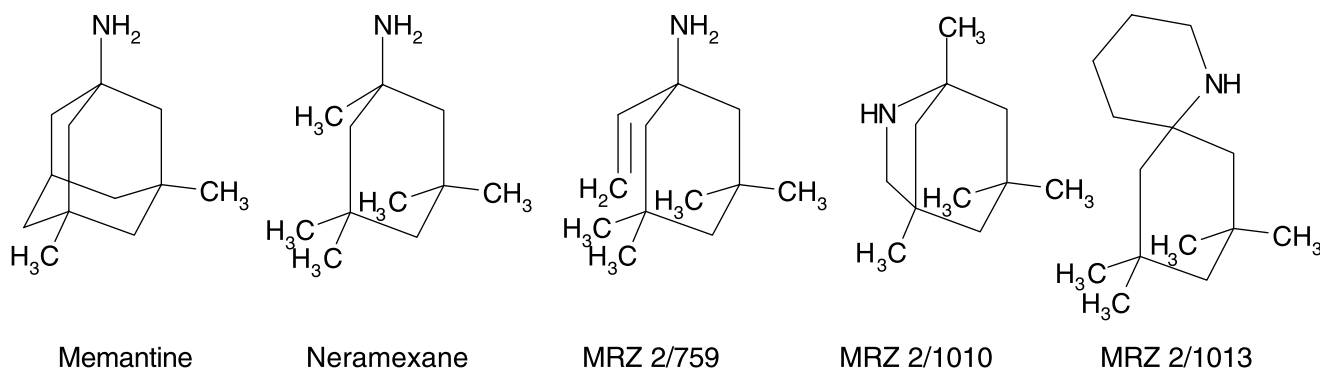


Fig. 1. Chemical structures of memantine, neramexane, MRZ 2/759, 2/1010, and 2/1013

and Wenk 2003; Johnson and Kotermanski 2006; Lipton 2006).

Indeed, memantine was recently registered in both Europe and the USA for the treatment of moderate to severe Alzheimer's disease (Reisberg et al. 2003; Tariot et al. 2004). Similarly, predicted therapeutic doses of neramexane were very well tolerated in male volunteers and this agent is presently in phase II clinical trials for 4 different indications (Alzheimer's disease, drug abuse, chronic pain and depression) (Danysz et al. 2002). Merz has developed a series of novel uncompetitive NMDA receptor antagonists based on an amino-alkylcyclohexane scaffold. The structures of MRZ 2/759 (1-ethenyl-3,3,5,5-tetramethyl-cyclohexylamine), 2/1010 (1,3,3,5-tetramethyl-6-azabicyclo[3.2.1]octane) and 2/1013 (8,8,10,10-tetramethyl-1-azaspiro[5.5]undecane) are shown in Fig. 1. Provisional data on these compounds were presented at the Society for Neuroscience meeting in 2003 (Jatzke et al. 2003).

The aims of this study were as follows:

1. To characterize the electrophysiological properties and the possible mode of action of the novel amino-alkylcyclohexanes MRZ 2/759, 2/1010 and 2/1013 on hippocampal neurons.
2. To compare these compounds with the well characterized moderate affinity NMDA receptor blockers memantine and neramexane.
3. To evaluate the potential of these compounds as useful therapeutics in combination with *in vivo* data.

Methods

[³H]-MK-801-Binding

The displacement of [³H]-(+)-MK-801 binding to cortical membranes is described in detail in (Parsons et al. 1999a).

Cell culture

Hippocampi were obtained from rat embryos (E20 to E21) and were then transferred to Ca²⁺ and Mg²⁺ free Hank's buffered saline on ice. Cells were

mechanically dissociated in 0.05% DNase/0.3% ovomucoid following 8 min pre-incubation with 0.66% trypsin/0.1% DNase. The dissociated cells were then centrifuged at 18 g for 10 min, re-suspended in minimum essential medium (MEM) and plated at a density of 150,000 cells cm⁻² onto poly-DL-ornithine/Laminin – precoated plastic Petri dishes. The cells were nourished with NaHCO₃/HEPES-buffered MEM supplemented with 5% foetal calf serum and 5% horse serum (Gibco) and incubated at 37 °C with 5% CO₂ at 95% humidity. The medium was exchanged completely following inhibition of further glial mitosis with ARAC (5 μM) after 5 days *in vitro*.

Patch clamp recordings

Patch clamp recordings were made from these neurons after 12–15 days *in vitro* with glass electrodes (3–5 MΩ) in the whole cell mode at room temperature with the aid of an EPC-7 amplifier (List). Test substances were applied by switching channels of a modified fast perfusion system (Warner instruments) with a common outflow (15–20 msec exchange times). The intracellular solution normally consisted of (mM): CsCl (120), TEACl (20), EGTA (10), MgCl₂ (1), CaCl₂ (0.2), glucose (10), ATP (2), cAMP (0.25) adjusted to a pH of 7.3 with CsOH. The extracellular solutions had the following basic composition (mM): NaCl (140), KCl (3), CaCl₂ (0.2), glucose (10), HEPES (10), sucrose (4.5). Neurons were pharmacologically isolated from one another by the addition of 0.3 μM tetrodotoxin (TTX) to block voltage-activated sodium currents and, in all cases except selectivity experiments, 25 μM bicuculline methobromide to block spontaneous γ-aminobutyric acid (GABA) mIPSPs. Test substances were added to this basic solution in concentrations detailed in results. In addition, the bath solution contained 1.5 mM CaCl₂ to aid formation of a “giga ohm seal”. D-serine (10 μM) was present in all solutions. All results from stable cells were accepted for inclusion in the final analysis i.e. showing at least 70% recovery of the responses to NMDA (200 μM) following removal of the antagonist tested. Despite this, the recovery from drug actions was not always 100% because of rundown in some cells. When present, this was compensated by basing the percentage antagonism at each concentration on both control and recovery and assuming a linear time course for this rundown.

Concentration-dependence

Experiments were performed by applying five, cumulatively-increasing concentrations of the Merz compounds (1/2 log intervals progression from 0.3 to 30 μM), each for 15 sec, in the presence of NMDA (200 μM) for 120 sec with a constant holding potential of –70 mV. IC₅₀ values were calculated according to the logistic equation (percentage control = 100%/1 + (concentration/IC₅₀)^S where S is the Hill slope) using GraFit 5.0.

Kinetics

Kinetic experiments were performed by applying various, separate concentrations of the novel compounds (1/2 log intervals progression from 0.3 to

30 μM for MRZ 2/759 and 2/1013 and from 1.0 to 100 μM for MRZ 2/1010) for 20 sec in the presence of NMDA (200 μM) for 70 sec in neurons held at a constant potential of -70 mV. Exponential fits were made using the program TIDA for windows. Most responses were better fit by a double exponential.

Such double exponential fits were integrated to single exponentials according to the following relationship $[(\text{tfast} \times \text{weightfast}) + (\text{tslow} \times \text{weightslow})] / (\text{weightfast} + \text{weightslow})$. Steady-state blockade was also determined from these recordings.

Selectivity

In selectivity experiments, NMDA (200 μM) was applied for 4 sec every 20 sec at -70 mV and this cycle was repeated nine times. Compounds were continuously present from records 4 to 6. An identical cycle was made with AMPA (100 μM , 2 sec) and GABA (10 μM , 4 sec). D-Serine (10 μM) was continuously present for all agonists.

Voltage-dependency

Block by MRZ 2/759, 2/1010 or 2/1013 (10 or 30 μM) at various holding potentials in cultured hippocampal neurons was used to determine the voltage-dependency of this effect. NMDA (200 μM) was applied for 55 sec every 65 sec at different holding potentials from -70 to $+70$ mV in 10 mV increments. MRZ 2/759, 2/1013 (10 μM) or MRZ 2/1010 (30 μM) were applied for 15 sec. Pooled data were fit by the following equation:

$$\text{Fractional current} = (1 - \beta) [1 + [\text{MRZ compound}] / \text{IC}_{50} (0 \text{ mV}) \exp(-z\text{dFV} / \text{RT})]^{-1}$$

Xenopus oocytes

The cRNAs encoding the NR1a and NR2A subtypes were a generous gift of Prof. J. P. Ruppersberg, University of Tübingen, Germany. Mature female *Xenopus laevis* were anaesthetized in 0.2% Tricaine on ice for 15 min prior to surgery. Oocytes were removed and incubated in 2 mg/ml collagenase (type II) in Ca^{2+} -free oocyte Ringer solution (mM) (NaCl (82.5), KCl (2), MgCl_2 (2), HEPES (5) pH 7.5) for 30 min at room temperature and washed thoroughly with OR-2 (NaCl (100), KCl (2), MgCl_2 (1) CaCl_2 (2), HEPES (5) pH 7.5). The remaining follicle cell layer was removed manually with fine forceps and the oocytes were kept in OR-2. The cRNA was dissolved in DEPC-treated, sterile distilled water. The NR1a subunit was mixed 1:1 with NR2A cRNA and fifty nanoliters was injected in the oocyte's cytoplasm using a Nanoliter Injector (World Precision Instruments). The oocytes were incubated at 19°C in OR-2 for the following 3–6 days.

Electrophysiological responses were obtained using the standard two-electrode voltage-clamp method (GeneClamp 500 amplifier), 3–6 days after injection. The electrodes had a resistance between 0.2 and 1 M Ω and were filled with 3 M KCl. The perfusion system was a modified Oocyte Carousel system which allows rapid wash in and washout of agonist and antagonist (exchange times less than 1 sec). The bath solution was prepared Ca^{2+} -free,

to avoid Ca^{2+} -induced Cl-currents: (mM) NaCl (100), KCl (2), HEPES (5), BaCl_2 (2), pH 7.35. NMDA receptor blockade by antagonists was determined by applying various concentrations (0.1–100 μM in a log 3 dosing regime) for 10 sec in the continuous presence of glutamate (100 μM and glycine 10 μM) at -70 mV for 100 sec.

Results

The three amino-alkyl-cyclohexane derivatives tested displaced [^3H]-MK-801 binding to rat cortical membranes and antagonized inward current responses of cultured hippocampal neurons with moderate affinity, comparable to memantine or neramexane.

MRZ 2/759, 2/1010, and 2/1013 displaced [^3H]-MK-801 binding with IC_{50} s of 2.59, 5.69 and 8.00 μM , respectively. The corresponding K_i values were 2.2 fold lower $1.18 \pm 0.20 \mu\text{M}$, $2.59 \pm 0.17 \mu\text{M}$, and $3.64 \pm 0.22 \mu\text{M}$ (values indicate means \pm SEM; $n = 3-6$, Fig. 2, Table 1). MRZ 2/759 had a very similar potency to memantine ($K_i = 0.97 \pm 0.11 \mu\text{M}$) and neramexane ($K_i = 0.80 \pm 0.09 \mu\text{M}$),

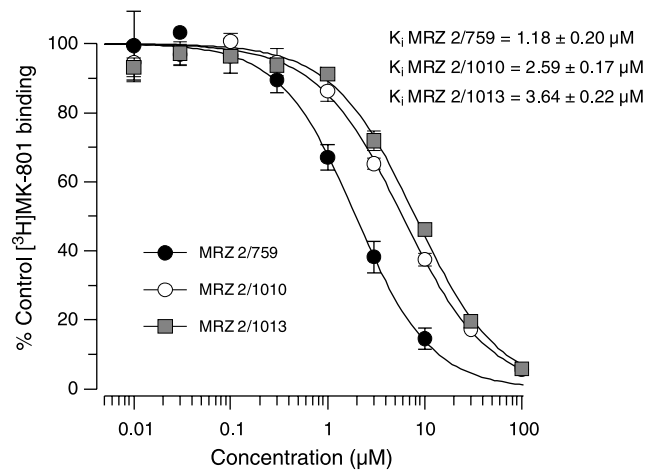


Fig. 2. Displacement of [^3H]MK-801 binding by MRZ 2/759, 2/1010, and 2/1013. MRZ 2/759, 2/1010, and 2/1013 displaced [^3H]-MK-801 binding with IC_{50} s of 2.59, 5.69 and 8.00, respectively. The corresponding K_i values were 2.2 fold lower $1.18 \pm 0.20 \mu\text{M}$, $2.59 \pm 0.17 \mu\text{M}$ and $3.64 \pm 0.22 \mu\text{M}$ ($n = 3-6$). MRZ 2/759 had a very similar potency to memantine ($K_i = 0.97 \pm 0.11 \mu\text{M}$) and neramexane ($K_i = 0.80 \pm 0.09 \mu\text{M}$), whereas MRZ 2/1010 and 2/1013 were somewhat less potent

Table 1. Summary of the potency of the Merz compounds at NMDA receptors

Substance	Cumulative DRC				Kinetic				[^3H]MK-801	
	IC_{50} (μM)	SEM	Hill	SEM	IC_{50} (μM)	SEM	Hill	SEM	K_i (μM)	SEM
Memantine	1.56	0.17	0.80	0.04	1.27	0.08	0.93	0.06	0.97	0.11
Neramexane	1.21	0.04	0.88	0.02	0.85	0.05	0.81	0.03	0.80	0.09
MRZ 2/759	1.51	0.04	0.92	0.02	1.15	0.11	1.00	0.09	1.18	0.20
MRZ 2/1010	3.06	0.31	0.99	0.08	2.84	0.23	0.90	0.06	2.59	0.17
MRZ 2/1013	2.20	0.28	1.02	0.10	4.29	0.35	1.14	0.09	3.64	0.22

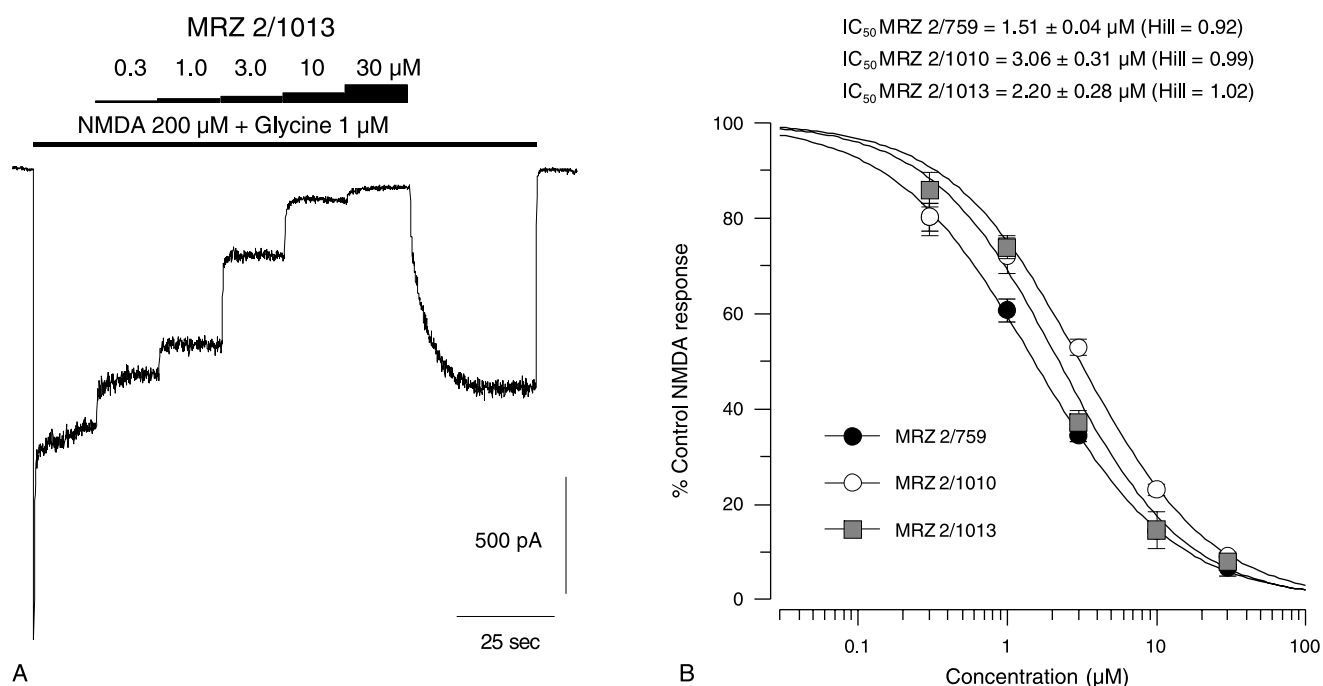


Fig. 3. Concentration-dependence of cumulatively increasing concentrations of MRZ 2/759, 2/1010, and 2/1013 on NMDA current of cultured hippocampal neurons at a holding potential of -70 mV . **A** Representative block of NMDA-mediated current by applying five, cumulatively-increasing concentrations of MRZ 2/1010 ($0.3\text{--}30\ \mu\text{M}$). **B** Concentration-dependence of the blockade of NMDA receptors by MRZ 2/759, 2/1010, and 2/1013. Plateau current responses were normalized to control levels and plotted as means ($\pm\text{SEM}$) against the antagonist concentration ($n=5\text{--}10$ per concentration)

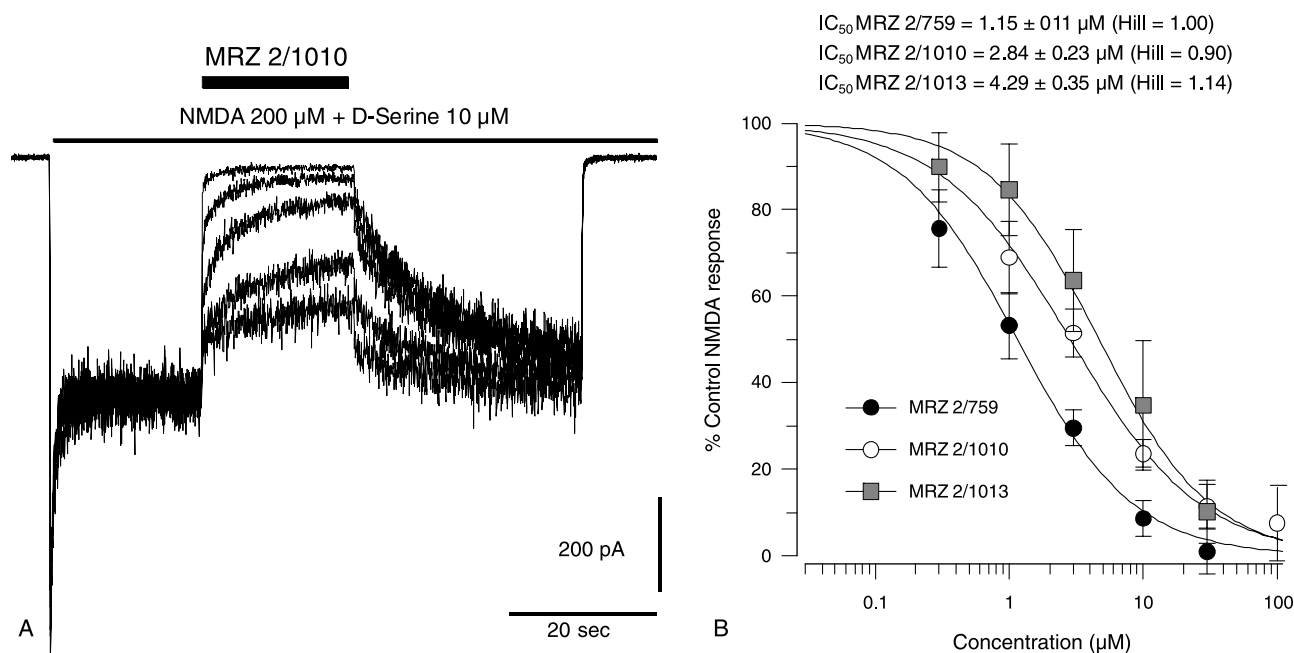


Fig. 4. Concentration-dependence of MRZ 2/759, 2/1010, and 2/1013 on NMDA current of cultured hippocampal neurons at a holding potential of -70 mV in kinetic experiments. **A** Fast, concentration-dependent block of inward current responses of a single cultured hippocampal neuron to NMDA ($200\ \mu\text{M}$) at a constant holding potential of -70 mV . Various concentrations of MRZ 2/1010 ($1\text{--}100\ \mu\text{M}$) were applied for 20 sec in the presence of NMDA in the continuous presence of D-Serine ($10\ \mu\text{M}$). **B** Steady-state blockade was determined by averaging the current responses over the last 1 sec of the relevant substance application section. Control currents were the average of NMDA-induced responses determined prior and following removal of MRZ compounds. All pooled data were fit using the logistic equation ($n=4\text{--}8$)

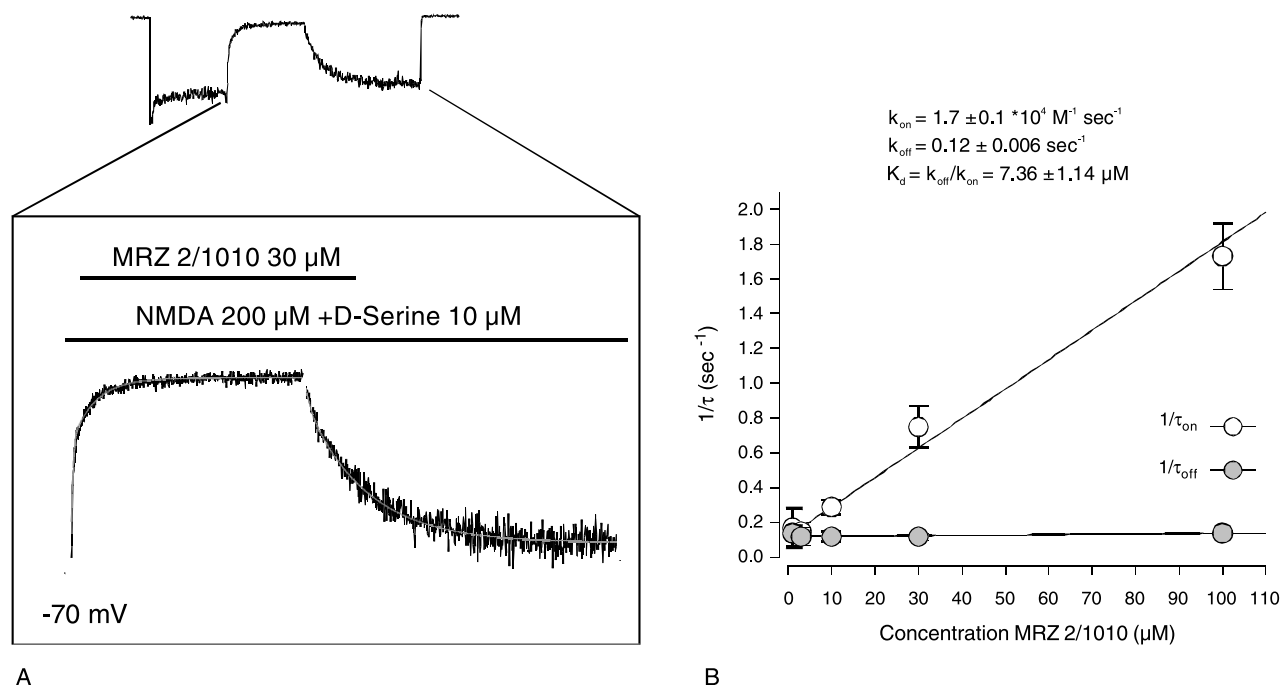


Fig. 5. Kinetics of the NMDA open-channel block by MRZ 2/1010. **A** Enlargement of the current response to NMDA by applying 30 μM MRZ 2/1010 at a holding potential of -70 mV. Exponential fits were made using TIDA for windows. Most responses were better fit by a double exponential. Such double exponential fits were integrated to single exponentials according to the following relationship $[(\text{tfast} \times \text{weightfast}) + (\text{tslow} \times \text{weightslow})]/(\text{weightfast} + \text{weightslow})$. **B** Pooled data for 7 neurons. $1/\text{toff}$ and $1/\text{ton}$ were plot against concentration of MRZ 2/1010 to determine k_{off} (y intercept) and k_{on} (slope), respectively. K_d is given by $k_{\text{off}}/k_{\text{on}}$

whereas MRZ 2/1010 and 2/1013 were somewhat less potent.

In patch clamp experiments, using cultured hippocampal neurons with cumulatively-increasing antagonist concentrations at a holding potential of -70 mV, all three agents showed a concentration-dependent block of NMDA-induced currents with similar potencies to those seen in the binding experiments, and the Hill coefficients were close to unity. MRZ 2/759, 2/1010 and 2/1013 had IC_{50} s of 1.51, 3.06 and 2.20 μM , respectively ($n = 5-10$, Fig. 3, Table 1). Again, these values were similar to those achieved with memantine (1.56 μM , $n = 9$) and neramexane (1.21 μM , $n = 10$) under identical conditions.

The kinetics of NMDA receptor blockade were addressed in different patch clamp experiments with applications of separate concentrations of the novel compounds (1/2 log intervals progression from 0.3 to 30 μM for MRZ 2/759 and 2/1013 and from 1.0 to 100 μM for MRZ 2/1010) for 20 sec in the presence of NMDA (200 μM) at -70 mV. This approach is technically somewhat more difficult than cumulative concentration-response curves because the neurons have to be recorded from for longer, but it has the advantage that receptor run-down is somewhat less of a problem and that the kinetics of blockade

can be addressed for each concentration tested. However, the potency of all three agents was very similar to that seen in the cumulative concentration-response curve experiments. MRZ 2/759, 2/1010 and 2/1013 had IC_{50} s of 1.15, 2.84 and 4.29 μM , respectively ($n = 6-7$, Fig. 4, Table 1) and under identical conditions, the values for memantine and neramexane were 1.27 μM and 0.85 μM , respectively ($n = 6$ each).

In these experiments, the kinetics of blockade were rapid and in line with the moderate affinity of these agents for the channel site. This is illustrated for MRZ 2/1010 in Fig. 5. For all compounds tested, the rates of onset were concentration-dependent, whereas the offset rates were fast but concentration-independent (Table 2). The onset kinetics

Table 2. Summary of the kinetics of NMDA receptor blockade by the Merz compounds

Substance	k_{on} ($10^4 \text{M}^{-1} \text{sec}^{-1}$)	SEM	k_{off} (sec^{-1})	SEM	K_d (μM)	SEM
Memantine	5.94	0.35	0.125	0.019	2.10	0.45
Neramexane	9.30	0.90	0.120	0.008	1.33	0.20
MRZ 2/759	7.90	1.11	0.129	0.026	1.63	0.74
MRZ 2/1010	1.69	0.13	0.120	0.006	7.36	1.14
MRZ 2/1013	2.63	0.20	0.239	0.009	8.87	1.29

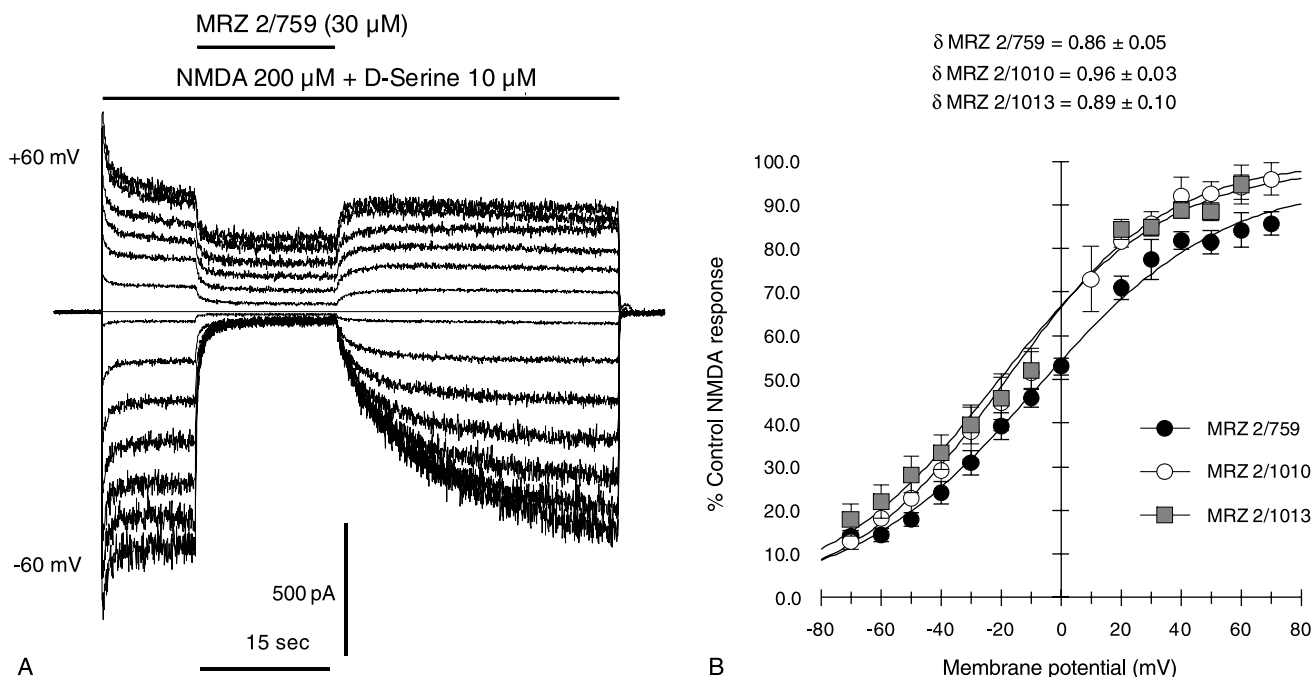


Fig. 6. Voltage-dependent block by MRZ 2/759, 2/1010, and 2/1013. **A** Original patch clamp data for a single cultured hippocampal neuron. NMDA (200 μM) was applied for 55 sec every 65 sec at different holding potentials from -60 to $+60$ mV in 10 mV increments. MRZ 2/1010 (30 μM) was applied for 15 sec as indicated by the bar. **B** Pooled data from at least 6 neurons were well fit by the following equation:

$$\text{Fractional current} = (1 - \beta)[1 + [\text{MRZ 2/1010}]/\text{IC}_{50}(0 \text{ mV}) \exp(-z\text{dFV}/RT)]^{-1}$$

For MRZ 2/1010, the fraction of voltage-independent sites (β) was 0.0 i.e. 0%, the fraction of the electric field sensed by the voltage-dependent site (δ) was 0.96 and the IC_{50} (0 mV) was 56.4 μM . Other parameters have their normal meaning

(k_{on}) for MRZ 2/759, 2/1010 and 2/1013 were 7.90, 1.69 and $2.63 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$, respectively, and similar to values for memantine ($5.94 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$) and neramexane ($9.30 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$). The offset kinetics (k_{off}) for MRZ 2/759, 2/1010 and 2/1013 were 0.129, 0.120 and 0.239 sec^{-1} . Values for memantine (0.125 sec^{-1}) and neramexane (0.120 sec^{-1}) were similar but somewhat slower than previously published for memantine and neramexane (around 0.2 sec^{-1}) but this might be due to the use of D-serine in the present study.

The calculated K_d values ($k_{\text{off}}/k_{\text{on}}$) were similar both to IC_{50} values determined at equilibrium in this set of experiments, and the IC_{50} values determined by applying the cumulative concentration response curve. K_d values for MRZ 2/759, 2/1010, 2/1013, memantine and neramexane were 1.63, 7.36, 8.87, 2.10 and 1.33 μM , respectively.

Further support for uncompetitive receptor antagonism was provided by the fact that the block of NMDA-induced responses was strongly voltage-dependent with comparable values of the fraction of the electric field sensed by the voltage-dependent site (δ) to memantine or neramexane (0.83–0.96). This is illustrated for MRZ 2/759 in Fig. 6

Table 3. Summary of the voltage-dependence of NMDA receptor blockade by the Merz compounds

Substance	IC_{50} (0 mV) (μM)	SEM	δ	SEM
Memantine	17.35	1.78	0.83	0.04
Neramexane	16.99	2.09	0.96	0.04
MRZ 2/759	13.12	1.57	0.86	0.05
MRZ 2/1010	59.35	4.22	0.96	0.03
MRZ 2/1013	20.82	4.65	0.89	0.10

and pooled values for all three agents are compared to memantine and neramexane under identical conditions in Table 3 ($n = 5-7$ each).

As with memantine and neramexane, the offset kinetics of all three novel compounds became faster at depolarized potentials and were around 1.0 sec at $+60$ mV. This voltage-dependence of offset kinetics was most pronounced for MRZ 2/759. This aspect is very important for the proposed mechanism of action (MOA)/tolerability of memantine – see Discussion. Onset kinetics were slower at positive potentials but this effect was less consistent and pronounced and is, in fact, of less significance for the proposed MOA. However, in the case of MRZ 2/759 and 2/1010, it was associated with an decrease in the weight of

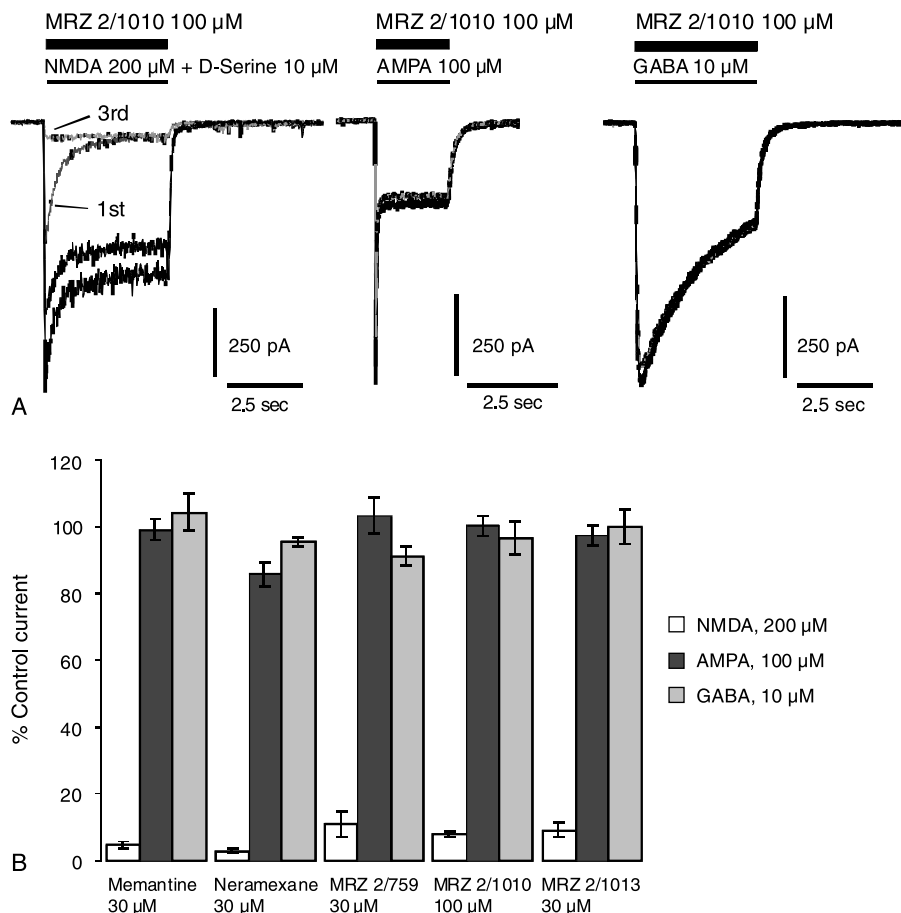


Fig. 7. Selectivity of memantine, neramexane, MRZ 2/759, 2/1010, and 2/1013 on cultured hippocampal neurons. **A** Selective NMDA antagonistic effect of MRZ 2/1010 on current responses of a single hippocampal neuron. NMDA (200 μM, 4 sec), AMPA (100 μM, 2 sec), or GABA (10 μM, 4 sec) were applied at a constant membrane potential of -70 mV. The black traces show control and recovery responses to the agonist tested. Grey traces show equilibrium effects of MRZ 2/1010. The open-channel blockade of MRZ 2/1010 is clearly apparent for the first NMDA induced response after 20 sec of pre-incubation with MRZ 2/1010 the peak current response was only partially blocked. The grey traces show agonist responses at the third applications of 100 μM MRZ 2/1010. **B** Equilibrium current responses in the presence of memantine (30 μM), neramexane (30 μM), MRZ 2/759 (30 μM), 2/1010 (100 μM), or 2/1013 (30 μM) as percentage of control. The current responses were averaged over the last second of agonist application. Pooled data from 4–7 cells are presented as means \pm SEM

the fast component of a double exponential fit and also with an absolute decrease in the speed of this component (not shown).

High concentrations of MRZ 2/759, 2/1010 and 2/1013 blocked NMDA-induced responses, but did not affect responses to AMPA or GABA. This selectivity is illustrated for a representative cell with MRZ 2/1010 in Fig. 7A. From this recording, the use-dependency of this open channel block is also apparent i.e. the peak of the first response to NMDA following a 20 sec application was not fully blocked, and antagonism developed during the course of this response giving the impression of receptor desensitization. This was unlikely to be due to interactions with the glycine_B site – antagonists of which induce receptor desensitization – because the third response to NMDA in the

continuing presence of antagonist showed equal block of both peak and plateau components. Also, the first response following removal of antagonist showed characteristic unblocking kinetics (not shown). Similar effects were seen with all three agents as well as with memantine and neramexane, and the pooled data are illustrated in Fig. 7B ($n = 5-7$ each).

Xenopus oocytes

Memantine and neramexane antagonized glutamate-induced currents at NR1a/2A receptors expressed in *Xenopus oocytes* with IC₅₀s of 1.05 ± 0.11 and 1.84 ± 0.19 μM, respectively. It should be noted that these values are somewhat different to those previously published – especially

those for neramexane. The rank order of the potencies of MRZ 2/759, 2/1010 and 2/1013 were also a little different to the values obtained in cultured hippocampal neurons with MRZ 2/1010 being the most potent – IC₅₀s were 3.29 ± 0.61, 0.76 ± 0.12 and 4.72 ± 0.75 μM, respectively. This may be indicative of somewhat different subtype selectivities of the three agents but this point has not yet been addressed.

Discussion

The properties of all three novel amino-alkylcyclohexanes were very similar to the open-channel blockers memantine and neramexane (Parsons et al. 1999a). All agents were selective, moderate affinity, uncompetitive NMDA receptor antagonists with strong voltage-dependency and rapid blocking kinetics. There was some variability in their potencies, as would be expected with any biological system. We took a somewhat unconventional approach and averaged the potencies from all assays used to come up with single values for memantine (IC₅₀ = 1.37 μM) and neramexane (IC₅₀ = 0.95 μM). From this analysis, it is apparent that MRZ 2/759 was the closest in potency (IC₅₀ = 1.28 μM) to memantine and neramexane whereas MRZ 2/1010 (IC₅₀ = 2.83 μM) and MRZ 2/1013 (IC₅₀ = 3.38 μM) were somewhat less potent.

Moderate affinity and associated strong voltage-dependency and rapid unblocking kinetics have been proposed to underlie the good therapeutic utility of memantine in the treatment of Alzheimer's disease. This agents has been hypothesized to act as a more potent surrogate for Mg²⁺ ions which gate the NMDA receptor channel under normal physiological conditions (Danysz and Parsons 2003; Parsons et al. 1999b). Central to this hypothesis is the assumption that under chronic but mild pathological conditions occurring in chronic excitotoxic diseases such as Alzheimer's disease, there is a continuous mild energy deficit which is associated with moderate plasma membrane depolarization and subsequent relief of NMDA receptor gating by Mg²⁺. This allows a continuous slow influx of Ca²⁺ into the cell via NMDA receptors which ultimately leads to cell death. Under these conditions, memantine can more effectively block the NMDA receptor because its voltage-dependency is not as pronounced as that of the divalent cation Mg²⁺. However, the voltage-dependency of memantine is sufficient and the offset kinetics are fast enough to allow it to leave the NMDA receptor during transient but strong membrane depolarization occurring during normal physiological release of synaptic glutamate. In contrast, high affinity antagonists

such as dizocilpine ((+)-MK-801) and phencyclidine (PCP) have weak apparent voltage-dependency, associated with very slow unblocking kinetics. They cannot leave the NMDA receptor during physiological activation and therefore cannot differentiate between pathological and physiological activation.

The novel agents tested in the present study have been shown to be effective anticonvulsants in the mouse maximal electroshock test (MES) with ED₅₀ values of 3.66, 5.99 and 9.39 mg/kg i.p. and their therapeutic indices, compared to ataxic/sedative effects, are 2.9, 3.0 and 3.7, respectively. These values are very close to those reported previously for memantine (ED₅₀ = 6.9 mg/kg i.p. and TI = 2.5–2.9) and neramexane (ED₅₀ = 3.6 mg/kg i.p. and TI = 2.9–5.0, see Parsons et al. 1999a) and are clearly superior to compounds like dizocilpine which have TIs close to or less than unity. If this hypothesis is true, then three novel agents should have superior therapeutic indices in various animal models of diseases involving disturbances in glutamatergic neurotransmission. This is indeed the case. All three compounds may therefore be useful therapeutics in the treatment of dementia and or other CNS disorders.

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